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Article *in* Advances in applied microbiology · February 2004

DOI: 10.1016/S0065-2164(04)55002-5 · Source: PubMed

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Fungal Contamination as a Major Contributor to Sick Building Syndrome

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I. Introduction

Fungi are heterotrophic eukaryotes producing exoenzymes and absorbing their nutrients by a network of hyphae and reproducing through development of spores. They belong to Kingdom Eumycota (Kingdom of Fungi) or Kingdom Chromista (Kendrick, 2000). However, there is one group of organisms, which are traditionally studied by mycologists, called pseudofungi (such as slime molds in myxomycetes), that belong to Kingdom Protozoa (Kirk *et al.*, 2001). Fungi are a very large, diverse, and heterogeneous group of organisms found in nearly every ecological niche (Alexopoulos *et al.*, 1996). They play a very important role in our ecosystem and our daily life. Fungi always play dual roles on the earth: (a) a positive one as food, medicine, key components in food processing, decomposers breaking down organic matters to recycle the nutrients in the ecosystem and to form symbiotic

ADVANCES IN APPLIED MICROBIOLOGY, VOLUME 55

0065-2164/04 $35.00

relationship with other organisms; (b) a negative one as pathogens to humans, plants, and animals; as allergens, producing secondary metabolites, mycotoxins, fungal volatile organic compounds (VOCs); and as glucans, which are detrimental to human health and building occupants (Batterman, 1995; Ezeonu *et al.*, 1994; Miller, 1992, 1993). A large number of fungi are saprophytes or decomposers, which mainly occur in natural environments (outdoors) such as soil and plant debris. Some of these fungi can be found in indoor environments. One key factor that we should keep in mind is that most indoor fungi originate from the outdoor environment. Certain indoor fungal contaminants pose a potential health risk to building occupants and may lead to sick building syndrome (Gravesen *et al.*, 1994; Miller, 1992, 1993; Samson *et al.*, 1994). Indoor fungi have attracted unprecedented attention because of their potential health effects on humans in the last decade. Public awareness of indoor fungi in return generates more research to elucidate their roles in indoor environments and human health. Indoor fungus is not only a scientific issue but is also becoming a social issue. Public awareness does not automatically mean a good understanding of the indoor molds. There are still many key questions that need to be answered to have a better understanding of the indoor mold issue.

This chapter reviews available literature on fungal contamination as a major contributor to sick building syndrome.

II. Effects of Indoor Fungi on Human Health

A. Fungal Allergies and Allergenic Respiratory Diseases

Allergy (Gk *allos*, other; *ergon*, work) is a disease or reaction caused by an immunoglobin E (IgE)-mediated immune response to one or more environmental agents, resulting in tissue inflammation and organ dysfunction, and an exaggerated and pathological variant of a normal immune mechanism (Klein, 1990; Middleton, Jr. *et al.*, 1988; Paul, 1989; Raven and Johnson, 1986). Fungal spores are a well known cause of allergic diseases (Chapman, 1999; Gravesen, 1979; Horwitz and Bush, 1997) and were identified as one of the major indoor allergens (Burr, 1999; Pope *et al.*, 1993; Ruotsalainen *et al.*, 1995). Allergy is common throughout the world. The prevalence of sensitivity to specific allergens is determined by both genetic predilection and geographic and cultural factors responsible for exposure to the allergen (Stites and Terr, 1991).

All fungi may be allergenic, depending on the individual, the exposure situation, and the dose (Ruotsalainen *et al.*, 1995). The genera

of fungi, which have been reported to be allergenic, are compiled in Table I. Since the late 1870s, when Blakeley developed symptoms of bronchial asthma and ''chest tightness'' after inhaling spores from *Penicillium* cultures, it has been believed that mold sensitization is an important cause of respiratory allergy (Barth, 1981; Karlsson-Borgå, 1989; Salvaggio, 1986).

Allergy is perhaps the most common human reaction to airborne fungal spores (including conidia). About 20% of the population are allergic individuals with a genetic predisposition to produce IgE antibody to allergens that are either inhaled or ingested (Kaplan *et al.*, 1991; Tizard, 1988). The percentages of populations allergic to molds vary from 2% to 18%, and around 80% of asthmatic patients are allergic to molds (Flannigan *et al.*, 1991). About 20% of the population are atopic and easily sensitized by concentrations usually found in the outdoor air spora (up to 10^6 spores/m^3). These people react immediately on exposure in the upper airways with hay-fever-like symptoms or asthma and may become sensitive to several of the allergens to which they are exposed. The remainder of the population requires more intensive exposure (10^6–10^9 spores/m^3) for sensitization (Lacey, 1981).

The incidence and prevalence of allergic diseases is increasing (Ruotsalainen *et al.*, 1995). Allergies affect as many as 50 million people in the United States, costing them up to $5 billion annually (Jaroff, 1992), and the number is obviously much higher at present. Asthma, rhinitis, hypersensitivity pneumonitis, and humidifier lung are allergenic respiratory diseases that, to a certain degree, may be related to exposure to airborne fungi.

Asthma is the most common chronic respiratory disease in all countries. Both the severity and prevalence of persistent asthma appear to be increasing, leading to urgency in the search for its causes (Woolcock, 1991). Four thousand people a year reportedly died from allergic asthma attack in the United States (Jaroff, 1992). In Australia, asthma mortality rates doubled from 1978 to 1988 (Young *et al.*, 1991).

Immediate-type asthma symptoms were produced with both whole spores and spore extracts of *Alternaria* and *Penicillium* (Licorish *et al.*, 1985; Salvaggio, 1986). Airborne fungal spores are ubiquitous (Howard, 1984) and are known in many cases to be allergenic, so it is not surprising that mold spores are an important cause of asthma. At present the relationship between mold spores and asthma is still poorly understood. In Madison, Wisconsin, in a series of 100 consecutive patients with allergic asthma, skin tests were uniformly positive to *Alternaria* (Reed, 1985). Most of these patients had asthma symptoms not only before and after the ragweed season (about August 10 to

TABLE I

FUNGAL GENERA REPORTED TO BE ASSOCIATED WITH ALLERGY

Fungus	Order	Division
Absidia	Mucorales	Zygomycota
Acremonium		Hyphomycetes
Acrogenospora		Hyphomycetes
Acrothecium		Hyphomycetes
Agaricus	Agaricales	Basidiomycotina
Agrocybe	Agaricales	Basidiomycotina
Alternaria		Hyphomycetes
Amanita	Agaricales	Basidiomycotina
Armillaria	Agaricales	Basidiomycotina
Arthrinium		Hyphomycetes
Aspergillus		Hyphomycetes
Aureobasidium		Hyphomycetes
Bispora		Hyphomycetes
Boletinellus	Boletales	Basidiomycotina
Boletus	Boletales	Basidiomycotina
Botrytis		Hyphomycetes
Calvatia	Lycoperdales	Basidiomycotina
Candida	Yeast	
Cantharellus	Aphyllophorales	Basidiomycotina
Chaetomium	Sordariales	Ascomycotina
Chlorophyllum	Agaricales	Basidiomycotina
Cladosporium		Hyphomycetes
Claviceps	Hypocreales	Ascomycotina
Coniosporium		Hyphomycetes
Coprinus	Agaricales	Basidiomycotina
Coriolus	Aphyllophorales	Basidiomycotina
Cryptococcus		Hyphomycetes
Cryptostroma		Hyphomycetes
Cunninghamella	Mucorales	Zygomycota)
Curvularia		Hyphomycetes
Dacrymyces	Dacrymycetales	Basidiomycotina
Daldinia	Xylariales	Ascomycotina
Debaryomyces	Saccharomycetales (Yeast)	Ascomycotina
Dicoccum (*Trichocladium*)		Hyphomycetes

(*continued*)

TABLE I (*Continued*)

Fungus	Order	Division
Didymella	Dothideales	Ascomycotina
Drechslera		Hyphomycetes
Epicoccum		Hyphomycetes
Epidermophyton		Hyphomycetes
Erysiphe	Erysiphales	Ascomycotina
Eurotium	Eurotiales	Ascomycotina
Fomes	Aphyllophorales	Basidiomycotina
Fuligo		Myxomycetes
Fusarium		Hyphomycetes
Ganoderma	Aphyllophorales	Basidiomycotina
Geastrum	Lycoperdales	Basidiomycotina
Geotrichum		Hyphomycetes
Gibberella	Hypocreales	Ascomycotina
Gliocladium		Hyphomycetes
Gnomonia	Diaporthales	Ascomycotina
Graphium		Hyphomycetes
Helminthosporium		Hyphomycetes
Hypholoma	Agaricales	Basidiomycotina
Inonotus	Aphyllophorales	Basidiomycotina
Leptosphaeria	Dothideales	Ascomycotina
Leptosphaerulina	Dothideales	Ascomycotina
Lycoperdon	Lycoperdales	Basidiomycotina
Malassezia		Hyphomycetes
Merulius (=*Phlebia*)		Basidiomycotina
Microsphaera	Erysiphales	Ascomycotina
Microsporum		Hyphomycetes
Monilia		Hyphomycetes
Mucor	Mucorales	Zygomycota
Mycogone		Hyphomycetes
Naematoloma	Agaricales	Basidiomycotina
Neurospora	Sordariales	Ascomycotina
Nigrospora		Hyphomycetes
Oidium		Hyphomycetes
Paecilomyces		Hyphomycetes
Papularia		Hyphomycetes

(*continued*)

TABLE I (*Continued*)

Fungus	Order	Division
Penicillium		Hyphomycetes
Phoma		Coelomycetes
Phycomyces	Mucorales	Zygomycota
Phytophthora	Peronosporales	Oomycota
Piptoporus	Aphyllophorales	Basidiomycotina
Pisolithus	Sclerodermatales	Basidiomycotina
Pleospora	Dothideales	Ascomycotina
Pleurotus	Aphyllophorales	Basidiomycotina
Podaxis	Podaxales	Basidiomycotina
Polyporus	Aphyllophorales	Basidiomycotina
Poria	Aphyllophorales	Basidiomycotina
Psilocybe	Agaricales	Basidiomycotina
Puccinia	Uredinales	Basidiomycotina
Rhizopus	Mucorales	Zygomycota
Rhodotorula	Yeast	Basidiomycotina
Saccharomyces	Endomycetales (Yeast)	Ascomycotina
Scleroderma	Sclerodermatales	Basidiomycotina
Scopulariopsis		Hyphomycetes
Serpula	Aphyllophorales	Basidiomycotina
Sphaerotheca	Erysiphales	Ascomycotina
Spondylocladium		Hyphomycetes
Sporobolomyces	Yeast	Basidiomycotina
Sporotrichum		Hyphomycetes
Stachybotrys		Hyphomycetes
Stemonitis		Myxomycetes
Stemphylium		Hyphomycetes
Stereum	Aphyllophorales	Basidiomycotina
Syncephalastrum	Mucorales	Zygomycota
Tetracoccosporium		Hyphomycetes
Thermomyces		Hyphomycetes
Tilletiopsis		Hyphomycetes
Tilletia		Basidiomycotina
Torula		Hyphomycetes
Trichoderma		Hyphomycetes
Trichophyton		Hyphomycetes

(*continued*)

TABLE I (*Continued*)

Fungus	Order	Division
Trichothecium		Hyphomycetes
Typhula	Aphyllophorales	Basidiomycotina
Urocystis		Basidiomycotina
Ustilago	Ustilaginales	Basidiomycotina
Verticillium		Hyphomycetes
Wallemia		Hyphomycetes
Xylaria	Xylariales	Ascomycotina
Xylobolus	Aphyllophorales	Basidiomycotina

Chapman (1986); Ibanez *et al.* (1988); Latgé and Paris (1991); Santilli *et al.* (1990); Shen *et al.* (1990); Smith (1990); Van Bronswijk *et al.* (1986).

September 20) but also during the time of year *Alternaria* spore counts are high (July through October) (Reed, 1985). *Cladosporium herbarum* has been shown to be a potential cause of allergic asthma and rhinitis (Malling, 1990).

In a recent study, the prevalence of most building-related symptoms was between 32% and 62%. Positive basophile histamine release (HRT), showing serum IgE specific to one or more of the molds, was observed in 37% of the individuals (Lander *et al.*, 2001). The highest frequency of positive HRT was found to *Penicillium chrysogenum* and then to *Aspergillus* species, *Cladosporium sphaerospermum*, and *Stachybotrys chartarum* (Lander *et al.*, 2001). Savilahti *et al.* (2000) showed that moisture damage and exposure to molds increased the indoor air problems of schools and affected the respiratory health of children.

Cladosporium, Alternaria, Penicillium, Aspergillus, and *Mucor* were reported to be the commonest allergenic fungi (Furuuchi and Baba, 1986; Malling *et al.*, 1985). *Cladosporium* is believed to be the most common one causing mold allergy (Malling *et al.*, 1985). However, the most prevalent airborne fungi are not necessarily the most potent allergens, at least as determined by prick testing (Terracina and Rogers, 1982). Spores of *Alternaria alternata* and those of the closely related genera *Stemphylium* and *Ulocladium* are considered to be the most important mold allergens in the United States (Hoffman, 1984; O'Hollaren *et al.*, 1991; Reed, 1985). *Penicillium* exposure was a risk factor for asthma, while *Aspergillus* exposure was a risk factor for atopy (a genetic trait of increased allergen sensitivity) (Garrett *et al.*, 1998).

Chow *et al.* (2000) characterized Pen n 13 as a major allergen of *Penicillium notatum* (a synonym of *P. chrysogenum*).

Aspergillus restrictus was demonstrated to be a potentially important causative agent in atopic diseases when using skin prick tests and radioallergosorbent test (RAST) on 24 patients (Sakamoto *et al.*, 1990). *Aspergillus* species and in particular *Aspergillus fumigatus* appeared to be the etiological agents in various lung diseases and allergens. Inhalation of low doses of *Aspergillus* spores may induce sensitization and asthma in sensitive patients, while inhalation of high doses may trigger alveolitis and farmer's lung (Wallenbeck *et al.*, 1991). Martinez Ordaz *et al.* (2002) of Mexico found that the association of skin reactivity and indoor exposure was significant only for *Aspergillus*.

Curvularia lunata was found to be a cause of allergic bronchopulmonary disease (Halwig *et al.*, 1985). *Epicoccum nigrum* was reported to be able to colonize nasal sinuses and cause allergic fungal sinusitis (Noble *et al.*, 1997). Sooty molds caused allergies ranging from rhinitis to asthma in the eastern United States (Santilli *et al.*, 1985).

Ascospores are important airborne allergens and present unique antigens (Eversmeyer and Kramer, 1987). Fifteen of 18 patients reportedly reacted to *Leptosphaeria* ascospores (Burge, 1986). About 40% of atopic patients reacted to at least 1 ascomycete preparation. *Chaetomium* species, particularly *C. globosum*, are important ascomycetes commonly found growing indoors on water-damaged paper and wood products.

Basidiospores of *Agaricus campestris, Coprinus micaceus, Lycoperdon perlatum, Scleroderma lycoperdoides*, and *Ustilago maydis* caused allergies ranging from rhinitis to asthma in the eastern United States (Santilli *et al.*, 1985). Basidiospores are antigenic and can elicit immediate skin reactivity in sensitive patients. Mushrooms and basidiospores are considered most likely to be of outdoor origin, although mycelia and conidia of wood decay fungi and, occasionally, mushrooms of the genus *Coprinus* and wood decay fungi have been identified in indoor environments with a chronic water-damage history.

In a military hospital building in Finland with severe, repeated, and enduring water and mold damage, the most abundant species was *Sporobolomyces salmonicolor*. Four new cases of asthma, confirmed by *S. salmonicolor* inhalation provocation tests, were found among the hospital personnel, one of whom was also found to have alveolitis (Seuri *et al.*, 2000). Seven other workers with newly diagnosed rhinitis reacted positively in nasal *S. salmonicolor* provocation tests. Skin prick tests of *Sporobolomyces* were negative among all 14 workers (Seuri *et al.*, 2000).

Several epidemiologic studies concerning water damage, fungal growth, and exposure to mold spores have been conducted in a number of countries. The occurrence of *Cladosporium, Aspergillus versicolor*, and *Stachybotrys* showed some value as an indicator of moisture damage. Presence of moisture damage in school buildings was a significant risk factor for respiratory symptoms in school children (Meklin *et al.*, 2002). The association between moisture damage and respiratory symptoms of children was significant for buildings of concrete/brick construction but not for wooden school buildings. The highest symptom prevalence was found during spring seasons, after a long exposure period in damaged schools (Meklin *et al.*, 2002).

Questionnaire surveys conducted in the United Kingdom, Canada, United States, and the Netherlands showed positive correlations between self-reported allergenic respiratory symptoms and self-reported water damage and indoor fungi problems (Andrae *et al.*, 1988; Brunekreef, 1989; Dales *et al.*, 1991a; Dekker *et al.*, 1991; Melia *et al.*, 1982; Strachan, 1988; Strachan and Sanders, 1989; Strachan *et al.*, 1990; Waegemaekers *et al.*, 1989). Most studies identified an association between airborne fungal spore concentrations and self-reported allergic symptoms in the United Kingdom, Sweden, and the Netherlands (Holmberg, 1987; Platt *et al.*, 1989; Strachan *et al.*, 1990; Waegemaekers *et al.*, 1989), but there is not always a correlation between indoor spore counts and symptoms found in research (Tobin *et al.*, 1987).

Yang *et al.* (1997) showed that the prevalence of respiratory symptoms was consistently higher in homes with dampness than in non-damp homes. Dampness in the home can be used as a strong predictor of and a risk factor for respiratory symptoms and is a considerable public health problem in Taiwan (Yang *et al.*, 1997). A significant relationship was found between dampness and work-related sick building syndrome in day-care-center workers in Taiwan (Li *et al.*, 1997). A significant association was found between most building-related symptoms (BRS) and positive basophil histamine release (Lander *et al.*, 2001). Jacob *et al.* (2002) found that mold spore counts for *Cladosporium* and *Aspergillus* were associated with an increased risk of allergic sensitization. Sensitized children exposed to high levels of mold spores (>90th percentile) were more likely to suffer from symptoms of rhinoconjunctivitis. Fungal allergies were more common among children exposed to *Cladosporium* or *Penicillium* in winter or to musty odor (Garrett *et al.*, 1998).

