

# Scientific Literature Review of Mold

A REPORT ON THE  
HEALTH EFFECTS OF INDOOR MOLD

EXHIBIT

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**NAHB**

National Association of Home Builders  
Washington, DC: September 2003

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The National Association of Home Builders, Washington, D.C., today announced that it is sending more than 244,000 letters as part of its homebuilding, remodeling and renovation program, which includes a survey, tracking, design, financing, insurance, building products, manufacturing and other services. The company and home computer and construction "Knowledge" President of the homebuilding industry, NAHB is offering to provide more than 800,000 and local home building associations around the country. NAHB's computer network will consist of about 80,000 and more than 10 million new homebuilding projects to 2005, and the homebuilding of the largest capacity of residential growth in the country.



August 22, 2003

Dear NAHB Member:

During the past few years the alleged link between indoor mold and serious human illness has been the subject of much debate by diverse groups including public health officials, federal and state legislators, homeowners and building occupants, attorneys, and the media. For builders, property owners, and property managers, the debate is not merely academic -- the alleged health effects of mold have been the subject of numerous lawsuits seeking thousands, and in some cases millions, of dollars in health-related damages.

Currently, there are no standards concerning "safe" or "unsafe" levels of exposure to indoor molds. The effects on health -- if any -- depend on the type of mold, the level of mold exposure, and the sensitivity of the person exposed. The most common reaction is allergic. According to the U.S. Environmental Protection Agency symptoms other than the allergic and irritant types are not commonly reported as a result of inhaling mold. Similarly, the Centers for Disease Control and Prevention has indicated that there are very few case reports that molds producing certain mycotoxins inside homes can cause unique or rare health conditions such as pulmonary hemorrhage or memory loss. Moreover, these case reports are rare, and a causal link between the presence of those mold products and serious health conditions has not been proven. Nonetheless, lawsuits and insurance claims continue to allege that mold causes brain cancer, cognitive impairment, memory loss, loss of intellectual capacity, and neurological problems, among others.

Trying to sort through the complex and often conflicting information regarding the health effects of mold can be a daunting task. The National Association of Home Builders (NAHB) is committed to assisting you as you confront these issues. To that end, NAHB convened a panel of experts in the fields of mycology, industrial hygiene, immunology, and toxicology. The panel was instructed to review the existing English-language scientific literature to determine whether it provides a reliable and reasonable scientific basis for one to conclude that there is a causal link between indoor molds and any medically recognized human ailment. The panel was also asked to identify future research that might be beneficial toward determining the links between indoor molds and their related health effects.

The report of the panel follows. The report includes an Executive Summary and three parts. Part I of the report provides the scientific background for studying mold based on the area of expertise of the four panelists. They follow in sequence: mycology, industrial hygiene, immunology, and toxicology. In Part II of the report, which follows the same sequence, each panelist reviews the relevant scientific literature pertaining to the question presented. Part III of the report contains short summaries of what the panelists considered to be the approximately 100 most important documents. A list of references is provided at the end of the report.

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August 22, 2003  
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The report confirms that molds are known to be a significant cause of allergic reactions in sensitized individuals. *However, the panel concluded that while various studies on humans have attempted to link molds and their components to a variety of non-specific problems (such as fatigue, nausea, depression), the evidence suggesting any link is weak, and there is no evidence of causation.* Also, there is little evidence to link inhalation exposures for environments such as offices, schools, hospitals, and residences with any form of cancer.

While the report provides a solid overview of recent work in this area – the authors reviewed over 500 citations of available animal and human data relating to indoor mold and health effects - it is not intended to be an exhaustive study of every aspect of this subject and should not be construed as such. No doubt some individuals may find disagreement with the panels interpretations and conclusions.

We trust that you will find this report helpful and that the report will contribute in a positive way to the public dialogue regarding the health effects of mold.

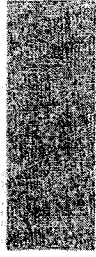
Best regards,

A handwritten signature in black ink, appearing to read 'C. Kent Conine', is positioned above the printed name.

C. Kent Conine  
2003 NAHB President

CKC/dj





# Acknowledgment

The National Association of Home Builders would like to thank Judith Steinman, PhD, McCarter & English, L.L.P. for her technical editing contribution to this Report.



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## Review Panel Members

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Scott D. Phillips, M.D., is a Clinical Associate Professor of Medicine and Surgery at the University of Colorado Health Sciences Center. He is also on the faculty of the Rocky Mountain Poison and Drug Center in Denver, Colo. Dr. Phillips is the Director of Toxicology Associates, Association of Occupational and Environmental Medicine Clinic.

Dr. Phillips is a Fellow of the American College of Physicians and the American College of Medical Toxicology. He serves on and chairs several committees, including the Board of Trustees of the American Academy of Clinical Toxicology, the Occupational and Environmental Toxicology Committee of the American College of Occupational and Environmental Medicine, the Occupational Toxicology Sub-Committee of the American College of Medical Toxicology, and the Emergency Response Planning Guideline Committee of the American Industrial Hygiene Association.

Dr. Phillips earned his medical degree from the American University of the Caribbean, and entered an internal medicine residency at the Framingham Union Hospital, in Framingham, Mass., where he served as the Chief Medical Resident. Dr. Phillips completed a fellowship in medical toxicology at the Rocky Mountain Poison and Drug Center in Denver. He is board certified in both internal medicine and medical toxicology.

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x Review Panel Members

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William F. Schoenwetter, M.D., is a Clinical Professor of Medicine at the University of Minnesota Medical School and a consultant in the Department of Asthma and Allergic Diseases at Park Nicollet Clinic in Minneapolis, where he is also Medical Director of the Asthma and Allergy Research Center.

Dr. Schoenwetter is a Fellow of the American College of Allergy, Asthma and Immunology, where he served as Co-Chairman of the Practice Standards Committee, and is a Fellow of the American Academy of Allergy, Asthma and Immunology, where he serves on several committees. He is former President of the Minnesota Allergy Society, as well as a member of the American Medical Association and the Editorial Board of *Annals of Allergy, Asthma and Immunology*. Dr. Schoenwetter is actively involved in research in the field of allergies and asthma, and his work has been published in several leading medical journals.

After earning a Bachelor of Science degree and a medical degree at the University of Wisconsin in Madison, Dr. Schoenwetter completed an internship at the Hennepin County General Hospital in Minneapolis and his residency in internal medicine at the University of Minnesota, Minneapolis. He completed a National Institutes of Health Fellowship in allergy and clinical immunology at the Hospital of University of Pennsylvania, Philadelphia. He is board certified in both internal medicine and allergy and clinical immunology.

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Dr. Wayne R. Thomann is Director of Occupational and Environmental Safety at Duke University/Medical Center. He is also an Assistant Clinical Professor in the Division of Occupational and Environmental Medicine, Department of Community and Family Medicine. Dr. Thomann received his Doctor of Public Health degree from the School of Public Health at the University of North Carolina in Chapel Hill. He has been researching the identification and control of bioaerosols for more than 20 years and is a member of ASHRAE Standard 62.1 Committee on "Ventilation for Indoor Air Quality." He also directs a graduate curriculum in Occupational Health and Safety within the Duke University Nicholas School of the Environment and lectures extensively about indoor air quality.



# Executive Summary

## Description of the Project

In order to present an understanding of the relationship between indoor mold and human illness, a panel of experts from four relevant disciplines convened to review, critique, and evaluate the existing scientific literature pertaining to this subject. The examined literature included, but was not limited to, controlled and uncontrolled studies, case reports, review articles, meta-analyses, animal studies, and unpublished research.

Prior to beginning the review process, the panel aimed to address two fundamental questions. 1) To what extent, if any—and with what limitations and caveats—does the existing scientific literature provide a reliable and reasonable scientific basis for one to conclude that indoor molds cause any medically recognized human ailment? 2) What future research might be beneficial toward determining the links, if any, between indoor molds and human health?

## Methodology of the Report

A search of the English-language medical literature was performed by an independent toxicology firm that collected available animal and human data relating to indoor mold and health effects. The materials were then distributed in full among the panel of experts (*e.g.*, mycology, industrial hygiene, allergy and immunology, and toxicology). Additionally, each panelist reviewed the literature in his field to identify and obtain any relevant articles. The collection of selected articles was reviewed by the panel to ensure that overlapping papers were assigned to the most appropriate specialist. This resulted in consideration of over 500 individual citations.

The appropriate panel expert independently reviewed the individual papers using scientific methodology. The literature was critically evaluated according to its individual merits and limitations, and panel members rigorously assessed each study's conclusions. The top 25 studies in each subject area were summarized and recorded. These studies were evaluated to produce an overall conclusion for each specific area relating to indoor mold exposure and human health. The results are summarized below.

## Panel Conclusions on Exposure to Fungi and Fungal Elements in the Indoor Environment

### *Molds in the Indoor Environment*

Studies have shown that indoor spore populations often resemble outdoor spore populations. The type and quantity of spores in the indoor environment depend primarily on the outdoor environment, but many other factors affect the indoor spore counts and population as well. Virtually all buildings, including their materials and surfaces, are naturally seeded with spores from the outdoor environment. The most common indoor molds are *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria* species. *Stachybotrys* species are found much less commonly in homes, and usually in much smaller concentrations compared with other molds. The presence of indoor mold in and of itself does not necessarily indicate the presence of mycotoxins, compounds produced by some fungi.

The literature indicates that spores of various *Stachybotrys* species occasionally can be found in the air samples of some homes. Most often, evidence of *Stachybotrys* species is found in structures with significant water damage and visible mold growth; however, these species generally represent a very minor proportion of the overall mold population (*i.e.*, compared to *Aspergillus*, *Penicillium*, or *Cladosporium*), and respirable spore counts are generally low.

Scientific evidence suggests that mycotoxins are not volatile, do not evaporate into the air, and do not penetrate through materials used in construction. Accordingly, exposure to mycotoxins occurs by inhalation or ingestion of the actual spores themselves, or when other fungal components or fragments are inhaled, ingested, or potentially make contact with the skin. Outbreaks of health problems associated with indoor air exposure to mycotoxins are rare; most reports are related to the ingestion of improperly stored grain or foodstuffs that had been grossly contaminated with mold. These molds are not commonly encountered in the indoor environment.

### *Possible Health Effects of Indoor Molds*

Theoretically, molds can cause human ailments through several potential mechanisms: by physically invading and infecting humans (*pathogenic*); by producing mycotoxins that, under certain circumstances, may be toxic (*toxigenic*); or by acting as or producing substances (allergens) that stimulate the human immune system (*allergenic*). Truly pathogenic (systemic) fungi do not originate indoors; however, numerous indoor opportunistic pathogens have caused human illness.

Various studies on humans have attempted to link fungi and their components to a variety of non-specific symptoms such as fatigue, weakness, nausea, vomiting, headache, eye, nose, or throat irritation, dry cough, dry or itchy skin, dizziness, difficulty concentrating, and depression. Collectively, these complaints have sometimes been referred to as "sick building syndrome." *Stachybotrys* species also have been implicated in sick building syndrome as well as other pseudonymous conditions that typically are characterized by multiple, subjective, ill-defined complaints; however, the evidence suggesting any link is weak, and there is no evidence for causation. Several of

the individual papers suggesting an association contain serious methodologic flaws or limitations.

In addition to the alleged role of mycotoxins in sick-building syndrome, numerous high-profile papers over the past two decades have also tried to associate mycotoxins with idiopathic pulmonary hemorrhage (*i.e.*, bleeding in the lungs) in humans, particularly in infants and young children. Most of these authors concluded that mycotoxins from various indoor molds, most notably *Stachybotrys*, were responsible. As has been noted by the federal agency, the United States Centers for Disease Control and Prevention (CDC), however, there are serious flaws in these studies, such that a causal relationship between mycotoxins and pulmonary hemorrhage in humans has not been demonstrated. There is currently insufficient evidence to conclude that indoor molds cause pulmonary hemorrhage as assessed by the CDC and this panel.

At this time, there remains insufficient toxicologic evidence to support a causal relationship between mycotoxin exposure from indoor mold and any human health condition. No study met all of the commonly accepted criteria for documenting causality after an indoor environmental exposure to mycotoxins. Other toxicologic reactions to indoor mold include only anecdotal evidence in the form of a single case report describing kidney failure that was purportedly related to mycotoxin exposure. The evidence reveals only a weak association, at best, between mycotoxins and neurologic effects in humans. The current aggregate data from the body of scientific and medical literature have not provided compelling evidence that the inhalation of mycotoxins causes health effects in humans at levels expected in most mold-contaminated indoor environments.

Lower respiratory ailments, such as asthma, are reported more frequently in damp buildings due, in part, to high humidity, which favors the growth of mites and certain molds. Thus, most studies to date support an association between indoor dampness and the occurrence or exacerbation of asthma, allergic rhinitis, and various respiratory symptoms. However, the strength of the association varies between studies, and a few studies found no association at all. Although, the literature does not necessarily support a causal relationship between the presence of indoor mold and the development of asthma in non-sensitized individuals, the literature is sufficient to conclude that indoor mold is associated with asthma in individuals who already have developed the condition. Because no evidence exists that demonstrates mold as the primary cause of asthma, more data are needed to prove a causal relationship.

At least two case reports in the literature have reported hypersensitivity pneumonitis with mold exposure in the home; however, a cause-effect relationship remains unproven, and the individual reports have significant limitations. No cases of hypersensitivity pneumonitis have been linked to *Stachybotrys* exposure.

The people most susceptible to mold-related infections are those with clinically significant immune dysfunction. Currently, there are no cases of direct human infection with *Stachybotrys* species. The fungal species that are characterized as opportunistic pathogens generally are acquired from exposure to the outdoor environment. The literature supports the position that the general (*i.e.*, immunocompetent) population is not affected by similar exposure.

The scientific data are currently insufficient to fully evaluate transient irritant effects potentially related to microbial volatile organic compounds (MVOCs). The



existing scientific literature does not support a relationship between MVOCs and allergic reactions. A few papers have claimed that specific chemical components of molds (e.g., glucans, MVOCs) might be the cause of human illnesses such as sick building syndrome, but the evidence associating them with human disease is weak, and far from proving a causal relationship.

Despite the long history of medicine and science dealing with fungal organisms, the current state of the science is insufficient to quantify exposures or dose relationships that are clinically important. Currently, there are no reliable standards for determining whether there is a specific "dose" of mold or any component of a fungal organism that can be related directly to health effects. Air and bulk sampling may aid in identifying whether a particular mold or component of a fungal organism is present, but the scientific literature does not support the use of these techniques for quantifying an exposure. No permissible exposure level (PEL) has been identified or validated for any fungal species.

### Summary of Overall Conclusions

- Exacerbations of certain respiratory conditions have been associated with fungal forms, including hypersensitivity pneumonitis, asthma, and allergic rhinitis.
- The scientific literature does not support a causal relationship between molds or mycotoxin exposure and idiopathic pulmonary hemorrhage.
- The scientific literature does not support a relationship between mycotoxins from indoor air and human illness, including systemic diseases such as renal failure.
- There are no reliable scientific data that support any relationship between exposure either to indoor molds or mycotoxins and neurological illness.
- From a toxicology standpoint, the evidence supporting an association between mycotoxins and sick-building syndrome is inconclusive.
- Arbitrary or "self-proclaimed" exposure limits are simply not acceptable. There are no reliable methods for quantifying exposure levels for fungal contaminants in the indoor environment.
- The scientific data are not sufficient to support a causal role for MVOCs, glucans, or any other mold component derived from indoor air in the induction or exacerbation of human illness.
- Attempts to interpret laboratory results in the absence of a site-specific perspective are not reliable.
- The evidence of the agricultural environment contaminated with mycotoxins should not be taken out of scientific context and extrapolated to the indoor living environment.
- Agriculture or industrial exposures such as Organic Dust Toxic Syndrome should not be used to extrapolate potential consequences to exposure in the indoor environment, nor should the dramatic exposure during a remediation of a mold contaminated building be used to extrapolate to the typical, undisrupted indoor environment.

### Future Research

There is a continuing, and arguably growing, need for more investigations into the relationship between indoor molds and human ailments. While there appears to be a rela-

tionship between indoor molds and allergic illnesses of the upper and lower respiratory tract, the strength and details of this relationship need to be further defined. Although a causal relationship is suspected, the relationship cannot be distinguished from the involvement of indoor dampness, dust mites, and other factors. There is a need for better investigations of all kinds, particularly those with more *objective* data on the exposure (*e.g.*, measurements of spore counts) and the health effects (*e.g.*, measurements of pulmonary function, serologic markers).

Only after these investigations are complete can we make adequate conclusions as to whether or not indoor molds are indeed the causes or merely bystanders of these illnesses. There is also a need for further development of various methods of mold detection and identification, and for further studies to validate these detection methods in order to improve and to standardize the interpretation of their results.

To better isolate the effect of molds on human health, it will be necessary to establish criteria to measure the presence of molds in buildings and to determine what levels are associated with toxic effects on health. Newer laboratory techniques are needed to measure presence of indoor mold focus on the use of biochemical and immunochemical markers that indicate the presence of a mold antibody in a patient. Also needed are widely available, affordable, and reliable methods to assess objectively the health effects of individuals who report symptoms related to their indoor environment.

Currently, there are numerous methods used for sampling molds or mold byproducts within the built environment; however, there are no accepted standards for mold sampling in indoor environments or for analyzing and interpreting the indoor sampling data in terms of human health. The variability in sampling methodologies makes it very difficult to compare the results in the scientific literature. In addition, most of the studies tend to be based on baseline environmental data rather than human dose-response data. Therefore, setting standards and/or guidelines for exposure levels for indoor fungal contaminants is not currently practical. Further research is needed to determine whether sampling for specific types of molds or mold byproducts has any bearing on human health. More controlled studies are needed to demonstrate whether or not there is sufficient consistency amongst the various sampling methods to allow comparisons to be made.

Further work is necessary in the area of mycotoxins and human illness, particularly, in the indoor air environment from the inhalation exposure pathway. Development of reliable methods for detecting mycotoxins is necessary before the hypothesis that airborne mycotoxins from indoor molds cause human illness can be tested.

Sampling for marker agents (*e.g.*, MVOCs, glucans) is an emerging area that requires additional research before any conclusions may be drawn as to whether there exists specific compounds that have any relationship with human health conditions.



# Scientific Background

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## Introduction

The kingdom classified as fungi consists of a large group of organisms that have cells bound by rigid walls, usually formed of chitin and glucans (Burge 1999). There are more than 100,000 different species of fungi, and they can be found in virtually every ecological niche. The scientific study of this diverse group of organisms is called "Mycology," from the Greek words *mykes* for mushroom and *logos* for discourse.

The systematic study of fungi is only 250 years old, but manifestations of this group of organisms have been known for thousands of years—ever since the first toast was proposed over a shell full of wine and the first leavened bread was baked (Alexopoulos 1996)—as both friend and foe of man. Beneficial activities associated with fungi include fermentations, production of antibiotics, cheese ripening, production of industrial enzymes, and decay of plant products. Some of the deleterious consequences of fungal activities include plant diseases, animal and human mycoses, spoilage of food, production of mycotoxins, and allergic manifestations. However, the benefits that fungi provide far outweigh the complications associated with our co-existence (Kendrick 1985).

## Characteristics of Fungi

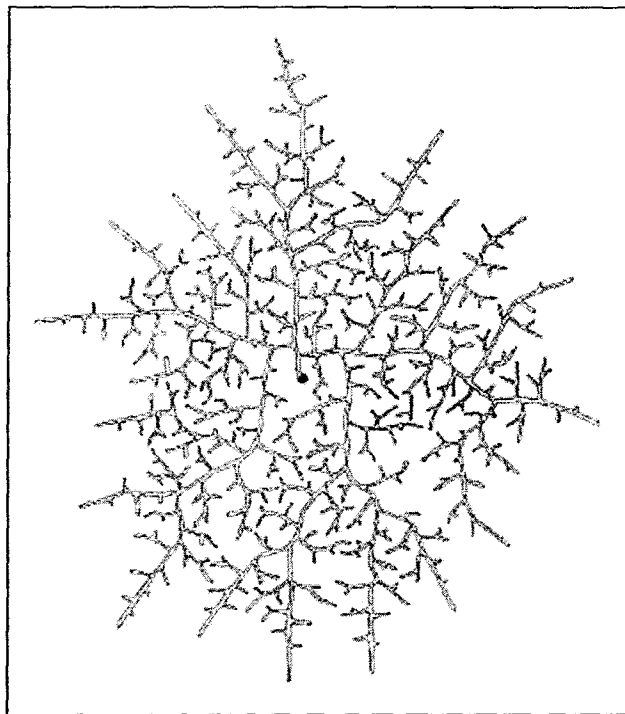
Fungi can be unicellular or multicellular. A few species of fungi will have both unicellular and multicellular forms and are called "dimorphic." Unicellular fungi are called yeasts. Through a process called budding, yeasts create daughter cells from the parent cell. This process produces opaque, creamy, or pasty colonies when grown on a substrate. Yeasts are generally not considered a significant problem in the indoor environment, but they can be associated with health effects as discussed elsewhere in this document.