In atopic children, total IgE showed a significant linear relation with age. Prevalence of specific IgE for *Cladosporium* ranked first, followed

closely by *Aspergillus* and *Alternaria* (Nolles *et al.*, 2001). Sensitization to fungi is prevalent in childhood, with an age-dependent distribution reaching maximum values at 7.7–7.8 years, followed by a decline for all fungal sensitization with increasing age (Nolles *et al.*, 2001).

Jaakkola *et al.* showed that the risk of asthma was related to the presence of visible mold and/or mold odor in the workplace but not to water damage or damp stains alone. The fraction of asthma attributable to workplace mold exposure was estimated to be 35.1% among the exposed (Jaakkola *et al.*, 2002). Large airborne fungal spore concentrations were recorded in association with musty odor, water intrusion, high indoor humidity, limited ventilation through open windows, few extractor fans, and failure to remove indoor mold growth in the homes in the Latrobe Valley, Victoria, Australia (Garrett *et al.*, 1998).

Aspergillus was associated strongly with work-related sick building syndrome in day-care-center workers (Li *et al.*, 1997). The diagnosis of sick building syndrome related diseases, such as asthma, rhinitis, and allergic alveolitis, can be very difficult. In the study of Thorn *et al.* (1996) the symptoms of a school teacher, who was working in a school that had indoor air quality problems on and off for several years, were first interpreted as pulmonary embolism and later as atypical sarcoidosis. However, 6 years later the diagnosis of the illness was revised to chronic allergic alveolitis.

It is important to understand that even correlations do not necessarily mean causal relations. Most studies on indoor airborne fungi were conducted without taking allergic symptoms into account. Several recent epidemiological studies have shown that long-duration indoor exposure to certain fungi can result in hypersensitivity reaction and chronic diseases. Mold spore levels comparable to outside background levels are usually well tolerated by most people. Normal or ''typical'' indoor molds may vary depending on diurnal and seasonal patterns of outdoor fungi, weather conditions, climate variations, and geographical regions (Li and Kendrick, 1995a).

There are other diseases caused by airborne fungal allergens, such as rhinitis, hypersensitivity pneumonitis, and humidifier lung (Burge, 1990b; Salvaggio, 1986). A number of occupational hypersensitivity diseases of the lung can be implicated by fungi (Table II). Hypersensitivity pneumonitis, also called *extrinsic allergic alveolitis*, is a well-recognized occupational disease. Hypersensitivity pneumonitis caused by inhalation of spores from the edible mushroom *Pholiota nameko* was documented by Nakazawa and Tochigi (1989). A diagnosis of hypersensitivity pneumonitis caused by an *Aspergillus* species was made by Jacobs *et al.* (1989). *Pleurotus ostreatus* was defined to be an

TABLE II

FUNGI-IMPLICATED OCCUPATIONAL HYPERSENSITIVITY DISEASES OF THE LUNG

Fungal agent	Disease	Source
Alternaria sp.	Pulpmill worker's lung	Moldy pulpwood
Aspergillus clavatus	Malt worker's lung	Moldy malt
Aspergillus fumigatus	Wood trimmer's disease	Moldy timber
Aspergillus sp.	Sawmill worker's lung	Moldy
Aspergillus sp.	Woodchip handler's disease	Moldy woodchip
Aureobasidium pullulans	Sauna taker's lung	Sauna steam
Aureobasidium pullulans	Sequoiosis	Moldy sawdust
Botrytis cinerea	Vinegrower's lung	Moldy fruit
Farnai rectivirgula	Potato riddler's lung	Straw
Cryptostrama corticale	Maple bark disease	Moldy maple bark
Graphium sp.	Maple bark disease	Moldy maple bark
Micropolyspora faeni	Farmer's lung	Moldy hay
Micropolyspora faeni	Mushroom worker's lung	Mushroom compost
Micropolyspora faeni	Woodchip handler's disease	Moldy woodchip
Mucor sp.	Woodchip handler's disease	Moldy woodchip
Penicillium casei	Cheese worker's lung	Cheese
Penicillium spp.	Suberosis, woodman's disease	Cork
Rhizopus sp.	Wood trimmer's disease	Moldy timber
Rhizopus (Mucor) stolonifer	Paprika worker's lung	Moldy paprika
Serpula (*Merulius*) *lacrymans*	Dry rot lung	Moldy building

allergen by Horner *et al.* (1988). Extrinsic allergic alveolitis caused by spores of *Pleurotus ostreatus* was reported by Cox *et al.* (1988).

In general, the adverse effects of fungal exposure by inhalation are related to duration and intensity. Many studies have shown that ''atypical'' mold spore levels in the indoor environment increase because of recurrent water leaks, home dampness, and high humidity, resulting in increases of allergies and respiratory problems (Burge, 1990a,b; Dales *et al.*, 1991; Flannigan *et al.*, 1991; Johanning *et al.*, 1993; Rylander, 1994; Solomon *et al.*, 1978; Strachan *et al.*, 1990; Streifel and Rhame, 1993; Tripi *et al.*, 2000). Path analysis showed that indoor total fungal spores, indoor *Aspergillus/Penicillium*, and the age of the residences had significant direct effects on allergic symptoms (Li, 1994).

There are still significant methodological problems in the preparation and production of reliable allergen extracts from fungi as

compared with those from cats, dust mites, and other better-characterized allergens. Extracts that are available correspond poorly with the fungi often found in indoor surveys (Horner and Lehrer, 1999). One of the technical difficulties is to produce enough spores for allergen extraction. Common practice in fungal allergen extraction is to use a mixture of spores and mycelia, which was believed to be a contributing factor to inconsistency in the low sensitivity of fungal allergenic tests. Because of the low sensitivity of some of the commercially available mold allergen extracts, false-negative results are not uncommon. Patients with an atopy are frequently allergic to multiple fungal species and manifest type I reactions (asthma, rhinitis, eczema, and hay fever). One of the reasons for the poor correlations is reportedly that fungal allergens are extracted from mostly vegetative hyphae grown in liquid cultures, not from spores. The differences in allergencity between hyphae and spores should be studied.

B. Infectious Diseases

Fungi are mostly known to cause not only allergies but also infectious diseases to the skin and other body organs (Table III). Infections caused by fungi are called *mycoses*. Mycoses are categorized into endemic and opportunistic. Endemic mycosis is caused by the inhalation of airborne fungal spores found in certain geographic regions where there is a higher frequency of such fungi because of unique soil and flora (Lacey, 1991; Pitt, 1979). Opportunistic fungal pathogens have a great public health importance, especially in immune system compromised individuals such as those with human immunodeficiency virus (HIV) and organ transplants (Keller *et al.*, 1999). These infections are not contagious, and the fungi are not obligatory pathogens. Immunocompromised patients may be at an increased risk for opportunistic infections if opportunistic fungal pathogens become airborne and their concentrations are significantly elevated in indoor air. The major fungi causing mycosis and their medical significance are listed in Table III.

Aspergillus fumigatus, *A. flavus*, and *A. niger* are among the fungi of significant concern. *Aspergillus fumigatus* is the most important airborne pathogenic fungus (Brakhage and Langfelder, 2003) because of its small respirable-size spores and its thermophilic nature (Klich and Pitt, 1988). This is the very reason why *A. fumigatus* could cause a significant problem in organ transplant wards in hospitals. Water damaged materials, houseplants, soil, bird and bat droppings, organic waste, or other organic substrates in buildings may be a source of these

TABLE III

PATHOGENIC FUNGI

Fungus	Classification	Disease	Affected area
Absidia sp.	Opportunistic Systemic mycosis	Zygomycosis (Mucormycosis, phycomycosis)	Face, sinuses, gastrointestinal tract, lungs
Cunninghamelia sp.			
Mortierella sp.			
Mucor sp.			
Rhizopus sp.			
Syncephalastrum sp.			
Basidobolus ranarum			
Rhizomucor sp.			
Conidiobolus coronatus			
Acremonium sp.	Cutaneous mycosis	Keratomycosis	Eye
	Subcutaneous mycosis	Maduromycetoma	
	Opportunistic Systemic mycosis	Systemic opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Alternaria sp.	Opportunistic Systemic mycosis	Systemic opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Arthrographis sp.	Subcutaneous mycosis	Dermatomycosis	Skin
Aspergillus fumigatus	Opportunistic Systemic mycosis	Aspergillosis	Lung, skin, mucocutaneous tissue, any of the body organs
Asp. flavus			
Asp. niger			
Asp. terreus			
Asp. ustus			
Aspergillus spp.			
Aspergillus sp.	Cutaneous mycosis	Outer cutaneous mycoses	Skin
		Onychomycosis	Nails
		Otomycosis	Ear
		Keratomycosis	Eye

(*continued*)

TABLE III (*Continued*)

Fungus	Classification	Disease	Affected area
Aureobasidium pullulans	Opportunistic Systemic mycosis	Systemic opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Basidiobolus sp.	Rare subcutaneous mycosis	Entomophthora basidiobolae	Smooth skin
Beauveria bassiana	Opportunistic Systemic mycosis	Systemic opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Blastomyces dermatitidis	Systemic mycosis	Blastomycosis	Primary infection in lung, may spread to all organs, skin lesions are common
Candida albicans	Cutaneous mycosis	Intertriginous candidosis	Moist skin areas: groin, glans penis, scrotum, folds of buttocks, under the breast, axilla, interdigital spaces
		Candida diaper rash	Diaper area
		Candidal granuloma	Hands, feet, face, and scalp
		Candida paronychia and onychomycosis	Nails and skin around nail
		Mucocutaneoius candidosis	Mucocutaneous areas
		Thrush	Mouth and tongue
		Perleche	Corners of mouth
		Vaginal candidosis	Vagina
		Candida balinitis	Glans penis
		Esophageal candidosis	Esophagus
		Perianal candidosis	Anal ara
		Chronic mucocutaneous candidosis	
Candida albicans, Candida spp.	Cutaneous mycosis	Onychomycosis	Nails

(*continued*)

TABLE III (*Continued*)

Fungus	Classification	Disease	Affected area
	Opportunistic Systemic mycosis	Systemic candidosis	Blood, heart tissue, kidney, bladder, mucocutaneous tissue (lungs are colonized, but rarely invaded)
	Cutaneous mycosis	Otomycosis	Ear
Candida sp.	Cutaneous mycosis	Keratomycosis	Eye
Cercospora apii	Opportunistic Systemic mycosis	Systemic Opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Chaetoconidium sp.	Opportunistic Systemic mycosis	Systemic Opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Chrysosporium parvum	Opportunistic Systemic mycosis	Systemic Opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Cladosporium carrionii	Subcutaneous mycosis	Chromomycosis	Skin surface, mostly lower extremities
Cladosporium trichoides	Opportunistic Systemic mycosis	Cerebral chromomycosis	Brain or central nervous system
Coccidioides immitis	Systemic mycosis	Coccidioido-mycosis	Primary infection in the lung may spread to other organs of the body; skin lesion may be produced
Coprinus sp.	Miscellaneous and rare mycosis	Basidiomycosis	
Cryptococcus neoformans	Systemic mycosis	Cryptococcosis	Lungs, central nervous system, skin, any organ of body
Curvularia geniculata	Opportunistic Systemic mycosis	Systemic Opportunistic fungal disease	Lungs, deep tissue, body organs, blood
Drechslera hawaiiensis	Opportunistic Systemic mycosis		
Entomophthora (conidiobolus) coronata	Rare subcutaneous mycosis	Entomophthoro-mycosis conididobolae	Nasal tissue and face

(*continued*)

TABLE III (*Continued*)

Fungus	Classification	Disease	Affected area
Epidermophyton floccosum	Cutaneous mycosis	Tinea cruris	Groin
		Tinea pedis	Feet, interdigital spaces, and soles
		Tinea manuum	Palms and fingers
		Tinea unguium	Nails
Epidermophyton spp.	Cutaneous mycosis	Dermatomycoses	Keratinized layers of body: skin, hair, nails
Exophiala (Phialophora) jeanselmei	Subcutaneous mycosis	Phaeomycotic Cyst	Smooth skin
Exophiala (Phialophora) spinifera	Subcutaneous mycosis	Phaeomycotic Cyst	Smooth skin
Exophiala jeanselmei	Subcutaneous mycosis	Maduromycetoma	
Fonsecaea compactum	Subcutaneous mycosis	Chromomycosis	Skin surface, mostly lower extremities
Fonsecaea pedrosoi	Opportunistic Systemic mycosis	Cerebral chromomycosis	Brain or central nervous system
Fonsecaea pedrosoi	Subcutaneous mycosis	Chromomycosis	Skin surface, mostly lower extremities
Fusarium sp.	Opportunistic Systemic mycosis		
Fusarium sp.	Cutaneous mycosis	Keratomycosis	Eye
Geotrichum candidum	Opportunistic Systemic mycosis		
Helmintho-sporium sp.	Opportunistic Systemic mycosis		
Hendersonula sp.	Subcutaneous mycosis	Dermatomycosis	Skin
Histoplasma capsulatum	Systemic mycosis	Histoplasmosis	Primary infection in lung

(*continued*)

TABLE III (*Continued*)

Fungus	Classification	Disease	Affected area
(*H. duboisii* in Africa)			Recticulorendothelial system is invaded; bone and kidney and other organs, including the skin, may be involved
Hortaea (*Phaeoannellomyces* or *Exophiala*) *werneckii*	Superficial mycosis	Tinea nigra	Thick stratum corneum, palms, and feet
Loboa loboi	Rare subcutaneous mycosis	Lobomycosis	Smooth skin
Malassezia furfur	Superficial infections	Pityriasis versicolor	
Microsporum audouinii	Cutaneous mycosis	Tinea capitis	Scalp
M. canis			
Microsporum spp.			
Microsporum canis	Cutaneous mycosis	Tinea corporis	Smooth body skin
M. gypseum			
Microsporum spp.			
Microsporum spp.	Cutaneous mycosis	Tinea barbae	Beard and coarse body hair
		Tinea favosa	Scalp, skin, and nails
Microsporum spp.	Cutaneous mycosis	Dermatomycoses	Keratinized layers of body: skin, hair, nails
Paecilomyces sp.	Opportunistic Systemic mycosis		
Paracoccidioides brasiliensis	Systemic mycosis	Paracoccidioidomycosis	Subclinical infection in lung, mucous membranes, and skin are involved
Penicillium sp.	Opportunistic Systemic mycosis		
Pseudallescheria (*Allescheria* or *Petriellidium*), *boydii*	Subcutaneous mycosis	Maduromycetoma	

(*continued*)

TABLE III (*Continued*)

Fungus	Classification	Disease	Affected area
Phialophora parasitica	Opportunistic Systemic mycosis		
	Subcutaneous mycosis	Phaeomycotic Cyst	Smooth skin
Phialophora repens	Subcutaneous mycosis	Phaeomycotic Cyst	Smooth skin
Phialophora richardsiae	Subcutaneous mycosis	Phaeomycotic Cyst	Smooth skin
Phialophora verrucosa	Subcutaneous mycosis	Chromomycosis	Skin surface, mostly lower extremities
Phoma hibernica	Opportunistic Systemic mycosis		
Phoma sp.	Subcutaneous mycosis	Phaeomycotic Cyst	Smooth skin
Piadraia hortae	Superficial mycosis	Black piedra	Scalp and beard
Pityrosporum orbiculare	Superficial mycosis	Tinea versicolor	Smooth body skin
Pseudallescheria (Allescheria or Petriellidium), boydii	Opportunistic Systemic mycosis		
Pythium	Miscellaneous and rare mycosis	Pythiosis	
Rhinosporidium seeberi	Rare subcutaneous mycosis	Rhinosporidiosis	Nasal mucosa
Schizophyllum commune	Miscellaneous and rare mycosis	Basidiomycosis	
Scopulariopsis brevicaulis	Opportunistic Systemic mycosis		
Scopulariopsis sp.	Cutaneous mycosis	Onychomycosis	Nails
Scytalidium sp.	Subcutaneous mycosis	Dermatomycosis	Skin

(*continued*)

TABLE III (*Continued*)

Fungus	Classification	Disease	Affected area
Sporothrix schenckii	Subcutaneous mycosis	Sporotichosis	Skin, primarily hands, arms, and legs
Torulopsis glabrata	Opportunistic Systemic mycosis		
Trichophyton concentriucum	Cutaneous mycosis	Tinea imbricate	Smooth body skin
Trichophyton rubrum	Cutaneous mycosis	Tinea manuum	Palms and fingers
T. mentagrophyte			
Trichophyton spp.			
		Tinea pedis	Feet, interdigital spaces, and soles
		Tinea unguium	Nails
		Tinea cruris	Groin
		Tinea corporis	Smooth body skin
Trichophyton schoenleinii	Cutaneous mycosis	Tinea favosa	Scalp, skin, and nails
Trichophyton spp.			
Trichophyton spp.	Cutaneous mycosis	Dermatomycoses	Keratinized layers of body: skin, hair, nails
Trichophyton tonsurans	Cutaneous mycosis	Tinea capitis	Scalp
Trichophyton spp.			
Trichophyton verrucosum	Cutaneous mycosis	Tinea barbae	Beard and coarse body hair
T. mentagrophytes			
Trichophyton spp.			
Trichosporon beigelii	Superficial mycosis	White piedra	Beard, scalp, pubic hair
Wangiella (*Phialophora*) *dermatitidis*	Opportunistic Systemic mycosis	Cerebral chromomycosis	Brain or central nervous system
	Subcutaneous mycosis	Chromomycosis	Skin surface, mostly lower extremities
		Maduromycetoma	
Wangiella mansonii	Superficial mycosis	Tinea nigera	Thick stratum corneum, palms, and feet

Compiled from Campbell and Stewart (1980); Henry (1984); Howard (2003); Rippon (1988).

fungi (Benenson, 1990; Burge 1990a; Larsen and Frisvad, 1995). These fungi can cause aspergillosis. In a hospital where an epidemic of aspergillosis occurred, the source of *Aspergillus* spores was attributed to a defective disposal conduit door and the dispersal of a contaminated aerosol from the ward vacuum cleaner, which had the highest measured concentrations of *Aspergillus fumigatus* in or around the building (65 colony forming units/m^3 as compared with 0–6 cfu/m^3 elsewhere). No further cases were identified in the hospital in the 2 years after relevant hygiene arrangements were incorporated (Anderson *et al.*, 1996).