Multicellular fungi are formed of microscopic, branched filaments called hyphae, which form after a spore germinates. The hyphae permit the thorough and intimate exploration and exploitation of newly available substrates. Figure 1 shows hyphae emerging from spores and looking for food. This growth represents a young colony of mold. The hyphae's strong, waterproof, chitinous extensions, its richly branched growth pattern, the digestive enzymes it secretes at its growing tips, and the hydrostatic pressures it can bring to bear all make it ideally suited for actively *penetrating*, *exploring*, and *exploiting* solid substrates in a manner that cannot be matched by bacteria, fungi's chief competitors in the recycling process (Kendrick 1985).

A colony is a visible mass of inter-woven hyphae that form a mycelium (Burge 1999). The "fuzzy" mass that you may see on foods, such as, bread, jelly, fruit, etc. is the colony formation of fungi. Colonies can grow as several different colors, including gray, brown, green, blue, black, yellow, red, or other colors. The mycelial fungi most commonly found growing in the indoor environment are often called "molds." Other common fungi include yeast, mushrooms, rusts, smuts, puffballs, bracket fungi, cup fungi, morels, and truffles – to name a few. These are also mycelial fungi, many of which form typical mold-like colonies in the laboratory. In the wild these fungi form large, sometimes edible, fruiting bodies (Burge 1999). The term "mold" is the lay term for multicellular fungi that grow as an entangled mass.

Lay terminology frequently refers to fungi growing on fabrics, windowsills, or bathroom tile as "mildew." However, mycologists use the term *mildew* to refer to cer-

**FIGURE 1. Hyphae Emerging from a Single Mold Spore (Kendrick 1985)**



rain species of fungi that cause plant diseases, such as downy mildew and powdery mildew.

Fungi do not have chlorophyll, so they cannot produce food through photosynthesis like plants. Rather, fungi feed by absorption of nutrients from the environment. They secrete digestive enzymes to break down the material (substrate) on which they are growing and then absorb the nutrients from that substrate. This process of feeding is crucial to the environment because it is what causes fungi to decay large amounts of plant debris, which may well make fungi one of the world's number one recyclers (Kendrick 1985). Unfortunately, fungi do not restrict their attention to naturally occurring dead wood and leaves. Where there is a trace of moisture, their omnipresent spores will germinate, leading them to attack food, fab-

ric, paper, paint, or almost any other kind of organic matter.

### ***Growth Requirements of Fungi***

In addition to an appropriate source of nutrients, fungi need certain other environmental conditions to grow. The most important conditions are temperature and water activity.

#### **Temperature Requirements**

Fungi will grow at a wide range of temperatures, but most species grow best between 25°C and 30°C (77-86° F) with the lower and upper temperature limits being roughly 10°C and 40°C (50 and 104° F). However, certain "thermophilic" fungi will grow at temperatures as high as 50° C (122° F) in composting habitats.

#### **Moisture and Water Activity**

Irrespective of the nutrient status of any material, the availability of water in the material dictates whether or not a microorganism can grow on or in the material. Every microorganism has its own particular moisture requirements. Its growth depends on the availability of enough "free" water (Flannigan 2001). Free water can be thought of as the absorbed water that is not held tightly in chemical union with the material itself. The term used to describe the amount of free or available water is "water activity."

#### 4 Scientific Background

Water activity is an important indicator of a material's ability to support microbial growth. Theoretical limits for microbial growth lie between water activity levels of 0.65 and 1.0, with 1.0 being saturated. Practically speaking, if water activity in materials is maintained below ~0.75, microbial growth will be limited; below a water activity of 0.65, virtually no microbial growth will occur on even the most susceptible materials (Shaughnessy 1999). Fungi found in indoor environments are able to grow at water activity from 0.70-0.80, and even some as low as 0.65 (Flannigan 2001). Other fungi, such as *Stachybotrys chartarum*, are hydrophilic (water loving) and prefer water activity levels greater than 0.90, which is a very wet environment.

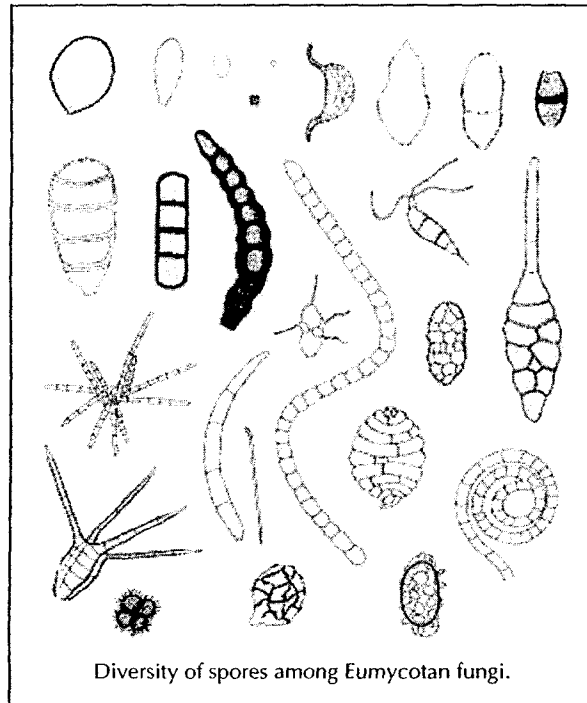
#### **Fungi Reproduction and Spores**

Reproduction is the formation of new individuals that have all of the characteristics typical of the species. Two general types of reproduction are recognized for fungi: sexual and asexual (Alexopoulos 1996). Typically, fungi reproduce both sexually and asexually, although not necessarily at the same time. The sexual and asexual reproductive units of fungi are called spores, which are usually enclosed in a rigid wall. Fungal spores differ from plant seeds because the spore does not contain an embryo (Moore-Landecker 1996). In general, asexual reproduction is more important because it results in the production of large numbers of individuals (Alexopoulos 1996). Asexual fungal spores include zoo-

spores, sporangiospores, and conidia and are of particular importance to indoor air quality because they are the reproductive units for species of *Cladosporium*, *Aspergillus*, *Penicillium*, *Ulocladium*, *Alternaria*, *Fusarium*, *Trichoderma*, and *Stachybotrys*.

Fungi are classified and identified primarily by the method of spore production, including the nature of the spore production process and morphology of the spore. Fungal spores span a wide range of colors from colorless to nearly black (Burge 2000). It is the spores that produce the color of the fungal colony we see growing on food products or wet building materials. Each spore may include one or more cells arranged in lengthwise chains or in three-dimensional arrays. As seen in Figure 2, spores come in a dazzling array of forms to fit specific functions. Spores are often produced very quickly (in a matter of days after the initial colonization of the substrate) and in enormous quantities (Kendrick 1985).

**FIGURE 2. Diversity of Spores in Common Molds (Kendrick 1985)**



Spores permit rapid dispersal and a kind of scattershot saturation of the biosphere—fungal spores are everywhere. Spores are dispersed by wind, by water, or by animal vectors and they can often survive long periods, sometimes even years, of unfavorable conditions such as freezing, starvation, or desiccation. This durability is an important characteristic for the survival of the species, but it also means that spores will remain viable in the environment for extended periods. The spores' resilience is associated with the mold growth that can occur in buildings that experience water infiltration. That is, spores may taint surfaces in a building and then just “wait” until conditions (*e.g.*, water activity) become right for them to germinate and grow.

## Aerobiology of the Outdoor and Indoor Environment

The science of aerobiology includes the study of bioaerosols (*i.e.*, the nature, prevalence, and distribution of airborne biologic-source particles) as well as the effect of airborne agents on people, animals, and plants. Biologic aerosols, including fungal spores, are controlled by the same principles as other aerosols. Among the many important physical aerosol parameters to consider are the aerodynamic particle size (related to diameter, shape, and density), the nature of the particle (hydrophilic, hydrophobic, soluble, etc.), electrostatic charges on the particles, and the concentration of the particle in air (instantaneous as well as variable over time) (Burge 1995). All of the factors work together to determine the distribution and exposure potential associated with any bioaerosol. Air movement dynamics are very important and yet are seldom evaluated properly.

Most fungal spores are released “passively” into the environment and rely on wind currents for dispersal. However, the dispersal of spores from the living mold colony is enhanced by specialized reproductive structures that “lift” the developing spores away from the surface on which mold is growing, and allow for more efficient erosion of the spores by wind currents (Mullins 2001).

Once the fungal spore is released into the environment, the length of time it remains airborne depends on the physical properties of the spore, including its size. Although airborne spores can range in diameter from <2 to >50 micrometers, most fall into the range of 2–10 micrometers and are thus physically capable of penetrating into the lower airways (respirable size particles) (Burge 2000). Therefore, the spore size affects the densities and distribution of airborne fungi (Mishra 1992). The physical state of the spore also has an impact on its movement in air (*i.e.*, the “dry” spores of *Penicillium* and *Aspergillus* will stay airborne longer than the “slimy” spores of *Fusarium*, *Verticillium*, and *Stachybotrys*).

## Mold Growth Within Homes

Mold growth has always occurred in buildings, even being referenced in Leviticus 14:37–57 of the Old Testament. The biblical citation notwithstanding, nearly everyone has dealt with “mildew” growing in some area of the house, on food products, or on houseplants. However, limited mold growth within a building does not represent a significant exposure risk for the occupants and therefore poses no serious threat to our well being. General hygiene principles have always recommended the immediate cleanup of visible growths through normal house cleaning.



There are, however, increasing numbers of reported health effects associated with continued or extensive mold growth in buildings. These reported problems are typically associated with extreme weather events (flood, hurricanes, and tornadoes) or chronic water problems in the building. Previously, most reported mold problems in buildings were thought to be associated with problems in the heating, ventilation, and air-conditioning (HVAC) systems. In the past decade, we have begun to recognize that significant mold growth problems may take place on many other building materials and components within our buildings. This occurs because most of the environmental conditions necessary to support mold growth already exist in buildings, *i.e.* building materials are already “seeded” with mold spores from natural infiltration, the temperature inside buildings is generally within the optimal range for mold growth, and many building materials serve as appropriate nutritive sources for fungi.

### Overview of Fungi with Potentially Adverse Health Effects

As previously discussed, most fungi are saprobic, feeding on dead or decaying material. However, many fungi are parasitic, feeding on living organisms without killing them, but causing deleterious health effects. Ergot, corn smut, and Dutch elm disease are all diseases caused by parasitic fungi. Other species of fungi may affect human health because of their toxigenic, allergenic, and pathogenic properties.

#### ***Potentially Toxigenic Fungi***

Some fungi have the potential to produce substances called mycotoxins. Mycotoxins are by-products of fungal metabolic processes. These compounds are considered secondary metabolites because they are natural products that are not necessary for fungal growth because they are derived from a few precursors formed during primary metabolism (Burge 1999). The chemical structures of mycotoxins are quite diverse and include polyketides, terpenes, and indoles. The function of fungal toxins has not been clearly established. However, they are considered to play a role in regulating competition with other microorganisms (Burge 1999).