Other clinically important fungal infections include candidiasis with local mucocutaneous or disseminated systemic organ manifestations and skin mycoses such as dermatophytoses, keratomycosis, tinea nigra, piedra, and malassezia-caused dermatitis. Invasive fungal diseases of the paranasal sinuses may also be associated with allergic sinusitis in atopic patients (Fatterpekar, 1999). *Aspergillus* species are frequently involved. Noninvasive forms may colonize body cavities and may be asymptomatic as long as some degree of immunological resistance can be maintained. *Cryptococcus neoformans* var. *neoformans* was isolated from 20 (13%) dwellings out of 154 dwellings in the metropolitan area of Rio de Janeiro, Brazil, comprising 5 (15.6%) of 32 dwellings of patients with AIDS-associated cryptococcosis (Passoni *et al.*, 1998).

Histoplasmosis is an intracellular mycotic infection of the reticuloendothelial system caused by the inhalation of conidia from the fungus *Histoplasma capsulatum* (Howard, 2003). *Histoplasma capsulatum* has a worldwide distribution, but the Mississippi–Ohio River Valley in the United States is a major endemic region, and the spore is occasionally found in certain indoor environments there (Collier *et al.*, 1998).

Coccidioides immitis causes coccidioiomycosis, a highly infectious upper respiratory disease, and infection is caused by inhalation of its airborne arthrospores (Howard, 2003). The disease is endemic in certain regions, mainly in desert soils and also in the air of endemic areas in North America (Cox and Wathes, 1995). Exposure to dustborne spores outdoors is the major risk factor of infection (Al-Doory and Ramsey, 1987).

C. Mycotoxins and Their Significance to Human Health

Another public health concern is mycotoxins produced by some indoor fungi (Table IV). Fungi are capable of producing a number of secondary metabolites (Nielsen, 2002). Most of these secondary

TABLE IV

COMMON MYCOTOXIGENIC INDOOR FUNGI

Fungus	Mycotoxins*
Alternaria alternata	Tenuazonic acid, alternatiol, alternatiol mononethyl ether, altertoxins
Aspergillus flavus	**Aflatoxin B_1**
Aspergillus fumigatus	**Gliotoxin**, verrucologen, fumitremorgceusins, fumitoxins, tryptoquivalins
Aspergillus niger	Naphthopyrone, malformins, nigragillin, orlandin
Aspergillus ochrrachceus	**Ochratoxin A** (a carcinogenic kidney toxin)
Aspergillus parasiticus	Aflatoxin B_1
Aspergillus versicolar	**Sterigmatocystin** and methoxysterigmatocystin
Aspergillus ustus	Austaminde, austdiol, austins, austocystins, kotanins X and Y
Chaetomium globosum	**Chaetoglobosins, chetomin**
Cladosporium cladosporioides	Cladosporin, emodin
Emericella (*Aspergillus*) *nidulans*	**Sterigmatocystin**, nudulotoxin
Fusarium culmorum	**T-2 toxin** (immunosuppressive)
Fusarium graminearum	**Zealralenone**
Fusarium verticillioides (= *F. moniliforme*)	**Fumonisins**
Memnoniella echinata	Trichodermol, trichodermin, dechlorogrisseofulvins, memnobotrins A and B, memenoconol, memnoconone
Paecilomyces variotii	Patulin, viriditoxin
Penicillium aurantiogriseum	Auranthine, penicillic acid, verrucosidin, **nephrotoxic glycopeptides** Mycophenolic acid
Penicillium brevcompactum	
Penicillium chrysogenum	Roquefortine C, meleagrin, chrysogin, penicillin
Penicillium expansum	Citrinin, patulin (nephrotoxic), cytotoxic metabolite of unknown origin
Penicillium polonicum	3-methoxyviridicatin, verrucosidin, verrucofortine
Penicillium verrucosum	**Ochratoxin A** (a carcinogenic kidney toxin)
Stachybotrys chartarum (syn = *S. atra*).	Macrocyclic trichothecenes: **satratoxins, verrucarins, roridins**, atranones, dolabellanes, stachybotrylactones, and lactams
Trichoderma harzianum	**Alamethicins**, emodin, suzukacillin, trichodermin
Wallemia sebi	Walleminols A and B, walleminone

*Toxins in boldface are of high potency. Compiled in part from Al-Doory and Domson, 1984; Frank *et al.*, 1999; Macher *et al.*, 1999; Samson, 2000; St-Germain and Summerbell, 1996.

metabolites are mainly to enhance the fitness of the fungi in nature. However, when some of these chemical compounds cause detrimental or toxic response in higher vertebrates at low concentrations, they are referred to as *mycotoxins* (Nielsen, 2002). Mycotoxicosis is defined as the disease resulting from exposure to a mycotoxin (CAST, 2003). Mycotoxicosis may be acute or chronic. More occult disease may occur when the mycotoxin interferes with the immune system and leads to a compromised immune system so as to make patients more susceptible to infectious diseases. Major mycotoxicoses include aflatoxicosis, ochratoxicosis, trichothecene toxicoses, citreviridin toxicosis, zearalenone toxicosis, fumonisin toxicosis, gliotoxin toxicosis, and immunomodulation.

Mycotoxins' detrimental effects on human health are at work when they are ingested (CAST, 2003; Matossian, 1989), inhaled (CAST, 2003; Croft *et al.*, 1986; Johanning *et al.*, 1993; Miller, 1993; Smoragiewicz *et al.*, 1993), or absorbed through skin contact (CAST, 2003; Dill *et al.*, 1997; Singh, 1994). Historically, human exposure to mycotoxins is mainly through ingestion of foodstuff containing or contaminated with mycotoxins (CAST, 2003). However, because of increases in public awareness of the health effects of indoor fungi, inhalation of mycotoxin-containing spores of indoor fungi has become a major public health concern in the indoor environment, and ingestion and dermal contact play a secondary role in indoor exposure. There are reportedly more than 200 mycotoxins produced by various common fungi, per the World Health Organization (WHO) Environmental Health Criterion 105 on mycotoxins (Yang *et al.*, 2002). Samson (1992) and Smoragiewicz *et al.* (1993) suggested that there are more than 400 toxic metabolites at present. The actual number of mycotoxins is not known, but the number of fungal toxic metabolites could be potentially in the thousands (CAST, 2003). With molecular masses between 200 and 800 kDa (Smoragiewicz *et al.* 1993), mycotoxins are not volatile at ambient temperatures (Tuomi *et al.* 2000). Schiefer (1990) considered that mycotoxins generally have low volatility, and therefore inhalation of volatile mycotoxins is not very likely. A task group of WHO concluded that an association between trichothecene exposure and human disease episodes is possible; however, only limited data are available (Yang *et al.*, 2002).

Major genera of toxigenic fungi include *Aspergillus, Penicillium, Fusarium, Stachybotrys, Memnoniella*, and *Claviceps* (CAST, 2003). There are other genera of mycotoxin-producing fungi. Species of 46 fungal genera have been reported to produce mycotoxins (Kendrick, 2000). Major classes of mycotoxins include aflatoxins, trichothecenes,

fumonisins, zearalenone, ochratoxin A, and ergot alkaloids (CAST, 2003). Major toxigenic fungi and the mycotoxins produced, as well as their health effects, are compiled in Tables V and VI. It should be pointed out that some of the fungi in Table V are often found indoors.

Mycotoxins are an integral part of the fungal spores or in association with dust particles when released into the substrates. Water-damaged building materials are often contaminated with fungi that produce detectable levels of mycotoxins (Nikulin, 1999), which may be aerosolized and contribute to pollution in indoor air. Sorenson *et al.* (1987) showed that aerosolized conidia of *S. chartarum* (syn. *S. atra*)

TABLE V

FUNGI IMPLICATING SOME HUMAN DISEASES BECAUSE OF INVOLVEMENT OF THEIR MYCOTOXINS

Etiologic agent	Disease	Natural substrate
Fusarium spp.	Akakabio-byo	Wheat, barley, oats, rice
Fusarium spp.	Alimentary toxic aleukia (ATA or septic angina)	Cereal grains (toxic bread)
Penicillium	Balkan nephropathy	Cereal grains
Aspergillus spp., *Penicillium* spp.	Cardiac beriberi	Rice
Sclerotinia	Celery harvester's disease	Celery (pink rot)
Dendrodochium toxicum	Dendrodochiotoxicosis	Fodder (skin contact, inhaled fodder particles)
Claviceps purpurea	Ergotism	Rye, cereal grains
Fusarium moniliforme	Esophageal tumors	Corn
Apergillus flavus, A. parasiticus	Hepatocarcinoma (acute aflatoxicosis)	Cereal grains, peanuts
Fusarium	Kashin Beck disease, ''Urov disease''	Cereal grains
Aspergillus flavus, A. parasiticus	Kwashiorkor	Cereal grains
Phoma sorghina	Onyalai	Millet
Aspergillus	Reye's syndrome	Cereal grains
Satchybotrys chartarum	Stachybotryotoxicosis	Hay, cereal grains, fodder (skin contact, inhaled haydust)

TABLE VI

MYCOTOXINS AND THEIR PATHOLOGICAL EFFECTS ON HUMANS AND ANIMALS

Mycotoxin	Substrates	Affected species	Pathological effects
Aflatoxins (B1, B2, G1, G2, M1, M2)	Peanuts, corn, wheat, rice, cottonseed, copra, nuts, various foods, milk, eggs, cheese, figs	Birds Duckling, turkey, poultry, pheasant chick, mature chicken, quail Mammals Young pigs, pregnant sows, dog, calf, mature cattle, sheep, cat, monkey, human Fish Laboratory animals	Hepatotoxicity (liver damage) Bile duct hyperplasia Hemorrhage Intestinal tract Kidneys Carcinogenesis (liver tumors)
Citrinin	Cereal grains (wheat, barley, corn, rice)	Swine, dog, laboratory animals	Nephrotoxicity (tubular necrosis of kidney) porcine nephropathy
Cyclopiazonic acid	Corn, peanuts, cheese, kodo millet	Chicken, turkey, swine, rat, guinea pig, human	Muscle necrosis Intestinal hemorrhage and edema Oral lesions
Ochratoxin A	Cereal grains, (wheat, barley, oats, corn), dry beans, moldy peanuts, cheese, grapes, dried fruits, wine	Swine, dog, duckling, chicken, rat, human	Nephrotoxicity (tubular necrosis of kidney) Porcine nephropathy Mild liver damage Enteritis Teratogenesis Carcinogenesis (kidney tumors) Urinary tract tumors
Patulin	Moldy feed, rotted apples, apple juice, wheat straw residue	Birds Chicken, chicken embryo, quail Mammals Cat, cattle, mouse, rabbit, rat, human	Edema Brain Lungs Hemorrhage Lungs Capillary damage Liver

(*continued*)

TABLE VI (*Continued*)

Mycotoxin	Substrates	Affected species	Pathological effects
		Others Brine shrimp, guppie, zebra Fish larvae	Spleen Kidney Paralysis of motor nerves Convulsions Carcinogenesis Antibiotic
Penicillic acid	Stored corn, cereal grains, dried beans, moldy tobacco	Mouse, rat, chicken embryo, quail, brine shrimp	Liver damage (fatty liver, cell necrosis); kidney damage; digitalis-like action on heart dilates blood vessels; antidiuretic edema in rabbit skin; carcinogenesis; antibiotic
Penitrem	Moldy cream cheese, English walnuts, hamburger bun, beer	Dog, mouse, human	Tremors, death, icoordination, bloody diarrhea
Sterigmatocystin	Green coffee, moldy wheat, grains, hard cheeses, peas, cottonseed	Mouse, rat	Carcinogenesis Hepatotoxin
Trichothecenes (T-2 toxin, diacetoxyscirpenol, neosolaniol, nivalenol, diacetylnivalenol, deoxynicalenol, HT-2 toxin, fusarenon X)	Corn, wheat, commercial cattle feed, mixed feed, barley, oats	Swine, cattle, chicken, turkey, horse, rat, dog, mouse, cat, human	Digestive disorders (emesis, diarrhea, refusal to eat), hemorrhage (stomach, heart, intestines, lungs, bladder, kidney), edema, oral lesions, dermatitis, blood disorders (leucopenia)
Zearalenone	Corn, moldy hay, pelleted commercial feed	Swine, dairy cattle, chicken, turkey, lamb, rat, mouse, guinea pig	Estrogenic effects (edema of vulva, prolapse of vagina, enlargement of uterus) Atrophy of testicles Atrophy of ovaries, enlargement of mammary glands Abortion

Compiled from CAST (2003).

contained trichothecene mycotoxins in the laboratory. The most common toxin was satratoxin H. Lesser amounts of satratoxin G and trichoverrols A and B were also detected, but less frequently. They also found that most of the airborne particles were within respirable range. Similar experiments, conducted by Pasanen *et al.* (1993), demonstrated that trichothecene mycotoxins were in airborne fungal propagules of *S. chartarum* (*S. atra*) and could be collected on membrane filters. Conidia of *A. flavus* and *A. parasiticus* were reported to contain aflatoxins (Wicklow and Shotwell, 1983). Miller (1993) also reported detection of two mycotoxins, deoxynivalenol and T-2 toxin, in conidia of *Fusarium graminearum* and *F. sporotrichioides*, respectively. These references suggest that inhalation exposure to conidia may also increase the chance of exposure to mycotoxins.

Studies indicated that some secondary metabolites of indoor airborne fungi could be responsible for health problems of occupants (Croft *et al.* 1986; Pieckova, 2002). Croft *et al.* (1986) identified several cases of mycotoxicoses caused by airborne exposure to the toxigenic fungus *S. chartarum* (syn. *S. atra*) in a residential building. Spengler *et al.* (1993) reported that a higher rate of upper respiratory tract and lung cancer occurred among workers with a high risk of inhalation of fungi in the grain and food handling industry.

Important indoor toxigenic fungi include *Stachybotrys chartarum* (syn. *S. atra*), *Memnoniella echinata, Aspergillus* species, *Penicillium* species, *Fusarium* species, *Trichoderma* species, and *Paecilomyces* species. These fungi are well documented to have associations with detrimental health effects in humans and animals by ingestion. However, many toxigenic fungi—such as *Stachybotrys chartarum* and species of *Aspergillus*, *Penicillium*, and *Fusarium*—have been found to infest buildings with known indoor air problems and sick building syndrome (Croft *et al.*, 1986; Flannigan *et al.*, 1991; Johanning *et al.*, 1993).

It should be pointed out that most mycotoxin studies were conducted on post-harvest stored or processed food. For better indoor environmental quality evaluation, it is important to know whether indoor fungi are able to produce mycotoxin in building materials or not and under what conditions. Tuomi *et al.* (2000) analyzed 17 mycotoxins from 79 bulk building materials collected from water-damaged buildings. Their results showed sterigmatocystin was present in 24% of the samples, trichothecenes in 19% of the samples, and citrinine in 3 samples. *Aspergillus versicolor* was found on most sterigmatocysin-containing samples, and *Stachybotrys* spp. were found on the samples in which satratoxins were present (Tuomi *et al.*, 2000).

Nielsen (2002) showed that *Stachybotrys chartarum* produced a number of mycotoxins on building materials at levels significantly higher than these products by other fungi. More importantly, he discovered that only 35% of the isolates of *S. chartarum* produced the extremely cytotoxic satratoxins. He opined that satratoxins might not be responsible for idiopathic pulmonary hemosiderosis (IDPH) in infants and that this disease may be caused by other mycotoxins produced by *S. chartarum* (Nielsen, 2002). Similar results showed that 39% of *S. chartarum* produced macrocyclic trichothecenes (Andersen *et al.*, 2002). The toxicity of the isolates producing macrocyclic trichothecenes is 1000 times that of other isolates, which produce atronones (Jarvis 2003, per. com.). However, a recent study in Belgium showed that in 6 IDPH cases, the isolates of *S. chartarum* recovered from patients' homes were all atronones producers (Nielsen, per. com.). This association further raised the question whether other mycotoxins and secondary metabolites are responsible for IDPH. To answer this question there is no doubt that more research is necessary.

Aflatoxins are toxins discovered in 1961 from *Aspergillus flavus* and *A. parasiticus* and considered human and animal carcinogens (CAST, 2003; International Agency for Research on Cancer, 1993). Aflatoxins are potent liver toxins. A sublethal dose from exposure may result in cancer (CAST, 2003). Aflatoxin-induced disease has been well documented and reviewed (Henry and Cole, 1993; International Agency for Research on Cancer, 1993; Kurup, 1999). *Aspergillus versicolor* produced the mycotoxins sterigmatocystin and 5-methoxysterigmatocystin, which are precursors of aflatoxins, in water-damaged materials under field conditions and experimental conditions (Gravesen *et al.*, 1999; Nielsen, 2002). Trichothecene toxins inhibit protein and DNA synthesis (CAST, 2003). The data of health effects on animals are ''inadequate evidence'' for humans (International Agency for Research on Cancer, 1993). Macrocyclic trichothecenes, such as satratoxin H, have not been classified. These toxins can cause alveolar macrophage defects and may affect phagocytosis. They have been investigated for use in cancer treatment (Goodwin *et al.*, 1978) but also in chemical-biological warfare.

In animal studies, all frequently isolated strains (*Penicillium* sp., *Aspergillus versicolor*, *A. flavus*, *Cladosporium sphaerospermum*, and *C. cladosporioides*) in Slovakia produced secondary metabolites with the strongest ciliostatic activity—their exo- and endometabolites stopped tracheal ciliary movement in chicks for 24 h (Pieckova, 2002). On building materials, *Penicillium chrysogenum* produced only few detectable metabolites or frequently none (Nielsen, 2002).

Toxic metabolites from isolates of *Trichoderma harzianum* isolated from the indoor environment of a building where the occupant was suffering serious building-related ill-health symptoms damaged the cell membrane barrier function of sperm cells (Peltola *et al.*, 2001). However, in Nielsen's studies, *Trichoderma* spp. did not produce detectable quantities of trichothecenes on building materials (Nielsen, 2002). Nielsen (2002) further showed that *Chaetomium globosum* produced high quantities of chaetoglobosins on building materials. *Wallemia sebi*, a common indoor xerophyllic fungus, was found to produce the mycotoxins walleminol and walleminone (Frank *et al.*, 1999). However, reports on the biological effects of most secondary metabolites are scarce, and very few of the studies evaluated the effects of inhaled secondary metabolites (Nielsen, 2003).