Mycotoxins may cause health effects because they are lipid-soluble and are therefore readily absorbed by the intestinal lining, airways, and skin. Mycotoxins are usually potent cytotoxins that cause cell disruption and interfere with essential cellular processes. Some mycotoxins are potent carcinogens, some are vasoactive, and some penetrate the blood-brain barrier to cause central nervous system damage (Burge 1999).

The vast majority of mycotoxins are not volatile and therefore mycotoxin exposure by inhalation is most likely to occur via inhalation of fungal spores (Sorenson 2001). Species of mycotoxin-producing molds include *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Stachybotrys* (Sorenson 2001, Burge 1999). A more detailed discussion of this relationship is presented in other sections of this document. (See background section on Medical Toxicology.)

#### ***Allergenic Fungi***

A vast number of fungi have been implicated in allergic illness. Airborne fungal spores have been widely recognized as major allergens capable of causing asthma and allergic

rhinitis in sensitized individuals, as well as other allergic manifestations. Interest in the association between the presence of fungal antigens in the environment and allergies or asthma has increased significantly in the past 10 to 15 years (Barnes 2001). Moisture problems in homes have been found to increase the risk of children having episodic and/or persistent allergic symptoms such as rhinitis, wheezing, and itching or redness of the skin (Rylander 1999). A more detailed discussion of this relationship is presented in other sections of this document. (See background section on Allergy and Immunology.)

### ***Pathogenic Fungi***

Only a few of the thousands of species of fungi actually cause human ailments, and then only under specific circumstances. Most species that do infect humans are limited by nutritional requirements and by host defense mechanisms that protect against invasion of the superficial skin and subcutaneous tissues. The species that are capable of invading the deeper tissues are, fortunately, quite few. Systemic fungal infections are acquired by accidental inhalation of airborne spores or by traumatic inoculation with contaminated soil or plant materials. These are uncommon except in certain geographical areas where a specific organism may infect almost 100% of the population, usually causing only mild illness (Cooper 1985). Many of the common fungal infections are not transmitted by the aerosol route, and are not directly relevant to this discussion. For example, ringworm is a fungal infection transmitted by direct or indirect contact. Another common fungal infection that is not connected with inhalation of inoculum from indoor sources is candidiasis. Candidiasis, which is generally caused by one of the yeasts in the genus *Candida*, is caused by yeast species that are part of the normal microbial flora of the mouth and gastrointestinal tract (endogenous infections). Some of the important infectious diseases caused by airborne fungal spores are discussed below.

## **Specific Health Effects Associated with Fungi**

### ***Systemic Fungal Ailments***

Classical systemic fungal diseases (mycoses) continue to be a significant health problem. The fungi classically referred to as the etiological agents of systemic mycoses include *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Histoplasma duboisii*, and *Paracoccidioides brasiliensis*. All of these fungi, except *C. neoformans* are diphasic; *i.e.*, they grow as complex mycelial elements in their "natural" state, and they grow as yeast in human or animal tissue. These fungi primarily cause pulmonary infections (Larsh 1985).

Soil is the natural habitat for the fungi that cause systemic mycoses, and the geographical distribution varies for each species. For example, *C. immitis*, which causes coccidiomycosis, occurs only in the arid and semiarid regions of North America, South America, and North Africa. *H. capsulatum* (causing histoplasmosis) can be isolated throughout the world, but it occurs primarily in the states bordering the Ohio, Missouri, and Mississippi river valleys in the United States. The growth and isolation

of *H. capsulatum* is most common from areas that have been “fertilized” by droppings of chickens, blackbirds, and bats.

*Cryptococcus neoformans*, as a cosmopolitan organism, is much more widespread geographically than *H. capsulatum* or the other agents of systemic mycoses. *C. neoformans* is frequently associated with bird droppings and can enter the indoor environment through windows and other openings. As with histoplasmosis, the presence of bird roosting activity increases the risk of exposure and, potentially, infection with *C. neoformans*. Fortunately, *C. neoformans* is much less virulent than *H. capsulatum* and a significant exposure is usually needed for even immunocompromised individuals to become infected (Summerbell 2001).

### Superficial Fungal Infections

In contrast to serious systemic infections caused by fungi, superficial fungal infections on the skin or mucosal surfaces are extremely common in normal subjects. These superficial infections include infection of the feet (*tinea pedis*), nails (*tinea onychomycosis*), groin (*tinea cruris*), and dry body skin (*tinea corporis*) and infection of the oral or vaginal mucosa. Some of the common organisms involved, e.g., *Trichophyton rubrum*, can be found growing as an indoor mold. Others, such as *Microsporum canis* and *T. mentagrophytes* can be found on indoor pets (e.g., dogs, cats, rabbits, and guinea pigs). As a common commensal on human mucosal surfaces, the yeast *C. albicans* can be cultured from more than half of the population that has no evidence of active infection. *C. albicans* infections are particularly common when the normally resident microbial flora at a mucosal site is removed by antibiotic use. Local factors, such as moisture in shoes or in body creases and loss of epithelial integrity, are important in the development of superficial fungal infections (Hardin 2002).

*Pityriasis (Tinea) versicolor* is a chronic asymptomatic infection of the most superficial layers of the skin due to *Pityriasis ovale* (also known as *P. orbiculare* and *Masassezia furfur*) and is manifest by patches of skin with variable pigmentation. This is not a contagious condition and thus is unrelated to exposures, but represents the overgrowth of normal cutaneous fungal flora under favorable conditions (Hardin 2002).

### Opportunistic Fungal Infections

There is an ever-enlarging group of fungi that, while not directly pathogenic, can cause environmentally acquired respiratory disease in the strongly immunocompromised patient, or rarely, in an individual who is both debilitated and heavily exposed, or, even more rarely, in an otherwise normal person (Summerbell 2001). These fungal pathogens are generally termed “opportunistic pathogens.” Opportunistic infections run the spectrum from otomycosis (fungal ear canal colonization), to allergic bronchopulmonary aspergillosis (colonization of the bronchial passages), to life-threatening invasive disease.

Many of the opportunistic fungi found in the indoor environment are typical inhabitants of outdoor environments and include *Aspergillus terreus*, *A. fumigatus*, *A. flavus*, *Rhizopus microsporus*, *R. oryzae*, *Fusarium* sp., *Trichosporon* sp., *Paecilomyces* sp.,

and *Alternaria* sp. (Summerbell 2001). Of this wide array of fungal species, *Aspergillus fumigatus* is the most common cause of opportunistic respiratory disease.

*Aspergillus* species are widely distributed fungi whose spores are present in the outside air throughout the year. Healthy people may inhale *Aspergillus* spores on a daily basis with no immediate health consequences, although inhalation of airborne spores of some *Aspergillus* species may cause various forms of ailments in susceptible individuals (Alexander 1999). Aspergillosis is a serious, invasive illness that generally only occurs in immunocompromised individuals. Most of our understanding regarding the pathogenic potential of *Aspergillus* species has been learned through investigation of hospital acquired infections, because the rate of transmission within the general population is so low. For hospitalized patients with severe immunocompromised conditions (*e.g.*, bone marrow transplants, AIDS, etc.), outbreaks of invasive Aspergillosis have been associated with building construction and renovation within or near the hospital. While many of the hospital patients will have the same exposure to the fungal spores that are disturbed and/or released during these activities, only the severely immunocompromised sub-set of patients will develop infections, thus characterizing the “opportunistic” nature of these pathogenic fungi.

### ***Stachybotrys chartarum***

There has been an exceptional focus of publicity and public awareness on the potential health effects associated with exposure to *Stachybotrys chartarum* in water-damaged buildings (Terr 2001, Vesper 2000). The allergenic and toxigenic potential of this mold will be discussed elsewhere in this monograph; however, it is important to note that there are no convincing cases of infection caused by *S. chartarum* (Terr 2001).

## **Conclusions Relating Fungi and Health Effects**

Data on human health effects of mycotoxins come primarily from literature on ingestion exposures, which do not have direct correlation with inhalation exposures (indoor air). Most of the information on the risk associated with inhalation of mycotoxins comes from occupational exposures, where there is clear evidence of health effects including cancer, skin inflammation, and skin scaling. However, there is little evidence to link inhalation exposures for environments such as offices, schools, hospitals, and residences with any form of cancer. Moreover, at this time, inadequate data exist to predict accurately the risk associated with human inhalation exposure to mycotoxins in the non-agricultural indoor environments (Burge 1999). Drs. Page and Trout of the CDC go even further in stating, “that there is currently no clear evidence documenting that mycotoxins cause health effects among building occupants” (Page 1998). Therefore, it appears that the scientific jury is still out on this issue, and more carefully controlled research is needed to characterize fully the toxigenic potential of inhaled mycotoxins. This conclusion even extends to the toxins produced by *Stachybotrys chartarum*, which have gained increasing interest because of health effects being identified in water-damaged buildings where high concentrations of *S. chartarum* spores have been isolated. The authors of a recent “position paper” concluded, “adverse health

effects from inhalation of *Stachybotrys* spores in water-damaged buildings is not supported by available peer-reviewed reports in the medical literature” (Texas Medical Association 2002). A similar conclusion was expressed by the American College of Occupational and Environmental Medicine, which stated:

*Molds growing indoors are believed by some to cause building-related symptoms. Despite a voluminous literature on the subject, the causal association remains weak and unproven, particularly with respect to causation by mycotoxins. One mold in particular, Stachybotrys chartarum, is blamed for a diverse array of maladies when it is found indoors. Despite its well-known ability to produce mycotoxins under appropriate growth conditions, years of intensive study have failed to establish exposure to S. chartarum in home, school, or office environments as a cause of adverse human health effects. Levels of exposure in the indoor environment, dose-response data in animals, and dose-rate considerations suggest that delivery by the inhalation route of a toxic dose of mycotoxins in the indoor environment is highly unlikely at best, even for the hypothetically most vulnerable subpopulations.*

## Summary

The fungi are a complex and diverse group of organisms. They are everywhere in the natural environment, and it is essentially impossible to avoid exposure to fungal elements and their by-products. Fungi become a natural contaminant within all buildings as a consequence of infiltration from the outdoors. Under certain circumstances, primarily water problems, these contaminants can begin growing within the building, which may have a negative impact on the indoor air quality and potentially affect the occupants of that building. While there generally are no untoward consequences related to exposures to fungi, some fungi are toxigenic, allergenic, and/or pathogenic and cause illnesses in certain individuals.