Samson (1992) divided the adverse effects into four categories: acute, chronic, mutagenic, and teratogenic. Symptoms related to mycotoxins or toxin-containing spores (particularly those of *S. chartarum*) include dermatitis; recurring cold and flu-like symptoms; burning sore throat; headaches and excessive fatigue; diarrhea; and impaired or altered immune function; as well as cough; irritation of eyes, skin, and respiratory tract; or joint ache (Singh, 1994; Tuomi *et al.*, 2000). Compromised ability of the body to resist infectious diseases may lead to opportunistic infections and possibly cancers. Certain mycotoxins, such as zearalenone, have been found to cause infertility and stillbirths in pigs (Matossian, 1989). Low-level, complex exposures from a mixture of mycotoxins may have synergistic effects and may result in central neuroendocrine-immune changes and consequently in complex health reactions of the endocrine and nervous systems (Ammann, 1999). Residents or occupants who were exposed to toxigenic fungi in water-damaged buildings might suffer from nonspecific symptoms (Tuomi *et al.*, 2000).

Although relationships were established to link inhalation exposure to mycotoxin-containing fungal spores and symptoms of mycotoxicoses in fungi-infested indoor environments (Croft *et al.*, 1986; Johanning *et al.*, 1993), other possible exposure routes such as ingestion and dermal contact are likely. Because fungal spores are ubiquitous in a contaminated environment, the chance of ingesting toxin-containing spores is likely to increase through eating, drinking, and smoking.

Concerns were raised that many of the data on exposures to toxigenic molds were derived from animal toxicity studies, and these are based primarily on ingestion (Assoulin-Daya *et al.*, 2002). Whether these results can be extrapolated to human health is questionable. In a review article, Robbins *et al.* (2000) argued that although evidence was found

for a relationship between high levels of inhalation exposure or direct contact to mycotoxin-containing molds or mycotoxins, and demonstrable effects in animals and health effects in humans, the current literature does not provide compelling evidence that exposure at levels expected in most mold-contaminated indoor environments is likely to result in measurable health effects.

Novotny and Dixit (2000) reported that two fungi, *Penicillium* (presumptively *Penicillium purpurogenum*) and *Trichoderma* sp., were cultured from surface samples collected in the residence where a 40-day-old male suffered pulmonary hemorrhage following exposure to indoor fungi and tobacco smoke. The authors thought the fungi to be mycotoxin producers and tried to link the fungi to the pulmonary hemorrhage. However, without properly identifying the fungi to species, it is premature to assume the fungi were mycotoxin producers and is difficult to establish the causal association between pulmonary hemorrhage, the fungi, and mycotoxins. The health effects of indoor molds can be inconsistent; the presence of fungi was reported to be a significant risk factor in Red Deer but not in Medicine Hat, Alberta, Canada (Hessel *et al.*, 2001).

Since mycotoxin production is species specific, it is crucial to inventory all fungi growing in a contaminated indoor environment and identify the fungi to species. Without knowing the fungal species that are present, determination cannot be made whether the species are toxigenic or not. In addition, the investigators will not be able to ascertain whether species are able to produce mycotoxins under the investigated conditions. Mycotoxin production can be influenced by substrates (medium composition), temperature, water activity, and other factors, and indoor fungi likely produce different mycotoxins on building materials (Nielsen, 2003). Therefore it is very difficult to evaluate the severity of indoor fungal and mycotoxin contamination.

D. Volatile Organic Compounds (VOCs)

Actively growing fungi produce a variety of volatile organic compounds (VOCs), which may produce a distinctive musty, moldy odor. Fungal VOCs may include 3-methylbutan-1-ol, 3-methylbutan-2-ol, fenchone, heptan-2-one, hexan-2-one, octan-3-one, octan-3-ol, pentan-2-ol, alpha-terpineol, and thujopsene. They emit these compounds into the indoor environment (Elke *et al.* 1999). The most prevalent compounds included xylene, toluene, 2-propanol, limonene, and heptane. Formaldehyde concentrations ranging from 1.7 to 13.3 microg/m^3 and mean acetaldehyde levels ranging from $<$3.0 to 7.5 microg/m^3

were reported (Reynolds *et al.* 2001). Larsen and Frivad (1995) studied the *in vitro* production of fungal volatiles from 47 *Penicillium* taxa and detected alcohols, ketones, esters, small alkenes, monterpenes, sesquiterpenes, and aromates. However, aldehydes were not among the VOCs detected.

Fiedler *et al.* (2001) studied VOC production by *Aspergillus fumigatus, A. versicolor, A. niger, A. ochraceus, Trichoderma harzianum, T. pseudokoningii, Penicillium brevicompactum, P. chrysogenum, P. claviforme, P. expansum, Fusarium solani*, and *Mucor* sp. More than 150 volatile substances derived from the fungal cultures have been analyzed by head-space solid-phase microextraction (HS-SPME) (Fiedler *et al.*, 2001). Each species had a defined VOC profile, which may be subject to considerable modification in response to external factors such as cultivation on different substrata. Cultivation on different substrata changes the number and concentration of VOCs (Fiedler *et al.*, 2001). Wilkins *et al.* (2000) studied the production of VOCs by mold species isolated from damp buildings. They were grown on sterile building materials and some synthetic media. Patterns of the volatile organic compounds were very media dependent, but media, which favor terpene biosynthesis, may give patterns unique enough for identification of dominant indoor molds (Wilkins *et al.*, 2000). It was proposed that species-specific volatiles may serve as marker compounds for the selective detection of fungal species in indoor environments (Fiedler *et al.*, 2001). Examination of VOCs from indoor air samples may become an important method in indoor air hygiene for the detection of type and intensity of masked contamination by molds (Fiedler *et al.*, 2001). Additional fungal VOCs are compiled and listed by Ammann (2) and Batterman (8). Almost all of the published information regarding fungal VOCs concerns species of *Penicillium* and *Aspergillus*.

Some of the fungal VOCs have an unpleasant odor (Gravesen *et al.*, 1994). The musty, moldy, and earthy odors are likely to come from 2-octen-1-ol and geosmin (1, 10-dimethyl-9 decalol) (Flannigan *et al.*, 1991). Ezeonu *et al.* (1994) identified ethanol, 2-ethyl hexanol, cyclohexane, and benzene from fiberglass air duct liners colonized by *Aspergillus versicolor, Acremonium obclavatum*, and *Cladosporium herbarum*. Acetone and 2-butanone were only detected on agar plate samples of *A. versicolor* and *A. obclavatum*. The 2-ethyl hexanol and cyclohexane are eye and skin irritants, and benzene is a generally recognized hazardous chemical. Other fungal VOCs associated with two common indoor fungi, *Penicillium* and *Aspergillus*, have been identified. They are 2-methyl-isoborneol, 2-methyl-1-propanol, 3-methyl-1-butanol, and 3-octanone (Gravesen *et al.*, 1994).

Ahearn *et al.* (1997) found that lower concentrations of volatile organics were released from air filter medium colonized by fungi than noncolonized filter medium in a water-damaged office building. However, the volatiles from the colonized filter medium included fungal metabolites such as acetone and a carbonyl sulfide-like compound that were not present and released from noncolonized filter medium. They suggested that the growth of fungi in air distribution systems may affect the content of volatile organics in indoor air (Ahearn *et al.*, 1997).

Fungal VOC levels indoors are normally low. Indoor concentrations of total volatile organic compounds (TVOCs) were reported ranging from 73 to 235 microg/m^3 (Reynolds *et al.*, 2001). However, microbial volatile organic compounds (MVOCs) and metabolites of fungi detected in indoor molds are considered to be a potential health hazard (Kreja and Seidel, 2002). Their toxicological relevance and health effect, however, is largely unknown, and data are rare (Kreja and Seidel, 2002).

Although VOCs produced by *Aspergillus, Penicillium*, and other fungi have been investigated extensively, little information exists on what VOCs can be produced by *S. chartarum* (Gao and Martin, 2002). Four unique VOCs—1-butanol, 3-methyl-1-butanol, 3-methyl-2-butanol, and thujopsene—were detected on rice cultures of *S. chartarum*, and only one of them (1-butanol) was detected on gypsum board cultures (Gao and Martin, 2002). For a given strain, VOCs were considerably different with different cultivation media (Gao and Martin, 2002). Concentration profiles of the volatile compounds varied among compounds; however, each compound exhibited corresponding concentration trends between the strains (Gao and Martin, 2002). In comparison with their previous studies of five *Aspergillus* species on gypsum board under the same experimental conditions, fewer unique VOCs were produced by *S. chartarum*, and they were significantly different. Gao and Martin (2002) indicated that VOCs produced by *S. chartarum* may represent a relatively small portion of the total volatiles present in problematic buildings where *Aspergillus* spp., *Penicillium* spp., and other fungi frequently coexist (Gao and Martin, 2002).

Elke *et al.* (1999) described a new, analytically valid procedure to assess the exposure of humans to the so-called microbial volatile organic compounds (MVOCs). The method can be used routinely for large sample numbers and is especially valuable as a basis for further research on the correlation between single MVOCs and indoor mold growth (Elke *et al.*, 1999). With the exception of 3-methylbutan-2-ol, fenchone, nonan-2-one, octan-2-one, and thujopsene, indoor air concentrations of all MVOCs under investigation were significantly higher

inside damp and moldy dwellings. It was found that 3-methylbutan-1-ol, hexan-2-one, heptan-2-one, and octan-3-ol were found to be most reliable indicators for mold growth (Elke *et al.*, 1999).

A correlation was also found between selected VOCs and the occurrence of mold species in mattress dust (Elke *et al.*, 1999). *Aspergillus* sp. correlated with heptan-2-one, hexan-2-one, octan-3-ol, octan-3-one, and alpha-terpineol, while the occurrence of *Eurotium* sp. was correlated with higher indoor air concentrations of 3-methylbutan-1-ol, 3-methylbutan-2-ol, heptan-2-one, hexan-2-one, octan-3-ol, and thujopsene (Elke *et al.*, 1999). However, the correlation raised the question whether mold species in mattress dust were spores or active growth. Spores are metabolically slow or inactive and are less likely to produce VOCs.

Indoor fungal VOCs have been suggested as possible contributors to adverse health effects. Symptoms related to exposure to fungal VOCs include nasal irritation and feelings of stuffiness (Flannigan and Miller, 1994). Possibly related mucous membrane and olfactory irritations may trigger an ''unpleasant odor reaction'' and annoyance (Yang and Johanning, 2002). Although exposure to molds can produce significant mucosal irritation, there are very few data to suggest long-term ill effects. More importantly, there is no evidence in humans that mold exposure leads to nonmucosal pathology (Assoulin-Daya *et al.*, 2002). In a recent study, MVOC-induced DNA damage was observed under conditions in which cytotoxic effects were observed but clastogenic and mutagenic effects could not be detected (Kreja and Seidel, 2002).

E. Glucans

The polyglucose (1 → 3)-β-D-glucan is a component of cell walls of fungi, some bacteria, and plants (Rylander and Lin, 2000). (1 → 3)-β-D-Glucan has been recognized as a potential proinflammatory agent responsible for bioaerosol-induced respiratory symptoms observed in both indoor and occupational environments (Gehring *et al.*, 2001; Milton *et al.*, 2001; Rylander and Lin, 2000). Relationships between the amount of (1 → 3)-β-D-glucan and the extent of symptoms, lung function changes, and inflammatory markers have been described. In addition, (1 → 3)-β-D-glucan can be used as a surrogate for measuring mold biomass in field studies (Rylander and Lin, 2000).

Milton *et al.* (2001) developed a specific enzyme immunoassay to quantify (1 → 6) branched, (1 → 3)-β-D-glucans in environmental samples. The assay was highly specific for (1 → 6) branched, (1 → 3)-β-D-glucans and did not show any response at 200 ng/ml to curdlan,

laminarin, pustulan, dextran, mannan, carboxymethyl cellulose, and endotoxins (Milton *et al.*, 2001). The detection level was 0.8 ng/ml for baker's yeast glucan and Betafectin. A coefficient of variation of 7.8% was obtained for (1 → 3)-β-D-glucans in house dust samples. It will be useful for the investigation of health effects from exposure to this class of biologically active molecules (Milton *et al.*, 2001).

Concentrations ranged from below the limit of detection to 19,013 microg/m^2 (22,588 μ/g dust) from living room floors of 395 homes of two German cities, Erfurt and Hamburg (Gehring *et al.*, 2001). Associations between (1 → 3)-β-D-glucan, housing characteristics, and occupant behavior were found for concentrations per square meter but not for concentrations per gram of dust (Gehring *et al.*, 2001). The following characteristics were associated with a significant increase in beta (1 → 3)-β-D-glucan levels: carpets in the living room, keeping a dog inside, use of the home by four or more persons, use of the living room for >180 hr/week, lower frequency of vacuum cleaning and dust cleaning, and presence of mold spots during the past 12 months (Gehring *et al.*, 2001).

III. Indoor Fungi

A. Fungal Identification

The modern concept of the Kingdom Fungi consists of four phyla, Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota. In addition, many fungi are conveniently placed in the form group deuteromycetes. Deuteromycetes include fungi that grow vegetatively and may reproduce by producing asexual spores. Species of Zygomycotina, Ascomycotina, Basidiomycotina, deuteromycetes, and myxomycetes have all been reported from the indoor environment or identified from indoor samples by the authors. Many important fungal traits, such as ecological preferences, the production of mycotoxins, secondary metabolites, VOCs, and the associated health effects, are species-specific. It is therefore extremely important that fungi be accurately identified to species. Miller (1991) stressed the importance of reliable fungal identification no matter what the identifications are used for.

Fungal classification and identification are based on morphological, biological, molecular, genetic, and physiological characteristics. Morphological characteristics, both macroscopic and microscopic, are conventionally the most important in fungal classification and identification. However, there are situations in which morphological characteristics cannot differentiate similar or closely related species in large fungal genera such as *Penicillium* or *Aspergillus*. Pitt (1979) and

Klich and Pitt (1988) incorporated physiological and other characters to facilitate speciation of *Penicillium* and *Aspergillus*. With the advances in molecular and genetic studies, different approaches have been tried. However, their practical uses have been limited and the current fungal identification is still morphology-based.

B. Airborne Fungi

Airborne fungal spores can originate from outdoor as well as indoor sources. Many studies have been conducted for indoor and outdoor airborne fungal spores in various parts of the world. In a well-built and properly maintained building without a history of water damage, its airborne fungal spores are likely from outdoor sources and should reflect outdoor spora qualitatively and quantitatively. Only if a building experiences a significant water damage problem and subsequent fungal growth do its airborne spores become a significant factor. The main health concern of indoor fungal growth is the exposure of occupants to airborne fungal spores and other byproducts of fungal growth from indoors.

A number of air samplers are available for airborne fungi analysis. However, two types of them are widely used: (a) spore trap type for total spore count; (b) sieve to agar type (cultural method) for examination of colonies. The first involves collecting fungal spores and fragments on sticky surfaces applied to a glass slide for direct fungal spore identification under microscopes. Results are expressed as fungal spores/m^3. This yields information of total airborne fungal structures including both viable and inviable. Such information is important for any allergen-related health concerns, since all fungal spores and fragments can be allergenic no matter whether they are viable or inviable (including some non-sporulated ones on cultural media, such as most basidiomycetes, obligate phytoplane fungi). However, most fungal spores can be identified to only genus level. The second method involves collecting fungal spores on growing media. After a 7-day incubation, fungi are identified from colonies developed on the media and enumerated as colony forming units (CFU)/m^3. The latter one, the culturable method, produces information very useful for assessment of pathogenic or mycotoxingenic fungi and their health effects. This method may not be able to quantify and characterize nonviable and non-sporulated fungi accurately, but the fungi developed on media can be identified to species level. The information of fungal species is essential for assessing the health effects of fungal infestation indoors, since pathogenicity and mycotoxingenicity are species-specific.

Molds growing indoors have been understood for some time as a source of aeroallergens (Pope *et al.*, 1993; Reed, 1985). Some fungi may cause infection or allergy, depending primarily on the susceptibility of the hosts (Morey and Feeley, 1990). Outdoor sources of spores are generally considered to be the major contributor to the indoor air spora (Flannigan *et al.*, 1991; Li and Kendrick, 1995a). Most of the fungi that contribute significantly to indoor airborne spores reproduce primarily by asexual spores (Burge, 1990a), although *Chaetomium globosum*, which produces ascospores, is a common contaminant of water-damaged paper and wood products. The indoor air environment may be considered a walled-in portion of the outdoor. It differs in patterns of air movement, humidity, temperature, and possibly also in gas composition. Air movement results from ventilation, convection, heating system, cleaning activities, and movement by occupants, but air does not move as continually—or as rapidly—over a surface as it does outdoors. Outside air movement influences movement indoors by forcing air through cracks on the windward side and sucking it out on the leeward, with the direction of airflow changing as the wind direction changes (Lacey, 1981). Artificial ventilation can rapidly circulate spores through a building, but convection can also be very effective, carrying spores from first- to fourth-floor halls within 5 min and into rooms within 20 min (Christensen, 1950).

During summer in North America, counts of airborne spores outdoors and indoors may roughly correspond when windows are open (Snelly and Roby, 1979). It has also been well known for many years that fungi readily invade and propagate in the interior of homes, and that perennial allergic symptoms can be attributed to high concentrations of such spores (Salvaggio, 1986).

Species of *Cladosporium, Alternaria, Mucor, Aspergillus*, and *Penicillium*, among others, are common and are capable of reproducing indoors when appropriate substrates and moisture are available (Morring *et al.*, 1983). Ten *Cladosporium* species, some of which are potentially allergenic, have been isolated inside houses in Córdoba, Spain. Only small differences were recorded among the spora in the different rooms (Infante-Garcia-Pantaleton and Dominguez-Vilches, 1988). The genus *Rhizopus* was isolated mostly indoors in Barcelona, Spain (Calvo *et al.*, 1980). Cooley *et al.* (1998) reported their studies in 48 schools in the United States that the fungal genera comprised over 95% of the outdoor fungi: *Cladosporium* (81.5%), *Penicillium* (5.2%), *Chrysosporium* (4.9%), *Alternaria* (2.8%), and *Aspergillus* (1.1%). At 20 schools, significantly higher concentrations of *Penicillium* species were found in the air samples from complaint areas than from

noncomplaint areas in the schools. Ren *et al.* (1999) found that in the United States, *Cladosporium* spp. was dominant in both indoor and outdoor air in summer, while *Penicillium* and *Aspergillus* were dominant in indoor air in winter. The dominant airborne fungal spores indoors in Southern Ontario, Canada, were *Cladosporium* (38.8%), *Aspergillus/Penicillium* (19.8%), *Leptosphaeria* (7.9%), unidentified basidiospores (6.5%), unidentified ascospores (2.8%), *Ganoderma* (2.6%), *Alternaria* (1.9%), *Coprinus* (1.8%), and *Epicoccum* (3.3%) (Li and Kendrick, 1995a).

Concentrations and compositions of airborne fungal spores are determined by many factors and variations. There are regional, yearly, seasonal, and diurnal variations of airborne fungal spore populations (Li and Kendrick, 1994). Chao *et al.* (2002b) showed that fungal spore concentrations in large office buildings varied significantly with season, with a summer peak, and that concentrations of airborne fungi were positively correlated with RH and negatively with CO_2 concentrations. The seasonal patterns of indoor fungi are closely correlated with outdoor fungi in residential buildings (Li and Kendrick, 1995a). Vegetation, landscape, land usage, meteorological factors, and other environmental factors, as well as biological characters of fungi, determine the concentration and composition of airborne fungal spores (Li and Kendrick, 1994). These factors ultimately influence the airborne fungi indoors. Better understanding of these variations or patterns and other important factors is crucial for proper sampling strategies and sample collection.

At present there are insufficient research data of dose/exposure level and response relationship to establish practical thresholds for making public health decisions. Although such thresholds are very important for indoor environmental quality evaluation and investigation, it is highly unlikely that a dose-response relationship can easily be established because of the complexity of fungal compositions and related allergens and secondary metabolites.

In 7 homes of patients with asthma bronchiale, the concentrations of mesophilic fungal spores of the indoor air ranged from 100 to 1000 CFU/m^3, and this was much higher than the mold counts simultaneously collected outdoors (Senkpiel, 1996). The major fungal species found indoors by the investigator were *Penicillium* sp. > *Aspergillus* sp. > *Cladosporium* sp., *Mucor* sp., *Chrysonilia* sp., *Verticillium* sp. > *Geotrichum* sp., *Trichoderma* sp. (Senkpiel, 1996). The main cause of fungal contamination was moist building materials on/in room walls, insufficient air ventilation, poor maintenance of the circulating air machines, and insufficient room hygiene (e.g., biological garbage in

the kitchen) (Senkpiel, 1996). However, the reliability of the fungal identifications is questionable. All fungi were identified to genera. In addition, some unusual indoor fungi, such as *Chrysonilia* sp. and *Geotrichum* sp., were reported. Although *Chrysonilia* sp. has been reportedly isolated from indoors (Samson *et al.*, 2000), it is highly uncommon. *Geotrichum* sp. typically produces slimy colonies. In fact, it is so unusual in the air that Haugland and Vesper (2001) used *G. candidum* to spike samples for quality control purposes for their Quantitative Polymerase Chain Reaction (Q-PCR) studies. Their detection and identification of such fungi in the air are highly unusual unless the fungi were misidentified. The primary characters for the identification of *Geotrichum* species are their production of arthrospores. Many airborne fungi produce arthrospores in culture and are likely mis-identified as *Geotrichum* species.

The fungal spore concentrations and compositions in indoor and outdoor air in Yokohama, Japan, were sampled and analyzed with a Reuter centrifugal sampler (RCS) and dichloran 18% glycerol agar (DG18) and compared with the levels assessed with potato dextrose agar (PDA) (Takahashi, 1997). In indoor air, the fungal concentrations were <13 to 3750 CFU/m^3. *Cladosporium* spp. predominated, followed by the xerophilic fungi such as the *Aspergillus restrictus* group, *Wallemia sebi*, the *A. glaucus* group, and *Penicillium* spp. The fungal concentrations in indoor air peaked in October. The concentrations of fungi were significantly correlated with the indoor temperature, indoor relative humidity, and the outdoor climatic factors, except for the average velocity of wind (Takahashi, 1997).

Studies of airborne fungi employed several different media and samplers. However, there are insufficient comparative studies to determine their efficiencies and differences. A number of studies were conducted within a short period of time (less than 1 year). Since airborne fungal spores have distinct seasonality and year-to-year variations, studies for less than 1 year may not be able to yield meaningful information for the studied area.

Khan *et al.* (1999) collected air samples on rose-bengal medium for 20 minutes by using a six-stage Andersen sampler. *Aspergillus* spp. were the predominant component (27.7%) of the outdoor aerospora of Kuwait, and *A. fumigatus* alone accounted for 21.3% of the total aspergilli (Khan *et al.*, 1999). *Cladosporium* spp. were the major component of airborne fungal spores indoors (22.8%), followed by *Aspergillus* (20.9%), *Penicillium* spp. (14.6%), and *Bipolaris* spp. (10.6%) (Khan *et al.*, 1999). Ismail *et al.* (1999) used the settle plate method with Czapek-Dox agar in Uganda from March through June 1998. The

most prevalent fungi outdoors and indoors were *Mycosphaerella*, yeasts, *Penicillium, Fusarium, Aspergillus, Cochliobolus*, and *Alternaria* (Ismail *et al.*, 1999). In Poland the concentrations of airborne fungi in dwellings without mold problems were between 0 and 1997 CFU/m^3, while in moldy homes they were 49 and 16,968 CFU/m^3, respectively. Dominant fungi were *Penicillium* spp., *Aspergillus* spp., and yeasts. There were as many as 167 microbial species isolated from the air of examined dwellings by Gorny and Dutkiewicz (2002). A study of airborne fungi in bedrooms in 485 houses was performed over 1 year in Melbourne, Australia. Fifty-five percent of the houses had viable fungal propagules exceeding 500 CFU/m^3, and *Cladosporium* and *Penicillium* were identified as the most prevalent and abundant fungal genera in indoor air (Dharmage *et al.*, 1999). Klanova (2000) reported the total concentrations of airborne fungi were much higher in moldy rooms than in the reference rooms, but health complaints did not correlate with the total concentrations of airborne fungi. All occupants of rooms where the average concentration was 2476 CFU/m^3 reported health complaints.

Sometimes samplings of airborne fungal spores may not reflect the fungal problems indoors. Hyvarinen *et al.* (2001) found that some fungal genera detected in moist building materials such as *Ulocladium* and *Chaetophoma* were not found in indoor air. This result showed that bulk samples of building materials provide additional mycota information in the building (Hyvarinen *et al.*, 2001).

Vujanovic *et al.* (2001) proposed that airborne fungi could be classified on the basis of the relationship between the two environmental factors and their combinations (i.e., temperature and water requirements [water activity, a_w]). Three different groups are proposed: (i), represented by *Emericella* (*Aspergillus*) *nidulans, A. niger*, and *A. ochraceus*, and characterized by sporulation that was more dependent on temperature than on water activity; (ii), represented by *A. flavus* and *A. versicolor*, in which sporulation was approximately equal and depended on both the temperature changes and a_w alterations; and (iii), represented by *Cladosporium* sp., *Penicillium cyclopium*, and *P. citrinum*, in which sporulation depended more on alteration of the a_w conditions than on temperature changes. Temperature and a_w for each of the three phases of fungal growth (i.e., germination, growth, and sporulation) could be important for the determination of the fundamental niche of each fungus and its ability to form or accumulate mycotoxins (Vujanovic *et al.*, 2001).

Recently Gorny *et al.* (2002b) reported their findings of the study of airborne fungal fragments. The study found that small fungal fragments of *Aspergillus versicolor, Penicillium melinii*, and *Cladosporium*

cladosporioides were released into the air simultaneously with conidia from agar and ceiling tile surfaces (Gorny *et al.*, 2002b). However, the results clearly showed that the release mechanisms for fungal fragments and conidia are different. The release of fungal propagules depended on the fungal species, the air velocity above the contaminated surface, and the texture and vibration of the substrates (Gorny *et al.*, 2002b). Gorny *et al.* (2002b) showed that fragments and conidia of *Aspergillus versicolor* and *Penicillium melinii* shared common antigens by using enzyme-linked immunosorbent assays with monoclonal antibodies. This study clearly showed the potential biological relevance of airborne fungal fragments. The presence of airborne fungal fragments at the clearance stage of any remediation in mold-infested buildings should not be overlooked or underestimated.

Shelton *et al.* (2002) evaluated 12,026 fungal air samples (9619 indoor samples and 2407 outdoor samples) collected from 1717 buildings located across the United States by using Andersen N6 single stage samplers. The culturable airborne fungal concentrations in indoor air were lower than those in outdoor air. However, *Stachybotrys chartarum* was identified in the air in 6% of the buildings studied and in 1% of the outdoor samples. The fungal levels were highest in the fall and summer and lowest in the winter and spring. Geographically, the highest fungal levels were found in the Southwest, Far West, and Southeast (Shelton *et al.*, 2002). However, the reliability of fungal identification and sampling quality control of such a large project must be scrutinized before the results and conclusions are accepted.

Viability and culturability of airborne fungi are influenced by many factors. Environmental factors are the predominant ones. However sampling methods, devices, and time may have significant effects on the viability of airborne fungi. The duration of air sampling when using Andersen, SAS, and RCS samplers varies from 1 to 10 min. Since most airborne fungi have well defined diurnal patterns (Li and Kendrick, 1995b) and such a short sampling time or ''snap shot'' for airborne fungi, an investigation may miss the peak periods of airborne fungi. This kind of air sampling does not fully reflect the exposure of occupants or workers to airborne fungi. This is the reason why filtration at composting facilities is a preferred method for a long duration of sampling for air fungal spores. In a recent study Durand *et al.* (2002) showed that increased sampling time (up to 6 h) did not affect the viability of airborne fungi collected on polycarbonate filters at 2 l/min.

At present there are a number of samplers available on the market for collecting culturable spores (Andersen, Burkard, SAS, RCS, AGI-30, Biosampler, etc.) and for trapping spores and hyphal fragments

(Burkard, Andersen, Air-O-Cell, Micro-5, Cyclex-D, Allergenco, Biosystem, AGI-30, BioSampler impingers, etc.) as well as ones used for both culturable and total spore collection purposes (bi-cassette, Button Sampler, polycarbonate filter, etc.). Several of these are new products. Further evaluation and validation of the new products are necessary. Comparative interpretation of the results collected with different samplers is impossible, since there is no standard sampling and analytical protocol, and different samplers have different collection characteristics. This is one more reason why determination of the exposure limits or thresholds of airborne fungi is difficult to impossible.

Gorny *et al.* (2002a) found that vibration of 1Hz/14W releases the highest number of fungal progagules of *Aspergillus versicolor, Penicillium melinii*, and *Cladosporium cladosporioides* into the air. Kildesø *et al.* (2002) found that release of conidia of *P. chrysogenum* and *Trichoderma harzianum* from wet gypsum board was not significantly influenced by repeated air disturbance on fungal growth. *Penicillium chrysogenum* reached maximal sporulation at 18 to 23 days on wet gypsum board, while *T. harzianum*, around 20 days (Kildesø *et al.*, 2002).

Several published studies have dealt with the adverse effects of airborne fungal spores indoors as related to residential characteristics such as presence of basement, stove, carpets, humidifier, and heating systems (Agrawak *et al.*, 1988; Su *et al.*, 1992). Specific indoor environments may have unique conditions that allow fungal growth to occur. Fungal species seem to develop preferentially in kitchens, followed by bathrooms. They occur less frequently in bedrooms, probably as a result of the lower humidity prevailing in these rooms (Infante-Garcia-Pantaleton and Dominguez-Vilches, 1988). Recorded spore concentrations in the air of some mold-affected houses during winter were equal to or greater than those expected outside in summer (Flannigan *et al.*, 1991). The predominance of these fungi in the indoor atmosphere has been attributed to their ability to grow on many household items such as foods, damp leather goods, paper and cotton fabrics, and almost any chronically damp surfaces (Vittal and Glory, 1985). Among indoor microhabitats known to favor mold growth are garbage containers, food storage areas, upholstery, wallpaper, house plants, books, papers, and areas of moisture such as damp basements, walls, ceilings, shower curtains, window moldings, air conditioners, humidifiers, and vaporizers (Morring *et al.*, 1983; Salvaggio and Aukrust, 1981).

Observations in one climate do not necessarily apply to other climates. Indoor molds may be more important in humid climates. Occasionally, in bathrooms or basements, a persistent damp area may support enough mold growth in otherwise dry houses to liberate

sufficient spores to cause disease (Reed, 1985). Li and Kendrick (1995a,c, 1996) used CANOCO and path analysis to show that most indoor fungi originated from outdoor sources, and both diurnal and seasonal patterns clearly showed the close correlations of airborne fungi indoors and outdoors. Different fungi possess different diurnal and seasonal patterns, and the diurnal patterns of ascomycetes and basidiomycetes are very different from hyphomycetes (Li and Kendrick, 1995a,b).

The significance of the diurnal and seasonal patterns in the evaluation of indoor airborne fungal spores has long been overlooked. Better understanding of the seasonal and diurnal patterns can help individuals who are doing mold exposure investigation to better understand population dynamics of airborne fungi at a specific time, location, and season and can assist individuals to determine the specific sampling strategy accordingly.

Compared with outdoor air, much less research has been done on indoor airborne fungal spores. It is partially due to the difficulty of obtaining access to suitable residences and patients to carry out a long-term study. In addition, there are many variables involved, such as the structure of the houses, the furniture, upholstery, ventilation and heating systems, and the cultural background and activities of residents. All these variables make it very difficult to design an experiment that accounts for all important factors and the interactions among them. Furthermore, the indoor niche is suitable for certain fungi to grow year around, which could blur the exposure/symptom relationship.

Four recent impactor air samplers were selected for study: Samplair (AES, Combourg, France); Air Test Omega (LCB, France); Air Samplair Mas-100 (Merck, France); and BioImpactor 100–08 (AES) (Nesa *et al.*, 2001). They were compared with one another at three different hospital sites with varying levels of air contamination. No significant difference in the efficiency of spore recovery was found between Air Test Omega, Mas-100, and BioImpactor, whereas Samplair was significantly less efficient (Nesa *et al.*, 2001). BioImpactor was then selected to represent the three superior impactors and was compared with the single-stage Andersen disposable sampler, the Collectron MD8 air sampler (Sartorius, France), and the High Flow Air Sample (BioTest, France), which are based on filtration and centrifugation methods, respectively. No significant difference was observed in terms of spore recovery (Nesa *et al.*, 2001). On the basis of their performance, unit sampling cost, autonomy, and simplicity of use, the authors concluded that Air Test Omega, Air Samplair Mas-100, and BioImpactor 100–08 are suitable for routine indoor evaluation of fungal contamination of air in hospitals (Nesa *et al.*, 2001).

Most research so far has been conducted with viable and culturable methods (which enumerate and identify fungal colonies) and with a variety of samplers because no standard method and instrument have been established. It is therefore very difficult to compare the results obtained with different protocols in a meaningful way. Research with sampling for culturable propagules tends to seriously underestimate actual spore levels. Airborne fungal spores may be viable, dormant, moribound, or dead. Burge (1986) believed that viable spores are highly selective in their cultural requirements. However, most fungi and fungal spores identified in various studies mentioned in the text are ready to grow on common fungal media unless they are dead or dormant. Some fungi, such as certain ascomycetes and basidiomycetes, are difficult to culture on the media normally employed. In any case, spores do not need to be viable to cause allergies. Some very important allergenic species would, therefore, have been ignored by most studies based on culturable methods. In the last several years several new spore-trapping samplers were developed in addition to Burkard, Allergenco, and Rotorod. The Burkard sampler, Allergenco, Air-O-Cell, Laro, and Cyclex-D samplers, etc., overcome the shortcomings of the culturable methods by permitting visual counting and identification of spores trapped on adhesive slides or tapes. However, there is a deficiency with these spore-trapping samplers—namely, that some fungal spores can only be identified to the generic or group level unless they are cultured from the spores. This problem points out the difficulty of identifying many similar-looking spores to the generic or species level even by highly experienced mycologists and the need to develop broad spectrum culture techniques or media. To a large extent, both problems remain unsolved.

C. Fungi Growing on Indoor/Building Materials

The detection of airborne fungi does not necessarily indicate growth or amplification of fungi indoors. However, it is generally believed that actively growing fungi indoors are the principal cause of the adverse health effects because of constant exposure to indoor sources of fungal allergens, mycotoxins, glucan, and fungal VOCs. Needless to say, it is important to characterize indoor fungi and at the same time to identify growth sites of fungi indoors.

Many common indoor fungi are strong deteriorating agents and have been reported from various building materials and systems. Many species of the genus *Penicillium*, commonly detected in indoor air sampling, have frequently been referred to as food spoilage and

bio-deteriorating agents (Gravesen *et al.*, 1994; Pitt, 1979). Pasanen *et al.* (1992) demonstrated that *Penicillium* was the most common mesophilic fungal genus in all the building materials studied (wallpaper, wood, plywood, gypsum board, and acoustical fiber board), comprising 70% of identified fungi. Raper and Fennell (1977) reported *Aspergillus* spp. from building materials such as textiles, jute, insulation materials, wallpaper, and other paper products. Gravensen *et al.* (1994) included a list of 13 fungal species as important molds in damp buildings. Samson *et al.* (1992) described 23 common fungal species in indoor environments.

In water-damaged building materials in Denmark, the fungal genera most frequently encountered indoors were *Penicillium* (68%), *Aspergillus* (56%), *Chaetomium* (22%), *Ulocladium* (21%), *Stachybotrys* (19%), and *Cladosporium* (15%) (Gravesen *et al.*, 1999). *Penicillium chrysogenum, Aspergillus versicolor*, and *Stachybotrys chartarum* were the most common species in water-damaged materials (Gravesen *et al.*, 1999). *Stachybotrys atra* was isolated with swab samples of visible growth under wet carpets, on wet walls, or behind vinyl wall coverings in 11 schools in the United States (Cooley *et al.*, 1998).