The field of industrial hygiene is concerned with whether or not exposure to certain environments is safe for people. Industrial hygienists use a procedure called *exposure assessment* to determine the concentration of a contaminant in an environment. Contaminant concentration is one of the critical elements in determining whether contaminants will cause a given health effect. The exposure concentration is related to the mass of the contaminant that is available to act on the biological system being studied. The dose is the ratio of the mass of the contaminant and the mass of the biological system that is absorbing the contaminant. Critical to determining exposure concentration and dose is the physical contact that occurs because there must be physical contact between the contaminant and the human system in order for a contaminant to affect an individual. If there is no contact, then the exposure is zero, the dose is zero, and there cannot be any health effects associated with the contaminant.

The pathways for potential exposures are affected by many physical characteristics and climatic conditions. These site-specific characteristics may affect the transport of fungal components between the growth area and, ultimately, the inhabitants. Therefore, information about physical, chemical, and climatic characteristics of the site is necessary. These characteristics may include the elevation of the site and the site's topography, which may affect surface water runoff and potential flooding. Soil characteristics and annual precipitation are critical in that these conditions can influence groundwater recharge, percolation, and transport. The nature of vegetation at the site can also have a bearing on surface water movement. Other factors that are important include the ambient temperature, prevailing wind velocity, and unnatural site characteristics such as retaining walls.

Within the structure, walls, ventilation, building materials, and other conditions may affect the potential movement of fungal materials from the site of growth to inhabitants. The source to inhabitant pathway via air is the most important because it can potentially reach individuals by inhalation, ingestion, or dermal contact.

## Approach to Assessing Indoor Contamination

Determining exposure to a contaminant is a multi-step process. The steps are:

1. Determine sources of contaminant emissions and characteristics;
2. Identify transport pathways for contaminant;
3. Identify the fate of the contaminants; and
4. Estimate potential receptor exposure/dose.

Figure 3 graphically states this with the following diagram.<sup>1</sup>

The exposure assessment considers the source, amount, frequency, duration, and routes of human exposure to agents in various mediums. The resultant assessment tool allows the investigator to consider the exposure in the context of potential human health effects. These assessments are multi-disciplinary appraisals that draw on the basic and health sciences. Each of the components listed in the exposure assessment

1. This diagram was derived from source material contained in Liroy PJ, Assessing total human exposure to human contaminants. Environ. Sci. Technol. 1990; 24: 938-945.

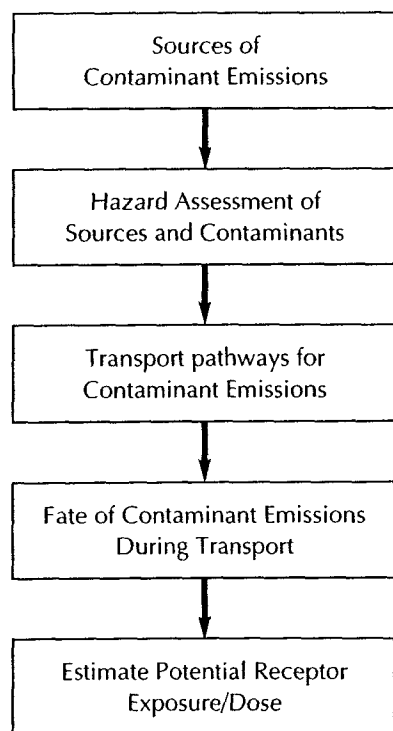
**FIGURE 3. Exposure Assessment**

diagram depicted above are critical elements and must be evaluated in the order noted.

In the cases relevant to this monograph, the sources of the contaminants of concern are fungal growths in homes; therefore, the challenge is to locate fungal growth, if present. Contamination by inhalation is generally considered the major transport pathway for exposure to fungal contaminants, but exposure can also occur from direct contact with surfaces on which the contaminant is present. Direct contact with surfaces that are contaminated can result in either absorption through the skin, ingestion, inhalation of disturbed particles, or all of the above. The fate of spores is important for the pathogenesis of opportunistic infections. However, it is important to remember that spores change in viability, not in composition. If an allergic individual is exposed to the type of mold to which they are allergic, it is inconsequential as to whether the mold is actively growing or dormant. When discussing markers such as microbial volatile organic compounds (MVOCs), however, the fate of the source is of paramount concern because volatile organic compounds may change in composition due to interactions with environmental factors (*i.e.*, sunlight, temperature). Their corresponding biological efficacy may, therefore, be altered.

The term “amplification” refers to active mold growth and is usually used to distinguish between proliferating and inactive molds. When amplification of fungal growth occurs in homes, industrial hygienists raise the following questions:

- Has fungal growth been amplified to a level that could pose a possible health risk to the individuals occupying the home?
- What types of fungi have been amplified?
- What are the locations and extent of the growths?
- Where are the moisture sources and conditions that could initiate an amplification of fungal growth in the home?
- If moisture sources are identified, what measures are necessary to eliminate the moisture source and to remediate the existing growths?

A host of tools is available to answer these questions. The remainder of this section will discuss the appropriate methods and tools for addressing these questions. Certified industrial hygienists and certain environmental professionals may be trained to use these tools appropriately. Effective interpretation of the results from these different methods stems from a combination of knowledge of the science involved and an understanding of the technology of building structures (*i.e.*, how ventilation systems are configured, what materials are used in different types of structures).



## Availability of Guidelines for Assessing and Remediating Indoor Mold Problems

Currently, there are no standards available to help in the process of assessment and remediation of fungal growth, although there are several guidance documents available. Some of the current guidance documents available on indoor fungal issues are:

*A Brief Guide to Mold, Moisture, and Your Home*: U.S. Environmental Protection Agency (EPA), Office of Air and Radiation, Indoor Environments Division (6609J) EPA Publication #402-K-02-003. October 2002.  
<http://www.epa.gov/iaq/molds/moldguide.html>

*Mold Remediation in Schools and Commercial Buildings*: U.S. EPA Office of Air and Radiation, Indoor Environments Division (6609-J) EPA 402-K-01-001. March 2001. [http://www.epa.gov/iaq/molds/mold\\_remediation.html](http://www.epa.gov/iaq/molds/mold_remediation.html)

*Update: Guidelines on Assessment and Remediation of Fungi in Indoor Environments*: New York City Department of Health and Mental Hygiene, Bureau of Environmental and Occupational Disease Epidemiology. January 2002. <http://www.ci.nyc.ny.us/html/doh/html/epi/moldrpt1.html>

*Occupational Safety and Health Administration (OSHA) Technical Manual*. Section III, Chapter 2, (TED 1-0.15A) - Indoor Air Quality Investigation. [http://www.osha.gov/dts/osta/otm/otm\\_iii/otm\\_iii\\_2.html](http://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_2.html). Also see <http://www.osha.gov/SLTC/indoorairquality/index.html>, updated December 2002.

These guidance documents are directed towards simple remediation of visible growths. The assessment of mold growth in a structure and the resultant exposure of individuals occupying the structure is still very much an emerging field, as indicated by the routine updates made to these various guidelines and as the state-of-knowledge regarding indoor mold has changed in recent years. Still, many of these documents are guidelines, and there currently are no specific regulations for identifying, assessing, or remediating indoor mold growth.

## Approach to Investigating a Building with Potential Mold Contamination: Assessing Structure Condition and Developing a Sample Protocol

The investigation of suspected mold growth is a multi-step process. The following items of information are the minimum that must be collected:

- A history of the structure;
- A history of the occupancy of the building;
- A history of occupant activities, including health histories of the building occupants;
- A review of any design or construction documents that are available;
- A visual inspection of the structure and its contents; and
- Any current health concerns that the occupants have.

Once these items are addressed, enough information should be available to make a determination as to whether or not further investigation is warranted. If further investigation is warranted, then a sampling protocol should be developed that answers the following questions as fully as possible (hypothesis testing):

- Where has mold growth been amplified?
- What genera and species of fungi have been amplified?
- What kind of exposure (*e.g.*, inhalation, ingestion, dermal contact) did the building occupants have?

Photographic or video documentation of the condition of the structure and the sampling operations should also be taken.

If there are signs of an indoor air problem, the sampling plan should address all spaces within a building. Because moisture is the key to fungal growth in the indoor environment, the moisture issue and fungal growth need to be addressed jointly. Moisture should be dealt with first. Water vapor travels wherever air exchange takes place. Air exchange may occur anywhere in the building envelope, including between interior wall spaces and the occupied areas of the building. Accordingly, a change in the relative humidity may reflect an increase in moisture in interior wall spaces or crawlspaces. These areas should be characterized by the sampling plan because sampling limited to occupied spaces may not reveal the source of moisture, depending on the conditions of the investigation. If there is evidence of increased ambient moisture in a specific area, then the industrial hygienist or environmental professional may choose to investigate whether the source is located in wall spaces or crawlspaces. If a building has repeated outbreaks of fungal growth, it may be due to a failure to locate outlying growths or growths related to other moisture release events that are not evident in the occupied space.

The sampling plan should also be designed with regard to how the results will be used. Each sampling location should be selected with a definite knowledge of how the information obtained will be integrated into the overall interpretation of the data.

### Sampling for Fungi in the Indoor Environment

Sampling is an essential element of exposure assessment and may be necessary for liability reasons. The U.S. Environmental Protection Agency (EPA) states that “sampling for mold should be conducted by professionals with specific experience in designating mold sample protocols, sampling methods, and interpretation of results” (EPA 2001). The purpose of the sampling is to determine whether or not fungal growth has undergone amplification; to identify the types of fungi that have been amplified; to estimate the exposure levels, if possible; and to identify the locations and extent of the growths.

To answer all of these questions, the implementation of a comprehensive sampling strategy may be necessary. At a minimum, the sampling plan should include air sampling collection of both a viable sample (*i.e.*, a sample taken to determine whether there are spores that can germinate or grow) and a non-viable sample (*i.e.*, a sample used to measure the total amount of collected material without regard for its amplification potential). Additionally, at least one viable and non-viable sample should be taken from an outdoor location. Depending upon the conditions, sampling may also include

methods that directly examine surfaces (*i.e.*, tape, bulk, swab sampling). Samples for markers, such as microbial volatile organic compounds (MVOCs) or (1-3)- $\beta$ -D-glucan, (Pasanen 2001, Rao 1996, Dillon 1999, Jantunen 1997) may be taken, although the scientific relevance of these fungal by-products as indices for mold amplification is currently unknown.

It is critical that the laboratory used to analyze the samples belongs to a certification program. The American Industrial Hygiene Association has an Environmental Microbiology Laboratory Accreditation Program (EMLAP), which is specifically for laboratories dedicated to identifying common microorganisms in air, fluid, and bulk samples.

Simple factors may have a dramatic impact on the outcome of sampling. For example, the physical orientation of the medium used to capture samples in relation to the direction of existing air currents will affect the air sampling results. The capture device should be positioned so that the existing air currents flow toward the intake of the capture device (Pasanen 2001). Failure to orient the device properly leads to results that are significantly skewed toward the collection of smaller-sized spores.