Morgan-Jones and Jacobsen (1988) studied moldy carpets, plasterboard, and wallpaper from three hotels in Florida and Georgia. The genera of fungi identified were species of the ascomycete genus *Chaetomium*; of the dematiaceous hyphomycete genera *Alternaria, Cladosporium, Stachybotrys*, and *Ulocladium*; of the moniliaceous hyphomycete genera *Acremonium, Aspergillus*, and *Penicillium*; and of the pycnidial genus *Phoma*. In the study, 14 species, including 2 new species of *Cladosporium*, in 11 genera were isolated and identified. In a study of toxicity of moldy building materials, Johanning *et al.* (1998) identified several groups of fungi from gypsum wallboard and other building materials. The fungi identified included those described by Morgan-Jones and Jacobsen (1988) and additional species of *Aspergillus, Paecilomyces*, and *Trichoderma*. Käpylä (1985) found that predominant fungus growing on wooden frames of insulated windows in Finland was *Aureobasidium pullulans*. In subartic areas, Pessi *et al.* (2002) found that indoor fungi occurred infrequently in the insulation inside insulated precast external concrete walls and that fungal infestation in the insulation was not found to influence indoor air in the region.

In Brazil in unusual situations, growth of *Cryptococcus neoformans* var. *neoformans* indoors was due to the presence of avians in the domestic environment or nearby the home. Higher incidences of cryptococcosis was reported among AIDS patients residing in the dwellings

from which *C. neoformans* var. *neoformans* was isolated than among AIDS patients from whose domestic environment the fungus was not detected (Passoni *et al.*, 1998).

In the United States, *Histoplasma capsulatum* and cases of histoplasmosis have been reported from indoor environments such as old houses, church attics, chicken houses, and barns (Collier *et al.* 1998; Lenhart, 1994). A primary source of *H. capsulatum* is soil, especially in regions of bird or bat habitats. While wind is probably the most important means of disseminating *H. capsulatum*, the fungus can survive and be transmitted from one location to another on the feet of both birds and bats (Rippon, 1988).

D. Fungal Biodiversity Indoors

One hundred sixty-seven microbial species were discovered from the air of dwellings in Central and Eastern Europe (Gorny and Dutkiecicz, 2002). Yang *et al.* (1993) reported that *Cladosporium, Penicillium, Aspergillus*, basidiomycetes, and *Alternaria* were identified, by frequency, as the top five fungal taxa both indoors and outdoors in the United States. An additional 50 fungal taxa were also identified. However, *Penicillium, Cladosporium, Aspergillus*, basidiomycetes, and *Alternaria* were the top five indoor fungal taxa by concentrations in a descending order, while *Cladosporium, Penicillium*, basidiomycetes, and *Aspergillus* were the top five outdoor taxa. Womble *et al.* (1999) of the US Environmental Protection Agency (USEPA) reported that nonsporulating fungi, *Cladosporium, Penicillium*, yeasts, and *Aspergillus* were the five most commonly found fungal taxa indoors and outdoors, based on the frequency of detection. The most common culturable airborne fungi indoors and outdoors in all seasons and geographic areas in the United States were *Cladosporium, Penicillium*, nonsporulating fungi, and *Aspergillus* in a descending order (Shelton *et al.*, 2002). In Taiwan the predominant genera of airborne fungi are *Cladosporium, Aspergillus, Penicillium, Alternaria*, and yeast (Su *et al.*, 2001b). *Stachybotrys chartarum* was identified in the indoor air in 6% of the buildings studied and in 1% of the outdoor air around the buildings studied in the United States (Shelton *et al.*, 2002).

E. *Stachybotrys chartarum* and Other *Stachybotrys* spp.

Stachybotrys chartarum is one of the major species occurring on cellulose-based building materials in indoor environments. This species has attracted the most attention because of its ability to produce

some of the most potent mycotoxins known and its association with infant pulmonary hemorrhage and hemosiderosis and adult nasal and tracheal bleeding (Dearborn, 1997; Vesper and Vesper, 2002; Vesper *et al.*, 2001).

Stachybotrys chartarum was isolated for the first time from the lung of a child diagnosed with pulmonary hemosiderosis in Texas (Elidemir *et al.*, 1999). Flappan *et al.* (1999) reported another case of infant pulmonary hemorrhage associated with the presence of *Stachybotrys atra* (=*S. chartarum*), and mycotoxin analysis demonstrated that the isolate was highly toxigenic. Vesper *et al.* (2001) characterized a hemolysin, later called *stachylysin*, from *S. chartarum* and analyzed its hemolytic activity and siderophore production. It was hypothesized that stachylysin could be a contributing factor to infant pulmonary hemorrhage and hemosiderosis (Vesper and Vesper, 2002).

Under field conditions, several trichothecenes were detected in each of three commonly used building materials heavily contaminated with *S. chartarum* (Gravesen *et al.*, 1999). Under experimental conditions, four out of five isolates of *S. chartarum* produced satratoxin H and G when growing on new and old, very damp gypsum boards (Gravesen *et al.*, 1999). In a preliminary study conducted in a Dynamic Microbial Test Chamber, Foarde and Memetrez (2002) showed that conidia of *Stachybotrys chartarum* released from gypsum boards at low air flow rate were positively related to air flow rate, but negatively related to relative humidity.

Enzyme immunoassay indicated 65 of 132 (49.2%) sera tested contained IgG against *S. chartarum* and 13 of 139 (9.4%) sera tested contained IgE against *S. chartarum* (Barnes *et al.*, 2002). Sensitivity to *S. chartarum* is potentially much more widespread than previously appreciated (Barnes *et al.*, 2002). This fungus may affect the asthmatic and the allergic population through both immunologic and toxic mechanisms (Barnes *et al.*, 2002).

Rao *et al.* (2000), using an animal model, showed that methanol extraction dramatically reduced the toxicity of *S. chartarum* spores, and a single, intense exposure to toxin-containing *S. chartarum* spores resulted in pulmonary inflammation and injury in a dose-dependent manner.

Initial spore concentrations were between 0.1 and 9.3 spores/m^3 of air, and the toxicity of air particulates was correspondingly low. However, the dust in the house contained between 0.4 and 2.1×10^3 spores/mg (as determined by hemocytometer counts) (Vesper *et al.*, 2000). Air samples taken postremediation showed no detectable levels of *S. chartarum* or related toxicity. Nine isolates of *S. chartarum* obtained from a

home were analyzed for spore toxicity, hemolytic activity, and random amplified polymorphic DNA banding patterns (Vesper *et al.*, 2000). None of the isolates produced highly toxic spores (>90 μg T2 toxin equivalents per gram wet spores) after growth for 10 and 30 days on wet wallboard, but three isolates were consistently hemolytic (Vesper *et al.*, 2000).

In addition to *S. chartarum*, several species of *Stachybotrys* have been isolated and identified from the indoor environment. *S. yunanensis, S. nephrospora, S. microspora, S. elegans*, and *S. chlorohalonata* were identified and present in samples from indoor environments (Li and Yang, 2003; Nielsen *et al.*, 2003). Cruse *et al.* (2002) examined 23 isolates collected around the United States by using markers for three polymorphic protein-coding loci and found that 2 cryptic species are present within the species of *S. chartarum*. The 2 cryptic species were indistinguishable morphologically. In another recent study, Andersen *et al.* (2002) stated that there were two chemotypes: one producing atranones, the other macrocyclic trichothecene, plus one undescribed taxon identified based on the analysis of morphology, growth, and more importantly metabolite production. The chemotypes and the undescribed taxon were all previously identified as *Stachybotrys chartarum* (Andersen *et al.*, 2002). Later the undescribed taxon was described as a new species, *S. chlorohalonata* (Andersen *et al.*, 2003). Taylor *et al.* (2003) developed a QPCR assay for the detection of *Stachybotrys elegans*.

Characteristic symptoms and immunological tests for antibodies (IgE and IgG) specific to *S. atra* and some other fungi strongly suggest exposure to the indoor fungi. Hemorrhagic lung disease in infants was highly associated with indoor *S. chartarum* exposure in a case cluster investigation in Cleveland (Dearborn, 1997; Montana *et al.*, 1995) and in a case-home investigation in the midwestern United States (Flappan *et al.*, 1999). An epidemiological study reported a high prevalence of pulmonary diseases among office workers of Florida court buildings following prolonged indoor exposure to *S. chartarum* and *A. versicolor* (Hodgson *et al.*, 1998). *Stachybotrys atra* was isolated from bronchoalveolar lavage fluid of a child with pulmonary hemorrhage (Elidemir *et al.*, 1999), and *S. atra* exposure was found in an infant that developed laryngeal spasm and hemorrhage during general anesthesia (Tripi *et al.*, 2000). In mouse studies with toxic *Stachybotrys* fungi, similar effects (inflammation and hemorrhage) were observed (Nikulin *et al.*, 1996). Some detailed information on the latest research of *S. chartarum*, mycotoxins produced, and its health effects can be found in the review article by Kohn and Ghannoum (2003).

F. PCR and Molecular Techniques

A new methodology, Quantitative Polymerase Chain Reaction (QPCR or sometimes called real time PCR), offers a new venue to rapidly detect and quantify fungi with an unprecedented accuracy. Vesper and Haugland of the US Environmental Protection Agency (US EPA) have developed and patented primers for over 100 indoor fungal species (personal communication) in the last several years. Detection and quantification of selected indoor fungi is now possible by using the QPCR. A fungus-specific PCR assay using only one primer set has been developed for detecting indoor fungi (*Alternaria chamydospora, Aspergillus flavus, Candida famata, Cladosporium fermentans, Penicillium chrysogenum*, and *Stachybotrys chartarum*) (Zhou *et al.*, 2002). Their results indicated that FF2/FR1 among the 4 sets showed no cross-amplification with non-fungal DNA. On the other hand, the primer set cannot differentiate different species of fungi. Haugland *et al.* (2002) evaluated three comparatively rapid methods for the extraction of DNA from fungi for use in QPCR and developed a simple bead milling method that provides sensitive, accurate, and precise quantification of target fungi in air and water samples. However, dust samples required further purification of the extracted DNA by a streamlined silica adsorption procedure (Haugland *et al.*, 2002).

However, further validation of the primers of each fungal species is necessary. Occasional cross-reactions against closely related species or fungi within the same genus have been observed in preliminary tests by the authors. In a recent study, Wu *et al.* (2002) evaluated 53 sets of primers developed by various researchers for commonly occurring fungi and bacteria and verified 28 sets of them. They also evaluated 7 sets of primers for specific detection of *Aspergillus fumigatus* and found only 4 sets to be specific to *A. fumigatus*. The primers and Taqman probes developed by Cruz-Perez *et al.* (2001) for detection of *S. chartarum* in a QPCR analysis were found not to be specific to *S. chartarum*, and they found that positive amplifications of various fragment sizes were obtained for several fungal species. This study is significant for the application of QPCR. Caution has to be taken when a set of primers is going to be employed for the detection of a particular fungal species. Verification of the specificity of the primers is a necessity for any application.

In a recent article, Brakhage and Langfelder (2003) reviewed significant developments made in the last several years in understanding the genetics of *Aspergillus fumigatus* and molecular techniques for the identification of virulence determinants and manipulation of the fungus.

IV. Ecological Factors of Fungi Indoors

Fungal ecology indoors is very important for the understanding of occurrences and population dynamics of indoor molds. Some physical/environmental factors are closely associated with fungal growth and spore populations (Li and Kendrick, 1994, 1995b, 1995c and 1996).

A. Physical Factors

1. *Water and Moisture*

Lawton *et al.* (1999) demonstrated that moisture sources were a significant factor for mold infestation in the houses. In indoor environments, most factors necessary for dispersal, development, and colonization of fungi—such as nutrients, temperature, light, air movement—are readily available. The only limiting factor for fungal growth indoors is water or moisture. Modern energy-efficient buildings are sealed, prone to moisture accumulation, and slow to dry out if water damage/intrusion or dampness occurs. Since other factors for fungal growth are present in buildings, fungi will thrive once moisture or water is available. Rudblad *et al.* (2002) observed that teachers suffering from nasal mucosal hyperreactivity in a water-damaged school persisted over years and only slowly recovered after the water-damaged school was successfully remediated.

Damp building materials, particularly cellulose-containing substrates, are prone to fungal amplification. Fungi commonly found are species of *Penicillium, Aspergillus, Chaetomium, Ulocladium, Stachybotrys*, and *Cladosporium* (Graveson *et al.*, 1999). In the United Kingdom alone it has been estimated that 2.5 million houses are seriously affected by dampness caused by condensation in 60% of cases. A further 2 million houses suffer to a lesser extent from condensation (Sanders and Cornish, 1982). Among tenants of houses in the public sector in the United Kingdom there is a concern that mold growth on damp walls may be a hazard to health (Flannigan *et al.*, 1990). Homes with a dampness problem showed higher average spore counts and higher prevalence of respiratory symptoms (Waegemaekers *et al.*, 1989).

Water activity (a_w) expresses the available water in a substrate as a decimal fraction of the amount present when the substrate is in equilibrium with a saturated atmosphere (an equilibrium relative humidity of 70% around the substrate means that the substrate has a water activity of 0.70) (Kendrick, 2000).

Grant *et al.* (1989) found that the minimal a_w for spore germination and growth of indoor fungi on building materials was much higher than on fungal growth medium (MEA). On building materials, fungal growth starts at a water activity near 0.8 (Table VII), but production of significant quantities of mycotoxins required an a_w of at least 0.95 (Nielsen, 2003). Xerophilic fungi, such as *Penicillium* spp. and *Aspergillus* spp., will begin to grow at a_w between 0.78 and 0.90, depending on the compositions of substrates of construction materials (Nielsen, 2003). The minimal a_w required was different for spore germination and growth of indoor fungi, and a_w for fungal growth is higher than for spore germination (Grant *et al.*, 1989) (Table VIII).

2. *T and RH*

Relative humidity (RH) and temperature (T) are the most important environmental parameters regulating spore production (Mallaiah and Rao, 1980; Smith and Crosby, 1973). Sufficient moisture is probably the most important factor in spore production (Lyon *et al.*, 1984). Spore

TABLE VII

FUNGI GROWING ON BUILDING MATERIALS AND THEIR a_w

Colonizer group	a_w range	Classification	Fungal example
Primary colonizers (storage fungi)	<0.80	Xerophilic/ Xerotolerant	*Penicillium chrysogenum*, and *Aspergillus versicolor*: the most common ones; *A. fumigatus, A. niger, A. sydowii, A. ustus, Eurotium* spp., *P. brevicompactum, P. commune, P. corylophilum, P. palitans, Paecilomyces variotii*, and *Wallemia sebi*
Secondary colonizers	0.80–0.9	Mesophilic	*Alternaria* spp., *Cladosporium* spp., *Epicoccum nigrum, Phoma* spp., and *Ulocladium* spp.
Tertiary colonizers (water-damage fungi)	>0.9	Hydrophilic	*Chaetomium globosum, Fusarium, Memnoniella echinata, Rhizopus stolonifer, Stachybotrys chartarum, Trichoderma* spp. (*T. atroviride, T. citrinoviride, T. harzianum*, and *T. longibrachiatum*)

Grant *et al.* (1989); Lubeck *et al.* (2000); Macher *et al.* (1999); Nielsen (2003).

TABLE VIII

MINIMAL a_w FOR SPORE GERMINATION AND GROWTH OF INDOOR FUNGI AT 20° TO 25°C ON GROWTH MEDIA

Fungus	Minimal a_w	
	For spore germination	For growth
Alternaria alternata	0.85	0.88–0.89
Eurotium (Aspergillus) repens	0.70–0.72	0.71–0.76
Aspergillus versicolor	0.75–0.81	0.78–0.80
Aureobasidium pullulans		0.89
Cladosporium cladosorioides	0.86	0.88
Cladosporium herbarum	0.85–0.88	0.90
Cladosporium sphaerospormum		0.85
Fusarium moniliforme	0.87	
Mucor plumbeus		0.93
Penicillium brevicompactum	0.78–0.84	0.81–0.82
Penicillium chrysogenum	0.78–0.85	0.79
Penicillium nigricans		0.79
Penicillium spinulosum	0.80	0.80
Phoma herbarum	0.92	
Sistotrema brinkmannii		0.97
Stachybotrys chartarum	0.85–0.95	0.94
Ulocladium chartarum		0.89
Ulocladium consortiale		0.89

Compiled from Grant *et al.* (1989).

release of hyphomycetes was correlated with increasing T and decreasing RH. The moisture content of the indoor air significantly affected all measurable airborne concentrations (Pessi *et al.*, 2002). Spore release of *Botrytis squamosa* was promoted largely by declining RH, increasing T, and rain but occasionally by increased RH (Sutton *et al.*, 1978). Leach (1975) found that spore release of *Drechslera turcica* and other fungi is affected by decreasing RH but not by temperature changes. Conidia of *Cercospora asparagi* were caught beginning at 07:00–08:00am when T increased and RH fell below 90%, and the number of spores increased drastically (Cooperman *et al.*, 1986). Positive correlations of total air spora, *Cladosporium*, and *Botrytis* with higher temperatures have been documented (Beaumont *et al.*, 1985).

Variations/fluctuations in indoor humidity and temperature have significant effects on fungal growth (Adan, 1994; Vitanen and Bjurman, 1995). In a bathroom situation in which transient high humidities are common, dominant mycota include *Alternaria, Aureobasidium, Cladosporium, Phoma*, and *Ulocladium* (Moriyama *et al.*, 1992; Samson *et al.*, 2000).

Basidiospores of *Paxillus panuoides* were released at temperatures above 0 °C, and daily peaks were usually correlated with increased T and decreased RH. Spore release increased from 2 °C to a maximum at 37 °C, then ceased at 45 °C. Light and RH treatments did not significantly affect spore release. Temperature was determined to be the stimulus for the natural spore release pattern (McCracken, 1987). The humidity factor was correlated with basidiospores (Beaumont *et al.*, 1985). The higher counts of basidiospores may have resulted from the higher relative humidity and lower sunshine in 1978 in Galway, Ireland (McDonald and O'Driscoll, 1980). Since temperature is correlated with relative humidity, the effects of both factors could not be defined separately in most studies.

3. *Light*

For a number of the perithecial ascomycetes, light is necessary to initiate ascospore discharge (Lyon *et al.*, 1984; Moore-Landecker, 1982). Sutton *et al.* (1978) suggested that light may affect the release of conidia of *Botrytis squamosa*. It is found that light triggers spore release in several fungi (Leach, 1975).

Concentrations of airborne spores are related to preceding conditions affecting spore production and release (Sutton *et al.*, 1978). In the conidial fungi, once the spores are produced, release is often influenced by wind velocity/air movement. In the Ascomycetes, radiation, minimum humidity, changes in humidity, and minimum wind velocity were all directly correlated with levels of airborne ascospores (Lyon *et al.*, 1984). These are some of the reasons why diurnal periodicity exists. On the other hand, spores of other fungi such as *Cladosporium, Alternaria*, and *Helminthosporium* are blown free by wind/air movement, and this type of ''dry spore'' increases in concentration with decreasing RH and increasing air movement. Thus these species are often abundant during midday periods with maximal sunlight.

The maturation and release of some spores such as basidiospores are also markedly affected by the presence of free water (Lyon *et al.*, 1984; Salvaggio, 1986; Salvaggio and Aukrust, 1981). Under some circumstances, circadian rhythms in humidity and temperature interact to

form diurnal patterns or nocturnal increases in spore concentrations, such as certain basidiospores (Salvaggio and Aukrust, 1981).

4. *Air Movement*

Air movement is the most unpredictable agent in the transport of fungal spores. Many fungal spores are adapted for aerial dispersal. It would appear that the horizontal distance over which individual microorganisms may be transported is largely determined by their ability to survive in the atmosphere (Tilak, 1984).

In hyphomycetes, dry spore release is often influenced by wind speed (Lyon *et al.*, 1984). *Botrytis squamosa* spores were apparently released at very low wind speeds (Sutton *et al.*, 1978). Maximum wind speed was negatively correlated with spore concentrations of *Cladosporium, Alternaria*, unidentified ascospores, and unidentified basidiospores; it was the only factor among 10 meteorological factors significantly correlated with all four groups. Minimum wind was directly correlated to spore counts, while maximum wind was inversely correlated (Lyon *et al.*, 1984). Wind/air movement plays an important role in basidiospore dispersal. After basidiospores are ejected from basidia and drop from between the gills of the basidioma, they are primarily dispersed by air movement (Moore-Landecker, 1982). Wind direction had a profound effect on the airborne fungi in Galway, Ireland (McDonald and O'Driscoll, 1980).

Although wind and air movement are known to assist in the release and dispersal of spores in nature, their importance in spore release and dispersal in the indoor environment has not been studied and is poorly understood. The primary air mover in a mechanically ventilated environment is the heating, ventilating, and air-conditioning system.

5. *Substrates*

Dust is ubiquitous in our daily environments and is a heterogeneous substrate. It can be found where surfaces (hard or porous) are present: furniture, walls, floor, ceilings, carpet, etc. It serves as a reservoir for fungal spores and fragments. It also provides certain nutrients for fungi to survive or grow, such as food crumbs, skin flakes, fibers, and other organic matter. Resuspension of fungal spores from dust into air could influence airborne fungal spore concentration and composition significantly and subsequently affect the exposure of occupants to the aerosolization. Individual differences in indoor conditions do not have much influence on the diversity of the fungal flora in dust. They do, however, influence its quantity (Rijckaert, 1981).

The most frequent fungal genera in dust were *Penicillium, Eurotium, Aspergillus, Alternaria, Epicoccum*, and *Cladosporium* (Oppermann *et al.*, 2001). A total of 41 different genera/species were identified (Oppermann *et al.*, 2001). In the Greater New Haven, Connecticut, area fungal composition and concentrations in house dust samples were not correlated with those present in the indoor air (Ren *et al.*, 1999). In dust samples, more *Mucor, Wallemia*, and *Alternaria* species were found in all seasons but fewer *Aspergillus, Cladosporium*, and *Penicillium* species were found (Ren *et al.*, 1999). Scott (2001) conducted a comprehensive study on indoor fungi from dust collected from 369 houses in Wallaceburg, Ontario, and found roughly 250 fungal taxa, with the 10 most common taxa being *Alternaria alternata, Aureobasidium pullulans, Eurotium herbariorum, Aspergillus versicolor, Penicillium chrysogenum, Cladosporium cladosporioides, P. spinulosum, C. sphaerospermum, A. niger*, and *Trichoderma viride*.

Engelhart *et al.* (2002) showed that 18% of total culturable fungi from carpet dust samples were *A. versicolor*, of which 49 of 50 isolates (98%) were found to be sterigmatocystin producers *in vitro*. Sterigmatocystin could be detected at low concentrations (2 to 4 ng/g of dust) in 2 of 11 native carpet dust samples by using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (Engelhart *et al.*, 2002).

Chao *et al.* (2002a) found that concentrations of total dust-borne fungi from floors were positively related to carbon dioxide and temperatures between 20° and 22.5 °C. A gradual increase in total fungal concentrations in floors was observed over a year (Chao *et al.*, 2002a). Total fungi isolated from chairs varied significantly by season, being highest in September and lowest in March, and were positively correlated with dust loads in floors (Chao *et al.*, 2002a). The results suggest the presence of seasonality of dust-borne fungi.

Higher numbers of mold isolates were associated with areas of high shade and high levels of organic debris near the home, along with poor landscape maintenance. Lower concentrations of mold isolates were associated with good dust control measures. No statistically significant correlations could be made between indoor mold isolates and any of the following factors: number and age of the occupants, age and size of home, month of survey, or the presence of plants (Banerjee *et al.*, 1987).

A wide range of fungi in floor and mattress dusts was reported in southwest Germany in winter and spring (Jovanovic *et al.*, 2001). The median value of CFU/g dust, collected from the floors, was 15,000 (range 0–700,000) and 28,000 (range 1500–1,350,000) for samples collected from mattresses. On the other hand, Oppermann *et al.* (2001)

reported total concentrations of fungi varied from 1.4×10^3 to 300×10^3 CFU/g of dust with a geometric mean of 26.5×10^3 CFU/g of dust. A total of 41 different genera/species were identified. Fungal spores were discovered more frequently in mattresses in humid flats.

Samples of settled dust were taken by vacuuming from the carpet in the living room of 405 homes in Erfurt (east) and Hamburg (west) and plated on DG18 agar. No significant difference could be shown for the total or for single genera (*Alternaria, Aspergillus, Cladosporium*, and *Penicillium*) in concentration of spores of viable fungi in settled house dust between Erfurt and Hamburg (Koch *et al.*, 2000). Seasonal variation of the fungi, with a peak in August, could be identified both indoors and outdoors. Koch *et al.* (2000) concluded that outdoor concentration is the main influence on indoor concentration of mold spores from June to October.

Hodgson and Scott (1999) studied 243 carpet dust samples collected from problem and control buildings in the United States. They found that fungal concentrations, analyzed with the extraction and serial dilution method, in dust samples from problem buildings were higher than control samples (1.8 million CFU/g v. 30,000 CFU/g). The most common dominant fungal group detected in problem buildings was *Pencillium*, followed by *Cladosporium* and *Aspergillus*. In the control buildings, *Cladosporium* was the most frequently detected dominant taxon, followed by *Penicillium, Phoma*, yeasts, and *Epicoccum nigrum* at equal percentages. *Aspergillus* was never the dominant taxon in the control buildings. Among individual *Aspergillus* species, *A. versicolor* was the most common species in samples from problem buildings. Other *Aspergillus* species of significance included *A. niger* and *A. sydowii*. They concluded that concentrations greater than 10^5 CFU/g, and certainly those greater than 10^6 CFU/g, were most likely associated with fungal contamination in buildings. However, major (20–50% of the concentration) or dominant taxa can also serve as an indicator of fungal contamination. *Penicillium* spp. and *Aspergillus* spp., especially *A. versicolor*, are generally the dominant taxa associated with problem building samples.

Scott (2001) studied genotypic variation of two common dust-borne *Penicillium* species, *P. brevicompactum* and *P. chrysogenum*. It was found that there were two genetically divergent groups of *P. brevicompactum* according to sequence analysis of the beta-tubulin (benA) and rDNA loci. Authentic strains of *P. brevicompactum* and *P. stoloniferum* clustered together in the predominant clade, accounting for 86% of isolates (Scott, 2001). At the same time, a clonal pattern of inheritance in *P. chrysogenum* was observed, and phylogenetic analyses of allele

sequences was found segregating the population into three divergent lineages, accounting for 90%, 7%, and 3% of the house dust isolates, respectively (Scott, 2001). Type isolates of *P. chrysogenum* and its synonym *P. notatum* clustering within the secondary lineage confirmed this synonymy. No available names for the predominant and minor lineages suggested that *P. chrysogenum* delineated previously is a species complex including three taxa. Further studies are necessary to determine the nomenclature status of the two unknown lineages of *P. chrysogenum*.

Although many fungal studies of household dust are available, Macher (2001) argued that no standard procedures for culturing fungi from dust have been adopted widely enough to ensure the validity of comparisons among studies. It is necessary to establish reference methods in environmental microbiology for use in the assessment of indoor environmental quality (Macher, 2001).

Separate collection of organic and nonorganic household waste is becoming a common form of waste management in Europe and North America. Household organic waste indoors is a good substrate, which might increase microbial growth in the home environment. A study in 99 homes in The Netherlands showed that increased microbial contaminant levels in homes are associated with indoor storage of separated organic waste when using mold beta (1 → 3)-glucans and fungal extracellular polysaccharides of *Aspergillus* and *Penicillium* species in house dust extracts as markers of microbial levels (Wouters *et al.*, 2000).

6. *Activities of Occupants*

It is generally believed that human activity in a building is a major contributor to the fungal population indoors. However, very few studies have addressed this issue. Sessa *et al.* (2002) conducted a study in a university auditorium in Rome, in an office of public buildings, and in an apartment in the presence and absence of the buildings' occupants, building materials, and furnishings by using a Surface Air System (SAS). In the presence of people and furnishings, the average concentrations of airborne fungi were much higher (University auditorium: 1256–1769 CFU/m^3; office: 858 CFU/m^3; apartment: 147–297 CFU/m^3) than in their absence (respectively: 301–431 CFU/m^3; 224 CFU/m^3; 102–132 CFU/m^3). In a recent study, Butterner *et al.* (2002) showed that after cut pile carpet was walked on for 1 minute, the airborne conidia concentrations of *P. chrysogenum* were significantly higher than the ones with vinyl tile and commercial loop pile carpet, and the differences in concentration were often $\geq$2 orders of magnitude.

B. Building Characteristics

Building characteristics and designs have significant influence on indoor aeromycota. The presence or installation of forced-air heating systems, humidifiers, air filters, and air conditioners can lead to reduced concentrations of airborne fungal spores (Li and Kendrick, 1995d). Concentrations of fungi were lower in day-care centers equipped with air conditioners/air cleaners than in centers that lacked such equipment (Li *et al.*, 1997). Lawton *et al.* (1999) found that low air leakage and natural ventilation were not associated with higher levels of mold growth. In the same study it was found that the presence of wood-burning stoves and fireplaces was significantly positively correlated to fungal infestation in residences (Lawton *et al.*, 1999).

Hirsch *et al.* (2000) showed that the installation of insulated windows and central heating systems in 98 apartments correlated with changes in the indoor environment over 7 months. The air-exchange rate decreased from 0.73 to 0.52 per hour, temperature increased from 13.4 to 17.5 °C, and absolute humidity increased from 4.6 g to 6.2 g H_2O/kg air in. In addition the abundance of *Aspergillus fumigatus* was found to increase from 20 to 60 CFU/g carpet dust (Hirsch *et al.* 2000). The presence of carpet was found to increase the concentration of airborne fungal spores in residential buildings (Li and Kendrick, 1995d).

The concentrations of airborne fungal spores vary significantly from room to room in some residential buildings. In the study of Li and Kendrick (1995d), results showed that numbers of airborne fungal spores were highest in living rooms, followed by family rooms, kitchens, bathrooms, and bedrooms. However, Ren *et al.* (1999) found no significant difference in concentration and composition of fungi between living room and bedroom or by season. Both concentration and type of fungi were significantly higher in basements than other indoor areas and outdoor air in winter. Significant seasonal variation in fungal type was observed in living rooms, bedrooms, and outdoor air but not in basements (Ren *et al.*, 1999).

Wan and Li (1999) showed that the levels of bacteria and fungi indoors were highest in day-care centers, followed by those in homes and office buildings. However, the prevalence of airway inflammation and systemic symptoms was higher for females in office buildings than for those in day-care centers. That means that there are other factors to be considered. A strong association was found between beta-1,3-glucan and symptoms of lethargy/fatigue (Wan and Li, 1999).

Floor dust and air samples from the bedrooms of 485 houses were collected over 1 year in Melbourne, Australia (Dharmage *et al.*, 1999).

The dust was analyzed for ergosterol, a marker of cumulative fungal biomass exposure. The median ergosterol level in bedroom floors was 3.8 μg/g of dust. Multivariate analysis showed that several factors were associated with lower total fungal propagules in bedroom air: a ceiling fan, absence of visible mold, frequent vacuuming, a solid fuel fire, and absence of pets. Total fungal propagules were lower when windows were closed during sampling. The presence of more than one cat had the greatest effect on total fungal propagules. Ergosterol levels were significantly lower in homes without old fitted carpets, visible mold, or pets and those with frequent airing and regular use of an extractor fan in the kitchen. Old wall-to-wall carpets had the greatest effect on ergosterol (Dharmage *et al.*, 1999).

Poor insulation of buildings may lead to a greater fluctuation in air temperature, condensation, and dampness in localized areas of buildings. A drop in the air temperature below the dew point often relates to fungal growth inside buildings, particularly in places such as badly insulated outer walls or room corners (Kaufhold *et al.*, 1997). This temporary fall below the dew point caused by temperature fluctuation probably resulted in the building materials and wallpapers becoming damp and subsequent fungal growth (Kaufhold *et al.*, 1997).

Installation and operation of germicidal UV lights in central heating, ventilation, and air conditioning (HVAC) systems of office buildings was found feasible, cannot be detected by workers, and does not seem to result in any adverse effects (Menzie *et al.*, 1999). However, the effectiveness of UV lights on fungal growth and contamination in the HVAC system was not convincing.

The number of CFU/m^3 air collected on MEA was significantly higher than on DG-18 (1033.5 and 846.0 CFU/m^3, respectively) from 1000 homes in the northeastern United States (Ren *et al.*, 2001). Temperature, relative humidity, seasons, and presence of cats indoors had a statistically significant impact on the presence of fungal propagules in indoor air. Ren *et al.* (2001) concluded that it is difficult to predict the presence of fungal propagules in indoor air reliably by home characteristics alone.

Pei-Chih *et al.* (2000) found that the fungal concentration for indoors was 8946 (4372–18,306) CFU/m^3 in winter and 4381 (1605–11,956) CFU/m^3 in summer. For outdoors, it was 11,464 (5767–22,788) CFU/m^3 in winter and 4689 (1895–11,603) in summer. In summer, the total fungal concentrations, both indoors and outdoors of suburban homes, were significantly higher than those of urban homes. The dominant fungi contributing to this difference were *Cladosporium* spp. indoors and *Penicillium* spp. outdoors (Pei-Chih *et al.*, 2000).

1. *Building Materials*

Building materials such as drywall, wallpaper, insulation materials, wood framing materials, carpet, wood flooring, and subfloor materials provide growth substrates for fungi to colonize and develop. They also serve as porous materials to hold water or moisture, which is another necessary factor for the growth of fungi.

From a score system assessing the bioavailability of building materials, the products most vulnerable to mold attacks were water-damaged, aged organic materials containing cellulose, such as wooden materials, jute, wallpaper, and cardboard (Gravesen *et al.*, 1999).

The growth of three fungal genera (*Cladosporium, Penicillium*, and *Stachybotrys*) was evaluated on cellulose-containing ceiling tile and inorganic ceiling tile (Karunasena *et al.*, 2001). The results show that inorganic ceiling tile did not support the growth of these three fungal genera while cellulose-containing ceiling tile did. The results demonstrated that inorganic ceiling tile could serve as an ideal replacement for cellulose-containing ceiling tile (Karunasena, 2001). In a controlled laboratory study, Chang *et al.* (1995) found that new ceiling tiles supported the growth of *Penicillium chrysogenum* and *P. glabrum* at a_w 0.85 and a corresponding moisture content >2.2%, and of *Aspergillus niger* at a_w 0.94 and a corresponding moisture content >4.3% on used ceiling tiles. The same study showed that used ceiling tiles were more susceptible to fungal growth than new ones (Chang *et al.*, 1995).

In a study on gypsum boards just off the production line, Doll and Burge (2001) showed that 11 fungal genera were present on new gypsum board without artificial inoculation. *Penicillium* spp. and *Aspergillus* spp. were found at 95% RH only on the paper sides and the number of fungi found on the new gypsum board increased with increasing moisture content. On one occasion *Stachybotrys* sp. was present on the gypsum boards (Doll and Burge, 2001). This study showed that new gypsum boards were not free from naturally occurring fungal spores.

Morgan-Jones and Jacobsen (1988) studied moldy carpets, plasterboard, and wallpaper from three hotels in Florida and Georgia. They found many fungi caused biodeterioration of paper, textiles, and plaster. The genera of fungi most often identified were the ascomycete genus *Chaetomium*; the dematiaceous hyphomycete genera *Alternaria, Cladosporium, Stachybotrys*, and *Ulocladium*; the moniliaceous hyphomycete genera *Acremonium, Aspergillus*, and *Penicillium*; and the pycnidial genus *Phoma*. In the study, 14 species in 11 genera were isolated and identified, including two new species of *Cladosporium*. In

a study of toxicity of moldy building materials, Johanning *et al.* (1998) identified several groups of fungi, detected satratoxin H and spirolactone/lactams, and demonstrated their cytotoxicity of the materials to cell cultures. The fungi were isolated from gypsum wallboard and other building materials. The fungi identified included those described by Morgan-Jones and Jacobsen (1988) plus additional species of *Aspergillus, Paecilomyces*, and *Trichoderma*. Käpylä (1985) found that the predominant fungus growing on wooden frames of insulated windows in Finland was *Aureobasidium pullulans*. In recent studies it was found that *C. sphaerospermum* out-competed with *P. chrysogenum* under fluctuating a_w on various plaster materials, paints, and plasterboards. However, under constant a_w, *P. chrysogenum* out-competed *C. sphaerospermum* (Nielsen, 2002, 2003).

Carpet is a good reservoir for fungal spores to survive and for their redispersal into air. The conidia of *P. chrysogenum* were much easier to be aerosolized from residential carpet (cut pile) by walking than from commercial type carpet (loop pile) and vinyl tile (Buttner *et al.*, 2002). High indoor fungal exposures were associated with infrequent ventilation or vacuuming, presence of pets, visible mold, and old carpets (Dharmage *et al.*, 1999).