The two primary sampling methods are culturable sampling and direct microscopic examination sampling, both of which will be explained further below. Although culturing a surface sample may help resolve a specific identification problem, when used alone such a culture may result in an inaccurate characterization of the sampled surface. Direct microscopic examination of a surface shows exactly what is there, without being affected by an organism's ability to compete and grow on sampling media. Other methods of sampling a surface may produce distortions of the data. Therefore, when direct information is needed as to the growth present on a surface, a bulk or tape sample should be analyzed using direct microscopic examination. These are the methods that produce the most reliable information about the true conditions present on the surface. Direct microscopic examination is also a useful qualitative approach to identifying the general type of mold that is present.

### **Culturable Sampling Methods**

Culturable sampling is currently the only air sampling method commercially available that produces reliable speciation data for *Aspergillus* and *Penicillium* molds. Speciation of these two genera provides information that may be helpful to the exposure assessment, depending on the medical history of the occupants. Culturable sampling methods require that the spores are intact at the time of sampling, remain viable throughout the sampling process, are able to germinate on the sampling media, and are able to compete with other species present on the growth media.

### **Air Sampling**

Air sampling consists of sampling for viable fungi or non-viable fungi. A 3-minute sampling period may produce useful results from a clean home, depending on sampling rate and system, but may yield unreadable samples in a home with visible mold growth, because the sample is overloaded with airborne particulates. Care should be taken to limit sampling times to a minimum, although changing the duration of the testing means that results cannot be quantified or compared to samples of a different duration.

An early and commonly used method of viable air sampling is the gravimetric/settling method. This procedure involves setting out agar plates for an extended period of time (typically 12 to 96 hours). The plates are then incubated and read. This method tends to skew the sampling results toward the fungi with large spores, since these spores readily settle out of the air. There is also no way to relate these results to exposure, as there is no relationship to concentration in air. As a result of these limitations, reputable investigations seldom use this method today and no longer recommend it (Dillon 1999).

Active air sampling can be of either the grab sample type or of a type designed to allow collection of samples over a period of hours or days. Instruments designed to collect long-term samples are not commonly used in the commercial business of sample collection and are seldom seen in the field, except for use at counting sites for outdoor pollen and spore collection.

Table 1 contains a list of the most commonly used grab sampling devices used today. Each type of sampling devices has its own advantages and disadvantages. Most of the advantages and disadvantages center on the capture efficiencies and the type of capture material. The capture efficiencies are related to the rate and volume of air flow over the capture surface, the design of the orifice, and the media chamber through which the air flows. Additionally, transference of the captured material to a different media can affect efficiency.

The Anderson single stage N6 and the Aerotech 6 Microbial Sampler are currently the most popular of the cascade impactors, and therefore the most commonly used field devices. The popularity of these samples is because the agar plate is directly loaded, which makes it very easy to load the samples and prepare them for shipment to the laboratory.

The samplers listed in Table 1 have a variety of media onto which the material can be captured. Different media types will produce different results even when used in the same device. For example, different concentrations of individual species will be

**TABLE 1. Commonly Available Active Sampling Devices Used for Indoor Culturable Fungi Sampling**

Sampler type	Company that manufactures
Cascade Impactor	Thermo Andersen, Atlanta, Ga; Aerotech Laboratories, Phoenix, Ariz.
Surface air system sampler	Pool Bioanalysis Italiana, Milano, Italy; Spiral System Instruments, Bethesda, Md.
Slit-to-Agar air sampler	Barramundi Corporation, formerly Mattson-Garvin Company, Homosassa Springs, Fla.
Centrifugal air sampler	Biotest-Serum-Institut GmbH, Frankfurt, Germany; Biotest Diagnostics Corporation, Denville, N.J.
47-mm Membrane filter air sampler	Gelman Sciences, Ann Arbor, Mich.
All-glass impinger-30 sampler	Ace Glass, Vineland, N.J.

reported if a cascade impactor is loaded with Dichloran Glycerol (DG-18) media as compared to corn meal agar. All samples collected with a specific device should be collected on the same type of media in order to make direct comparisons among the samples. This will reduce the variation in sample counts. Direct comparison of colony-forming units (CFUs) should not be made between samples collected with different types of sampling devices or collected on different sampling media. These samples are difficult to analyze statistically because of the variability of the results, even when collected with the same sampling device and onto the same media type (Dillon 1999). Table 2 provides descriptions of many of the commonly available media and some of their applications for culturable sampling.

### Surface, Settled Dust, and Debris Sampling

Surface samples can be taken by tape lift, swabbing the surface with a culturette swab, collecting settled dust or debris from the surface using a vacuum or sweeping, or submitting a bulk sample of the suspect surface.

**TABLE 2. Commonly Available Commercial Media Used for Fungal Culture**

Culturable Media Type	Use	Advantages
Malt Extract Agar (MEA)	For the isolation of broad-spectrum environmental and pathogenic fungi	Excellent wide-spectrum medium that gives reasonably uniform results
Dichloran-Glycerol Agar (DG-18)	Fungal isolation	Inhibits growth of rapid-growth fungi such as <i>Rhizopus</i> and <i>Mucor</i>
Rose Bengal Agar (RBA)	Fungal isolation	Inhibits growth of rapid growth fungi such as <i>Rhizopus</i> and <i>Mucor</i>
Cellulose Agar (CEL)	Fungal isolation	Excellent growth medium for <i>Stachybotrys</i>
Modified Cellulose Agar	Fungal isolation	Excellent growth medium for <i>Stachybotrys</i>
Potato Dextrose	For the isolation of broad-spectrum environmental and pathogenic fungi	Commonly used for culturable dust samples; May help induce sporulation of fungi in dust
Cornmeal Agar (CMA)	Fungal isolation	Excellent growth medium for <i>Stachybotrys</i>
Tryptic Soy Agar (TSA)	Bacterial isolation	General bacterial culturing
TSA w/ 5% Blood (BAP)	Isolation of broad-spectrum, fastidious bacteria including actinomycetes	Excellent for general pathogenic bacterial culturing

Culturing of any of these methods of sampling can lead to a skewed representation of the sample population. It is well established that dilution-plating does not give reliable data about the species that are active in the ecosystem (Dillon 1999). The results of dilution-plating are affected by the preference of individual organisms for particular media. The results may also be affected by an individual mold's ability to survive aggressive sampling techniques. These types of samples, when cultured, misrepresent the species that are actually active in the area, resulting in erroneous data that produce inaccurate interpretations of data.

## Direct Microscopic Examination Sampling Methods

### *Air Sampling*

Numerous spore trap designs are available. Allergenco/Blewstone Press makes a programmable spore trap impactor that collects slides at regular intervals over a time period that can vary from hours to days. This device accepts standard glass slides, which are greased by the user. Burkard Manufacturing makes a spore trap that collects samples on a revolving drum, which rotates over either a 1-day or a 7-day period. Samples are collected on an adhesive-coated transparent plastic tape attached to the rotating drum. Another company, Zefon Analytical Accessories manufactures a disposable spore trap called Air-O-Cell Cassettes. The popularity of Zefon Air-O-Cell cassettes results from their relatively low cost and small size. BIOS makes a pump for these Air-O-Cell Cassettes that can be programmed to collect short samples over multiple intervals for averaging airborne spore counts over relatively long time periods. All of these samplers use glass slides coated with adhesive.

Spore traps allow the collection of both spores and other particulates in the air. Spore traps allow the identification of spores that might be present in the environment, but may no longer be viable or not culture well. Spore trap samples that are caught concurrently with culturable samples provide combined results that are more useful than if collected at different times. However, direct comparisons between results from spore trapping and culturable sampling are not appropriate under any circumstances.

Concurrent spore trap and culturable sampling is beneficial because of the limitations of each type of sample. The direct examination of spore traps cannot identify many of the small colorless spores such as those from *Aspergillus* and *Penicillium* species. Culturable sampling, if collected concurrently with spore trapping, can identify the species of spores present. The spore traps can yield information about total spore types that could be trapped and, in combination with the results from the culturable sample, an estimate may be made of the number of viable spores present compared to the total number of spores present by group.

For example, a spore trap may yield a count of 11,000 spores per cubic meter of air of *Penicillium/Aspergillus* type spores. A culturable sample is taken concurrently on malt extract agar (MEA) and the culturable air sample has a raw count of 5 spores per cubic meter of air of *Aspergillus versicolor* and raw count of 7 spores per cubic meter of air of *Penicillium chrysogenum*. With a total spore count of 11,000 *Penicillium/Aspergillus* type spores per cubic meter and only 12 viable *Penicillium/Aspergillus* type spores per cubic meter, we can conclude that the vast majority of *Penicillium/Aspergillus* type spores

present are non-viable. This information can be coupled with other information provided by the spore traps, such as the presence of spores that do not culture well. This type of information may be helpful for the investigator to establish that the spores in the environment are not undergoing amplification.

A host of additional information can be provided by concurrent culturable and spore trap sampling. When conducted properly, the correlation of culturable and spore trap sampling can be useful in predicting the locations of mold growths, the extent of hidden mold growths, and the rise or decline of moisture levels.

### Surface Sampling

The major purpose of a direct microscopic examination of the material removed from a surface is to determine if mold is growing on the surface. If mold is present, then it is necessary to ascertain the types of mold. Both hard and soft surfaces collect a mixture of spores, which normally exist in the environment. However, when mold is growing, at times it is possible to note a skewing of the normal distribution of spore types. For example, many more spores of a particular genus are typically found trapped on surfaces near the area of the mold growth, so the increased number of spores indicates the mold growth. These spores can be in forms that indicate recent spore release, such as spores in chains or clumps. Marker genera are those spore types that normally occur in very small numbers, but which multiply indoors when conditions are favorable for growth. These include mold varieties that prefer food sources rich in cellulose (cellulose digesters) such as *Chaetomium*, *Stachybotrys*, and *Penicillium*.

#### *Tape Samples*

A tape sample, which allows for the direct examination of material that is located on a surface, is most suitable for relatively smooth hard surfaces. A tape sample is taken by cutting a clear single-sided sticky tape into 1- to 2-inch lengths. The tape is handled only by its edges and the adhesive side of the tape is positioned over the sampling location and pressed firmly against the sampling site. The tape is then lifted off and attached to a clean microscope slide. The advantages of tape samples are that they are inexpensive to collect and do not have any delay time in obtaining results, as with culturable samples. They also do not require any expensive specialized equipment.

#### *Swab and Surface Debris Samples*

Swab samples are collected by rubbing a swab over the surface to be sampled. Surface debris samples are collected by sweeping debris from the surface to be sampled. Swab and debris samples should be collected using a template, a sampling device designed so that a known area can be swabbed, and the swabbing or sweeping action should be of a type and pattern that can be repeatedly reproduced. A swab sample collected without properly controlled methods cannot be quantitatively compared and is not of use even for qualitative comparison. The results of swab and debris sampling are not highly reproducible even when performed properly, and the issue of differences in surfaces must be taken into account. Direct microscopic examination is difficult due to destruction of growth structures from the collection method. Culturing these samples is one approach to enhancing the information that may be gleaned from microscopy. See the section above entitled *Surface, Settled Dust, and Debris Samples* under Culturable Sampling Methods for a discussion of the issues involved when culturing these samples.

### *Vacuum Samples*

Vacuum samples are usually collected on an adhesive slide surface or a filter. This type of sampling can be useful for sampling soft surfaces such as fabrics. This type of sampling is qualitative in nature and can be used to determine if spores are present on a material. Vacuum samples should be collected using a template of known area and the vacuuming action should be of a type and pattern that can be repeatedly reproduced. The results from vacuum samples should only be used for qualitative comparison and generalized comparisons to other vacuum samples.

### *Bulk Samples*

Bulk sampling consists of physically detaching a piece of the material. Bulk sampling can be used for microscopic identification of fungal species if growth is present on the materials or if there is surface contamination from settled spores. Bulk samples should not be cultured. There are procedures for performing bulk dust sampling viewing of an array of substances, including allergens such as dust mites, pet dander, and cockroach debris. See the section titled *Surface, Settled Dust, and Debris Samples* under Culturable Sampling Methods for a discussion of the issues involved when culturing these samples.

### ***Sampling for Marker Agents***

Sampling for marker agents is an emerging area. In some cases, the marker agents may even be agents of potential health concern, such as certain MVOCs or (1-3)- $\beta$ -D-Glucan. (1-3)- $\beta$ -D-Glucan has been implicated as a possible allergen, although there is not sufficient evidence to support this hypothesis at the present time.

At today's knowledge level, these types of marker agents can be used only as confirmatory evidence to help support other sampling techniques. A large amount of additional research is needed in the area before it can be concluded that this type of sampling is of value.

### ***Sampling Using Polymerase Chain Reaction (PCR) Technology***

Polymerase Chain Reaction (PCR) technique is a relatively new analytic method that is based on species specific fungal (or other organisms) deoxyribonucleic acid (DNA) identification. This methodology uses technology that enables specific DNA sequences to be identified and amplified. Multiple copies of the nucleic acid sequence are produced, making the organism easier to identify from small samples. If a DNA sequence is known to be specific to the organism, this may then serve as a template for identification. It is beyond the scope of this monograph to detail the PCR methodology. A recent development in PCR technology is the use of messenger ribonucleic acid (mRNA). Messenger RNA is present only in living organisms. This later methodology is not available for a wide variety of organisms.

### ***Sampling for Mycotoxins***

There are no established methods for sampling mycotoxins. Additionally, there are no commercial laboratories performing a validated analysis for mycotoxins.



As a general rule of thumb, there is an inverse relationship between the molecular weight of a molecule and its vapor pressure, such that the higher the molecular weight of a substance, the lower the vapor pressure will be. Most of the mycotoxins that have been identified are of high molecular weights. This means that most of the identified mycotoxins have relatively low vapor pressures. As a result, it is unlikely that a person would receive a significant dose of mycotoxins from inhalation in the indoor environment, since only a relatively small amount of mycotoxins is produced and what is produced has a very low vapor pressure. Mycotoxins may also exist on fungal spores and other non-fungal particulates, but due to insufficient mass of mycotoxin available, it is not clinically relevant. For mycotoxins, physical contact or ingestion must be identified as the routes of exposure, unless there is exposure to extremely high concentrations of aerosolized mycotoxin.

### Data Analysis and Interpretation of Sampling Results

Even if samples are collected and read properly, interpretation of the resulting information can be a daunting task. To properly interpret the data, the interpreter needs to have a thorough understanding of the following items:

- Aerobiology of the agents of concern;
- Fate and transport of small aerosol particulates in the atmosphere;
- Knowledge of the atmospheric dynamics present in the area sampled;
- Knowledge of the construction techniques and materials used in the structure;
- Knowledge of the sampling technologies, including both their strengths and limitations;
- Knowledge of statistics; and
- Understanding scientific methods and use it in selecting sampling sites and interpreting data.

Each method of sampling (*i.e.*, culturable, direct microscopic examination, and marker agents) should be interpreted separately and the results correlated to produce conclusions. This is only possible if the sampling plan has been well designed. Sampling data that are confusing and conflicting is one indicator of a poorly designed or executed sampling plan. Physical inspection may greatly affect the interpretation of the data. A physical examination of the affected structure is critical to the proper design of a sampling protocol and interpretation of the results.

Moisture intrusion is an important factor in the data analysis and result interpretation. This is one of the most important factors that affect indoor fungal growth. No reliable data exist that establishes direct links between relative humidity in occupied areas of the structure and fungal growth. Moisture is the controlling factor, but moisture can result from leaking pipes, ground water, condensation, human activities in the structure (*i.e.*, showering, washing), and many other activities. It is quite common for relative humidity levels to rise to greater than 30% inside of interior wall spaces due to leaking pipes or condensation on high-capacity thermal conductors that pierce walls, while relative humidity in the occupied areas of the structure remains at levels below 15%. Due to these types of situations, it is very difficult to predict fungal growth issues from relative humidity levels in occupied areas of the structure.

## Do Indoor Molds Cause Asthma and Other Respiratory Problems?

Over the past several years, closer attention has been paid to the possibility that indoor air can be harmful to people's health. Reports on "sick-building syndrome" are being increasingly reported in both the scientific literature and the popular press. Government officials are also concerned. Several years ago, the EPA requested that the National Academies of Science (NAS) assess the relationship between asthma and indoor air quality, including exposure to mold (NAS 2000).

Although many medical conditions are commonly reported to be associated with poor indoor air quality (see Table 3), many of these reports remain unsubstantiated by scientific evidence.

**TABLE 3. Commonly Reported Allergic Reactions Thought to be Associated with Indoor Molds**

- 
- Upper and Lower-Respiratory Symptoms and ailments—asthma, bronchitis, coughing, wheezing, rhinitis
  - Skin problems
  - Gastrointestinal problems
- 

Despite the extensive research and reporting on the strong link between molds and ill health, proving that molds *cause* ill health is difficult. Part of the difficulty comes from the fact that other indoor pollutants may also affect health, such as dust mites, domestic pets, cigarette smoke, high humidity levels, and pollens. The difficulty of distinguishing the source of ill health associated with polluted indoor air is compounded by the diffi-

culty of validating the reports of people who report adverse health effects. Furthermore, standardized measurements to evaluate the presence of molds in homes are lacking; and even with a valid measurement, there still are no data on the level of mold needed to cause sickness, partly because this is dependent on the variability of peoples' reactions to molds. The threshold may be different for various types of molds. One of the major topics addressed in the NAS report (2000) was the methodological issues involved in evaluating the evidence.

The Allergy and Immunology section of Part II of this report looks at the scientific evidence behind the reports of allergic reactions to indoor molds, with a particular focus on respiratory-related conditions. It provides a brief description of the different kinds of molds and where they may arise in indoor environments. Part II also discusses key studies, which provide much of the evidence on the association between indoor molds and ill health, and finally, offers suggestions about what still needs to be done to better understand the connection between indoor mold, high humidity, and ill health.

Although everyone is likely to be exposed to a variety of indoor and outdoor molds during their usual daily activities, only about 10% of the population have allergic antibodies to fungal allergens. Only one-half of these people, or about 5% of the general population, are expected to experience clinical illness. Outdoor molds generally are more abundant and important in airway allergic diseases than indoor molds, leaving the latter with a minor but important overall role in allergic airway disease (Hardin 2002). Table 4 presents which indoor molds are frequently associated with adverse health.

Elevated levels of indoor dampness, usually above 50% relative humidity, are strongly implicated as the main reason that molds grow abundantly (Bornehag 2001). Five sources of dampness are:

TABLE 4. Indoor Molds Most Frequently Reported to be Associated with Disease

Type of Mold	Conditions Associated with Inhaling Spores	Conditions Associated with Ingestion
<b>Aspergillus</b>	Asthma, allergic rhinitis, sinusitis, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis	Liver cancer with or without hepatitis B, liver cirrhosis, Reye's syndrome
<b>Cladosporium</b>	Allergic rhinitis, asthma	
<b>Penicillium</b>	Allergic rhinitis, asthma	
<b>Fusarium</b>		Alimentary toxic aleukia
<b>Stachybotrys</b>	Chest/upper respiratory symptoms, pulmonary hemosiderosis and hemorrhage in infants	Fever, dermatitis, leukopenia

Adapted from Robbins 2000, Burr 2001.

- *Outdoor sources*, such as by rain or snow leaking into the building or up from the ground;
- *Indoor sources*, from activities such as bathing, cooking, and breathing;
- *Building sources*, such as moisture within building materials caused by improper protection against rain and snow while in the construction phase;
- *Accidents*, such as pipes breaking; and
- *Condensation*, on cold surfaces.

Distinguishing the source of dampness is not always easy. Emissions in the air from both moisture in building structures and humidity in indoor air may cause irritating symptoms or allergic reactions. Humidity also increases the risk of household dust mites, another common allergen. In fact, developing an allergic reaction (sensitization) to mites is far more common than it is to molds. This has led some people to believe that mites are the main source of ill health associated with indoor air (Bornehag 2001, NAS 2000). It is not always easy, therefore, to know if an allergic reaction to indoor air is caused by molds or other indoor allergens such as mites, since both may be associated with increased indoor dampness and often coexist.

### Ways that Indoor Molds Reportedly Cause Respiratory Problems

There are four basic ways that molds can affect the lungs and cause respiratory problems:

- I. Immunologic: Reaction that activates a response by the immune system (three types):
  - A. Hypersensitivity pneumonitis (also known as extrinsic allergic alveolitis):  
Pneumonia caused by an allergic reaction of the immune system to inhalation

of fungal or bacterial organisms, generally limited to occupational settings. Other causes include animal proteins (*e.g.*, bird serum or droppings), vegetable matter (*e.g.*, red cedar wood dust), or a variety of chemicals. Hypersensitivity pneumonitis is not induced by normal or even modestly elevated levels of mold spores, but is caused by inhalation of exposure to very large quantities of fungal or other proteins.