All fungi found to colonize building materials are either saprophytes, biodeteriorating agents, or both. Some fungi—such as species of *Aspergillus, Chaetomium, Chrysosporium, Stachybotrys*, and *Trichoderma*—are known to be capable of degrading cellulose fibers. Although a few fungal species have been reported in the literature, it is very likely that any saprophytic, biodeteriogenic, or cellulolytic fungi can grow indoors if opportunity arises and conditions are met (Burge, 1999).

Although species of the deuteromycetes are commonly detected in moldy building materials, species of Ascomycota (such as *Peziza* spp. and *Pyronema domesticum*) and Basidiomycota (such as species of *Cryptococcus, Rhodotorula*, and *Sporobolomyces*) are occasionally found on damp materials in buildings. In addition, basidiomata (fruiting bodies of basidiomycota) of *Coprinus* spp., *Pleurotus*, and *Poria* from various building materials, from ceiling tiles to wood products, have been identified (Yang, unpublished data). Mycelia and hyphae with clamped connections, indicating basidiomycetes, are frequently detected colonizing water-damaged wood structures and paper products. Wood-inhabiting basidiomycetes are often wood decay fungi. Most recently, wood decay fungi, *Merulioporia incrassata* and *Serpula lacrymans*, were identified and detected from decayed wood samples by using the quantitative PCR method (Yang, Li, and Lin, unpublished).

2. *Heating, Ventilating, and Air-Conditioning Systems (HVAC)*

Heating, ventilating, and air-conditioning systems (HVAC) play an important role (both positive and negative) in fungal infestation and dissemination, as follows: (1) as a dispersal pathway for airborne fungal spores; (2) as growth locations for fungi in the system (such as drip pans, chill coils, and insulated or soiled duct works), if the system is not properly maintained; and (3) as a filtration mechanism in a properly maintained system that can remove some airborne fungal spores and reduce the airborne fungal population indoors.

Fungi have been known to grow in the HVAC system. Window air-conditioners (AC) appear to substantially reduce indoor allergen levels by maintaining the isolation of enclosed spaces from particle-bearing outdoor air (Solomon *et al.*, 1980). Central air conditioning may reduce numbers of fungal spores in houses by 50% or more, and central electrostatic filtration also gives significant reductions (Flannigan *et al.*, 1991). However, fungal growth is known to occur in poorly maintained HVAC systems. Contaminated humidifiers and heating equipment are also sources of allergenic fungi (Flannigan *et al.*, 1991). Respiratory diseases caused by contaminated air-conditioning systems have been reported from the United Kingdom and the United States. *Acremonium, Paecilomyces, Aureobasidium, Phialophora*, and *Fusarium* are regularly found in humidifiers (Samson, 1986).

Heinemann *et al.* (1994) studied contamination of HVAC by fungi, bacteria, and thermophilic actinomycetes. They sampled surfaces of filters and fans with RODAC contact plates and used the serial dilution method for humidifier water. A wide variety of fungi were identified. However, some of the fungi identified were likely spore contaminants instead of fungal growth. Kemp *et al.* (1995) studied fungal growth on filters of the HVAC system and reported isolation of *Aspergillus niger, A. fumigatus, Alternaria, Cladosporium, Mucor* sp., *Aspergillus* sp., and *Penicillium* sp. However, when filters were directly examined under the microscope, they could only confirm growth of *Aspergillus* sp., *Cladosporium* sp., and *Penicillium* spp. Buttner *et al.* (1999) found that fungal growth may occur on a variety of duct materials, including bare metal, provided soiling and moisture are present. Yang (1996) examined microscopically as well as cultured 1200 Fiberglas insulation liners from HVAC systems in the United States and found fungal colonization and growth in approximately 50% of the samples studied. Fungal types were differentiated based on water and humidity conditions. Species of *Cladosporium* and *Penicillium* were primarily from areas with high relatively humidity.

Cladosporium cladosporioides, C. herbarum, C. sphaerospermum, and *P. corylophilum* were the primary species identified. Species of *Acremonium, Aureobasidium, Exophiala, Fusarium, Paecilomyces, Phoma, Rhodotorula, Sporobolomyces*, and yeasts are expected in areas subjected to frequent wetting, such as cooling coils and drain pans.

Graudenz *et al.* (2002) reported a higher prevalence and a strong association of building-related worsening of respiratory symptoms and symptoms of rhinoconjunctivitis in the group with ventilation machinery and ducts with >20 years of use in Brazil. The authors found that total viable fungal spores were higher outdoors than in the indoor samples. However, they did report that fungal growth was detected in the ventilation systems.

Ahearn *et al.* (1997) studied secondary air filters in the air-handling units on four floors of a multi-story office building with a history of fungal colonization of insulation within the air distribution system. Fungal mycelium and conidia of *Cladosporium* and *Penicillium* spp. were observed on insulation from all floors and on both sides of the air filters from one floor.

C. Succession and Changes in Indoor Fungi

In a chronically water-damaged environment, fungal populations are likely to change over time. Fungi are no different from any living organisms. They grow in an environment and compete with other fungi and organisms. The fungal population and composition as a whole evolve and change depending on environmental factors, interaction with other fungi and organisms, and the biology of the fungi. The evolution of organisms in an environment is called *succession*. Fungal succession in the indoor environment is largely unknown at this time. However, some postulations of indoor fungal succession have been discussed (Grant *et al.*, 1989; Singh, 1994). A knowledgeable mycologist can use various information and means to evaluate fungal succession in a given indoor environment. Defining fungal succession in an indoor environment follows a forensic approach. Information and evidence gathering is very important and should be as comprehensive as possible. This section discusses various observations, environmental parameters, and the biology of various molds that are useful in conducting an assessment of fungal succession in a water-damaged environment.

Fungal growth is always the consequence of a water damage or humidity control problem. Information regarding any water damage

history can provide an important clue of fungal growth. Observation of water-damaged building materials can also shed some light regarding a water damage history. Water is a good and common solvent and can dissolve many substances. A building material subjected to repeated water damage can show signs of deterioration. A ceiling tile may become soft and deformed due to repeated water damage. Nails and carpet strips may become rusty, stained, and weakened. If growth of cellulolytic fungi develops, cellulose-containing building materials can further be weakened because of degradation of cellulose fibers and paper.

Properly identified fungi can tell a story. Fungi are no different from plants. Water-loving plants, such as water lily or cattails, grow only in water or swamps. On the other hand, cacti grow in dry arid environments. Identifications of moisture-loving fungi suggest the environment was wet or water damaged. Moisture-loving fungi include species of *Acremonium, Chaetomium, Fusarium, Trichoderma*, *Ulocladium, Stachybotrys chartarum*, and *Memnoniella echinata*. Wet fungi—such as species of *Aureobasidium, Rhodotorula, Phialophora, Exophiala*, and *Sporobolomyces*—generally require that substrates be wet or water logged. On the other hand, detection of xerophilic fungal growth suggests sufficient but low water activity in the substrates. The condition to achieve low water substrates for growth of xerophilic fungi is usually persistent high humidity conditions. Xerophilic fungi include all species of the genus *Eurotium, Aspergillus restrictus, A. penicillioides*, and *Wallemia sebi*. The two most commonly encountered indoor fungal genera, *Aspergillus* and *Penicillium*, consist of both moisture-loving and xerophilic fungi. *Aspergillus versicolor* and *A. ustus* are commonly detected in water-damaged building materials. Although *A. versicolor* can grow at a water activity as low as 0.80, its optimal water activity is 0.97 (Smith and Hill, 1982).

Although all indoor fungi are essentially saprobes, many of them have unique environmental niches. Some fungi, such as species of *Aspergillus* and *Penicillium*, are called *sugar fungi* (Singh, 1994) because they exploit the sugar content of the substrates. Fungi that can break down complex carbohydrates (such as cellulose and lignin) or complex organics (such as wood) are usually late colonizers of the substrates. In addition, fungi can be classified into primary colonizers, secondary colonizers, or tertiary colonizers depending on the water activity for their growth (Grant *et al.*, 1989). The primary colonizers are defined as those that grow at water activity below 0.80; secondary colonizers grow at between 0.80 and 0.90; tertiary colonizers grow at greater than 0.90. Therefore, moisture-loving fungi are tertiary

colonizers. Common fungal colonizers of the indoor environment, as defined by Grant *et al.* (1989), are listed in Table VII. Although the categorization of various fungi based on their water activity is useful, further refinement is necessary. Many of the strongly xerophilic fungi have an ecological niche that does not usually overlap with the hydrophilic and mesophilic fungi. The authors have observed that strong xerophilic fungi thrive in conditions subjected to long-term high humidity conditions but not to water damage (Yang, personal communication).

In a water-damaged environment, more than one species of fungi are likely encountered. The types of fungi are likely to increase if the wet conditions persist for a long time. Fungal diversity is likely to increase when the water-damage conditions persist.

Fungi as well as mites, insects, and other arthropods are attracted to water sources. It is commonly observed in the laboratory that some insects and mites feed on fungal matter (Flannigan *et al.*, 2001). These insects and mites follow water trail and fungal growth. They also assist in transporting and dispersing fungal spores. The presence of insects, mites, and their fecal pellets and molt casts is a good indication of active insect and mite colonies and long-term fungal growth.

Fungi develop different mechanisms to release and disperse their spores (Kendrick, 2000). Some fungi—such as species of *Aspergillus, Cladosporium*, and *Penicillium*—produce dry spores, which are released by air movement and disturbance. Some fungi can actively discharge their spores into the air. Another group of fungi—such as species of *Acremonium, Stachybotrys*, and *Trichoderma*—produces wet, slimy spores. Water, insects, mites, or other arthropods and small animals often assist their release and dispersal. Dry-spored fungi are much more effective in the dispersal and dissemination of their spores.

Most fungi produce spores within a 2–10 μm size range. However, a few are slightly smaller than 2 μm, and many are larger than 10 μm. Fungi (such as species of *Aspergillus* and *Penicillium*) that produce smaller spores are much more prolific in their spore production. They produce a much larger number of spores. Fungi (such as *Stachybotrys chartarum, Alternaria alternata*, etc.) that produce comparatively larger spores produce fewer of them. Different species of fungi grow at different rates. Fast-growing fungi tend to produce more spores. Fungi that produce a larger number of spores have the advantage of disseminating to a wider territory, thus increasing their chance of finding the niche to colonize and grow.

Some fungi can cause wood decay, from brown rot, to white rot, to soft rot. The wood decay is a slow process caused by tertiary

colonizers. Both brown and white rots are caused by wood decaying basidiomycetes. Soft rot is caused by microfungi and ascomycetes (Wang and Zabel, 1990; Zabel and Morrell, 1992). If wood structures of a building show decay, it is an indication of a long-term problem caused by chronic or repeated wet conditions.

V. Recent Studies on Limits/Exposures of Indoor Fungi

In southwestern Germany between November 1997 and May 1998, the number of colony forming units was 135 CFU/m^3 (range 5–17,000) in indoor air and 145 CFU/m^3 (range 15–2900) in outdoor air (Jovanovic *et al.*, 2001). The data suggest that an indoor-outdoor difference exceeding 500 CFU/m^3 indicates an elevated mold concentration in indoor air compared with background (Jovanovic *et al.*, 2001). The authors suggested that this value could be used as a temporary reference value for southwest Germany in the winter season (Jovanovic *et al.*, 2001).

The CFU/g dust, collected from floors, was 15,000 (range 0–700,000), and that collected from mattresses was 28,000 (range 1500–1,350,000) (Jovanovic *et al.*, 2001). Spores of mold fungi were discovered more frequently in mattresses from humid flats (Oppermann *et al.*, 2001). Total concentrations of mold fungi varied from 1.4×10^3 to 300×10^3 CFU/g of dust with a mean of 26.5×10^3 CFU/g of dust (Oppermann *et al.*, 2001).

In extracts of *Aspergillus fumigatus* culture filtrate, two antigens were found to be produced under all studied growth conditions (common antigens), and in the extracts of the water-soluble portion of the mycelium, one common antigen was found (Wijnands *et al.*, 2000). The three common antigens may serve as marker antigens for exposure to *Aspergillus fumigatus* and its products (Wijnands *et al.*, 2000). In view of the simultaneous presence of two of these common antigens with *Aspergillus fumigatus* allergens, these two marker antigens may be useful for estimating exposure to allergens of *Aspergillus fumigatus*.

Gas chromatography–mass spectrometry was used to determine the microbial contents of building materials subjected to water damage in a laboratory experiment and of materials collected from houses affected by water during a flood in Klodzko in southwestern Poland (Szponar and Larsson, 2000). Ergosterol was examined as a marker of fungal biomass in southwestern Poland (Szponar and Larsson, 2000).

The amount of ergosterol was higher in materials that had been exposed to water than in unexposed ones (Szponar and Larsson, 2000). The marker was stable in the building materials for at least 6 weeks at room temperature and could thus be used to reveal microbial

contamination (Szponar and Larsson, 2000). Direct measurement of 3-hydroxy fatty acids and ergosterol in human environments could be a useful monitoring method for potentially harmful microorganisms and microbial constituents (Szponar and Larsson, 2000).

A fungal index or fungal detector, a simple device using spore germination of 1 to 3 fungal species as sensors for moisture, was used to assess indoor climate and potential for fungal growth (Abe *et al.*, 1996) or to monitor drying of water-damaged buildings (Morey *et al.*, 2002).

The following residential limit values (RLV) for dwellings and communal premises are proposed in Poland for the concentration of airborne bacteria, fungi, and bacterial endotoxin: 5×10^3 CFU/m^3, 5×10^3 CFU/m^3, and 5 ng/m^3 (50 EU), respectively (Gorny and Dutkiewicz, 2002).

Klanova (2000) proposed that any concentration of fungi in indoor air above 2000 CFU/m^3 could be a serious risk factor for health of occupants. The proposed values for occupational exposure limit (OEL) of bacteria, fungi, and bacterial endotoxin for industrial settings contaminated by organic dust are $100 \times (3)$ CFU/m^3, $50 \times (3)$ CFU/m^3, and 200 ng/m^3 (2000 EU), respectively (Gorny and Dutkiewicz, 2002).

Allergy thresholds to common molds have been reported (Gravesen, 1979), but variations in sampling strategies and methodological limitations make these very unreliable in practical settings (Dillon *et al.*, 1999; Miller, 1993). Therefore, the consensus is that acceptable safe threshold limits for exposures to indoor fungi cannot be established (Ammann, 1999; Macher *et al.*, 1991). It is generally recommended to avoid or minimize unnecessary exposures to indoor fungi (Anonymous, 1994).

Airborne fungi could be classified on the basis of the relationship between the two environmental factors and their combinations (i.e., temperature and water requirements) (water activity a_w) (Vujanovic *et al.*, 2001). One type involves three different groups of molds, selected on the basis of the quantitative and qualitative information about the ability of fungi to sporulate under different environmental conditions: group (i), represented by *Emericella* (*Aspergillus*) *nidulans, A. niger*, and *A. ochraceus*, and characterized by sporulation that was more dependent on temperature than on water activity; (ii), represented by *A. flavus* and *A. versicolor*, in which sporulation was approximately equal and depended on both the temperature changes and a_w alterations; and (iii), represented by *Cladosporium* sp., *Penicillium cyclopium*, and *P. citrinum*, in which sporulation depended more on alteration of the a_w conditions than on temperature changes (Vujanovic *et al.*, 2001).

Another type is characterized by four sporulation rates with two risk levels of mycotoxin accumulation in the spores (conidia) of each mold species: large (Ia) and moderate (Ib) sporulation rates with a risk of mycotoxin accumulation ($a_w > \text{or} = 86$; $t > \text{or} = 12\,^{\circ}C$); rare sporulation (IIa) and absence of sporulation (IIb), without risk of mycotoxin accumulation ($a_w < \text{or} = 86$; $t < \text{or} = 12\,^{\circ}C$) (Vujanovic *et al.*, 2001). In conclusion, providing a useful guide for two dimensions, temperature and water activity, for each of the three phases of fungal growth (i.e., germination, growth, and sporulation) facilitates the determination of the fundamental niche of each fungus and its ability to form or accumulate mycotoxin (Vujanovic *et al.*, 2001).

VI. Conclusions

Fungi are ubiquitous in nature. Fungal spores are very common both indoors and outdoors. Fungal spores are considered to be allergens. Some fungi are opportunistic pathogens and occasionally cause infectious diseases in susceptible or immunocompromised people. Fungi can readily grow on building materials, furniture, and other substrates in buildings experiencing water damage/intrusion or dampness problems without immediate repairs. Subsequent proliferation of fungi poses adverse effects on human health in the buildings.

Causal factors and agents of sick building syndrome are very complex. Indoor fungi are one of the agents associated with SBS. Clinical diagnoses of mold allergies and fungal infections are generally easier and less complicated than emerging health concerns stemming from such fungal metabolites as $(1 \rightarrow 3)$-β-D-glucan, mycotoxins, and fungal VOCs. Diagnosis of SBS can be difficult and challenging. The correct diagnosis may rely on cooperation of medical professionals with multiple other professions so as to determine the causes of SBS.

Synergistic inhalation effects of fungal byproducts—such as mycotoxins, β-glucans, or perhaps fungal VOCs—are potentially irritating, toxic, teratogenic, carcinogenic, and immune-suppressive. Risk assessment for human exposure to fungi and their byproducts is complicated, because it involves multiple agents, hypersensitivity reactions, and different disease consequences. Exposure to fungi and their byproducts at various concentrations is omnipresent in indoor environments. The sensitivity of humans to fungi and related byproducts varies from individual to individual. The health effects of inhalation exposure to many fungal metabolites are not well understood. There is a great need to better understand the adverse health effects of short-term and

long-term exposures to the fungal metabolites and to determine whether the health effects are reversible or not. It is, however, prudent to avoid unnecessary exposures to excessive fungi and their byproducts. At the same time, long-term research involving extensive cooperation among several related professions is needed. Controlling indoor fungal growth conditions is the most economical and practical approach to maintaining a healthy environment. Indoor fungal ecology is at present poorly studied. Future studies on indoor fungal ecology are necessary to better understand fungal development, colonization, and succession in the indoor environment. The information yielded from such studies will be useful to design better buildings that are less prone to fungal infestation.

Since the energy crisis, buildings were designed and built to be energy efficient. At the same time it is a common practice to use new and inexpensive materials. As a consequence, the modern buildings are conducive to fungal growth once they experience water damage or humidity control issues.

ACKNOWLEDGMENTS

The authors are very grateful to Mr. Brian Kerin and Mrs. Jenalynn Greer for their assistance in preparation of the manuscript.

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