- B. Allergic bronchopulmonary aspergillosis: Lung injury, which is often permanent, resulting from an exaggerated immune response to fungi found in the lower airways. The fungi actually grow within the patient's airway, and the condition generally occurs in individuals who have airway damage from previous illnesses leading to bronchial irregularities that impair normal bronchial drainage. This condition is always associated with a history of asthma and occurs more frequently in the United Kingdom than in the United States.
  1. Allergic fungal sinusitis: More recently, it has been recognized that a similar process may affect the sinuses. This condition also occurs in people with underlying allergic disease and, because of poor drainage, a fungus colonizes in the sinus cavity.
  2. There is no evidence to link specific exposures to fungi in home, school, or office settings to the establishment of fungal colonization that leads to allergic bronchopulmonary aspergillosis, or allergic fungal sinusitis.
- C. Allergic reactions: Asthma and allergic rhinitis. Molds are known to be a significant cause of allergic reactions. Individuals who have inherited the genetic background necessary to respond to low-dose antigens characteristically make immunoglobulin E (IgE) antibody responses to pollens, fungi, and the wide range of foreign proteins found inside houses. This process is called sensitization, which is the induction of an acquired sensitivity or allergy in genetically susceptible persons. Once sensitization has occurred, these sensitized individuals can manifest allergic disease (*e.g.*, allergic rhinitis, asthma, eczema).
- II. Mycotoxicosis: Toxic symptoms caused by massive exposure in agricultural settings have been studied (Emanuel 1986). The potential role of toxin exposure causing ailments in indoor environments will be discussed in other chapters. (See the section on Medical Toxicology in Part II.)
- III. Infections: The development of opportunistic diseases generally is limited to susceptible populations, such as people with AIDS or other compromised immune systems (*i.e.*, patients with acute leukemia, other cancers, or uncontrolled diabetes). In addition, patients receiving intense chemotherapy, bone marrow transplantation, or organ transplantation with potent immunosuppressive drugs are considered susceptible to fungal infections. The role of fungal associated infectious diseases in indoor environments will be discussed in other chapters. (See the section on Medical Toxicology in Part II.)
- IV. Irritant effect: Development of symptoms, such as headache, fatigue, or eye, nose, and throat irritation may be associated with exposure to a variety of mold by-products known as microbial volatile organic compounds (*i.e.*, alcohols, aldehydes, and ketones that can be detected as "moldy odors").

Other sections in this *Review* will address mycotoxicosis and infections. The section reviewing the immunology literature will briefly describe two studies that have attempted to link hypersensitivity pneumonitis, which reflects an immune reaction, to home environments, but will focus primarily on reported effects of indoor molds on asthma and other respiratory illnesses.

Fungi produce many substances that are beneficial to their existence. One group of these substances is termed the mycotoxins (*myco*—fungus and *toxin*—noxious or poisonous). Over 400 different mycotoxins have been identified thus far, although not all have harmful effects. In fact, some mold products are responsible for beneficial contributions to health such as the development of antibiotics. The use of penicillin, an antibiotic produced by a fungus from the *Penicillium* family, has saved millions of lives since it was introduced in the 1940s. Transplant medicine would not have reached its present achievements without the discovery of cyclosporine (*Tolypocladium inflatum* Gams), a fungus-derived medicine designed to combat the body's rejection of transplanted organs.

## Methods of Medical Toxicology

Certain basic scientific principles assist physicians (and others), in evaluating whether or not a given exposure may cause a health risk. Establishing causality, or a cause-effect relationship, between a substance and any illness in humans (*e.g.*, between tobacco smoke and lung cancer) requires a rigorous and formalized process known as causation analysis. Causation analysis requires that scientific weight of certain criteria be met in order to prove sufficiently that a cause-effect relationship exists. Even when there is some evidence of a scientifically established relationship in the general population, this same causation analysis needs to be applied to determine whether the effect in any given *individual* is the result of an exposure to that substance.

First, both an *exposure* and a *dose* need to be determined and quantified, if possible. In order for there to be any human reaction to a substance, there must be an *exposure*—or actual physical contact (*i.e.*, on the skin, by inhalation, by ingestion, etc.)—between the individual and the substance. The frequency, duration, intensity, and route of exposure determines the dose, amount, to which the individual is exposed. It is the industrial hygienist (IH) or environmental scientist who generally assesses the sources and the exposure or potential for exposure. For all substances—be it chemicals, drugs, toxins, medications, etc.—there is a *dose-response* relationship. This means that as the *dose* of the substance (the amount of substance that a person is exposed to) increases, so too does the likelihood that the exposure will cause a *response* (or clinical effects) in a given individual. The dose also generally affects the type and severity of any resulting effects. In addition, for almost all substances there is a *threshold dose*—a dose below which no one will become ill. Only doses above the threshold will therefore result in any human effects. In the case of the allergic individual, specifically sensitized to a certain allergen(s), the threshold provocative dose is lower than for non-sensitized persons.

If both an exposure and a sufficient dose have been established, then a measurable health effect must be documented that is consistent in severity, quality, and time *course*, with the effects that have been previously established by the scientific evidence. Other causes for the medical condition or illness must be ruled out, as best as is possible with a differential diagnosis. This last part, the medical evaluation, is generally done by the physician, using the traditional medical approach of history-taking, physical examination, and a variety of supporting laboratory tests, radiographic readings, or other studies. Subjective data (obtained from the patient) should always be correlated with objective data (obtained by the physician through various tests or examinations).

Sometimes, when there is not a well-established relationship between the substance and a disease, establishing individual causality can be much more difficult. In such circumstances, physicians and scientists often depend on other data such as animal studies or even biological or chemical plausibility to make an assessment.

Published data on a relationship between an agent and human disease may take several forms. *Epidemiologic studies* compare large populations of people (*e.g.*, exposed vs. not exposed, or those with disease vs. those without disease) to find factors that may be associated with disease. Epidemiologic studies have a number of limitations, the most significant of which may be that they can only show an *association* between an agent and a disease—they cannot prove *causation* (*i.e.*, that the agent causes disease). *Investigative trials* attempt to show that an agent causes a disease by administering it to subjects—limitations include the obvious fact such trials cannot ethically be performed on humans, and thus animal studies often take their place in this setting. *Literature reviews* pool the data from a number of separate but related studies to draw stronger conclusions. The limitations of literature reviews are that their conclusions depend heavily on the methodology of the review. *Case reports* and *case series* are detailed reports of individuals or groups of individuals and their disease. Unfortunately, these reports are probably the least helpful in determining the relationship between an agent and a disease, and they generally cannot prove causation.

## Elements of Medical Toxicological Assessment

### ***Dose-Response Relationship***

The concept that the dose makes the poison is the most basic rule in medical toxicology. This is also referred to as the biological gradient, or the dose-response relationship. This means that as the dose increases, the severity of an illness increases in an individual, as does the number of individuals who may become symptomatic. Within the dose-response relationship, however, there exists a threshold below which no effects occur. The threshold is the dose below which the probability of an individual(s) responding should be zero.

### ***Requirement For Physical Contact with Substance***

In order for either local or systemic toxicity to occur, there must be physical contact between the chemical and the biological system. If an exposure has not occurred, then there is no opportunity for physical contact. If there is no contact, there is no dose and no resultant associated medical illness. In the instance of indoor mold, inhalation is the potential pathway of exposure.

### ***Target Organ Specificity***

Chemical substances may cause local or systemic effects if of sufficient dose. Local effects occur at the first point of contact between the chemical and the biological system, such as the skin. The substance, its chemistry and pathway of exposure determine the degree of local injury. Systemic effects may occur only after sufficient dose has been

achieved. In systemic toxicity, not all organs are affected to a similar degree. Rather, one or two organ systems are the target of the substance. These are the “target organs” and specific substances are therefore said to have “target organ specificity.”

## Cause-and-Effect Relationship

Cause-and-effect analysis is a formalized process based on the principles and methods in toxicology, which have been refined with the progression of scientific knowledge. A physician asked to assess a cause-and-effect relationship between an exposure and a clinical outcome must follow a general methodology that is scientifically valid and defensible. This methodology must then be applied to the facts in each specific case. Causation analysis is not a matter of personal opinion or estimation in the absence of a defined methodology. The physician needs to consider such factors as the individual's opportunity for exposure, the amount and duration of such exposure, the dose received, the precise nature of the clinical condition being considered and its differential diagnosis, and the biologic plausibility and consistency of the evidence.

There is an accepted scientific methodology for establishing a cause-and-effect relationship between potential exposure to a substance in the environment and the development of a medical illness or the establishment of a risk for development of injury in the future. This methodology requires an assessment of the exposure pathway (see the Industrial Hygiene section), review of all relevant medical circumstances, and knowledge of the host's susceptibility.

Even when it is known that a chemical is capable of causing a particular health condition, under some circumstances, it is the dose (amount at the target tissue) of the chemical received by an individual that will largely determine whether the chemical is likely to have caused or have contributed to the health condition. For example, many compounds that may be toxic when received at large doses produce no toxic effects, or may actually be beneficial, when received in smaller doses, such as aspirin and even water.

The dose absorbed by an individual depends on numerous factors including exposure (*i.e.*, the concentrations of the chemicals, frequency and length of time of contact, and the pathway of the substance), the physical chemistry of the chemical, and numerous other susceptibility factors relevant to the individual. Thus, to reach an acceptable scientific conclusion, one must unavoidably involve an analysis of the pertinent toxicologic data (*i.e.*, the exposure, dose if any, and medical condition or estimated risk of developing a medical condition). Additionally, it is crucial to utilize the accepted criteria for establishing causation of the medical condition (or risk of its development in the future). These criteria include the nature of the effect, its dose-response relationship, time-course relationships, alternative causes evaluated by performing a differential diagnosis, and the coherence of the evidence including its consistency and biologic plausibility.

A medical complaint alone cannot be used to determine dose, exposure, or a source. If an individual has had the opportunity to contact a substance (exposure), has received a dose of a sufficient quantity, and a recognized medical condition develops, then a differential diagnosis must be performed to consider all possible etiologies known to present with the objective findings. A differential diagnosis is the process of



collecting subjective and objective data from a patient, then determining the most likely diagnosis of the patient. Causation analysis must be used to determine if the illness is the direct result of the substance. Typically, the causation criteria put forth by Sir Austin Bradford Hill are used to determine causation (Hill 1965). The elements of this analysis of causation have been promulgated in the "Hill Criteria."

These "criteria" are a set of widely accepted points articulated by Sir Hill in his Presidential Address to the Royal Society of Medicine, in London, England, on January 14, 1965. This widely accepted model involves the consideration of nine points that should be examined in the analysis of any possible association between the "causative" substance and a disease entity. It is not necessary for all nine criteria to be met, rather, the conclusion should be based on the weight of the scientific evidence. These criteria comprise a series of steps in causation analysis, which may be applied to a theorized association between a substance and disease in order to determine whether a causal relationship exists. The Hill Criteria are:

*Strength of Association:* Pivotal to the establishment of a causal relationship is the observation of increased incidence of the disease in the exposed population that is greater than in the unexposed population. In order for an association between a substance and a disease to be considered more common in the exposed group, the possibility of a chance difference based simply on the statistical possibility of a randomly occurring false association must be evaluated. To determine if an epidemiological association exists, formal studies must be considered on similarly exposed individuals without illness. The formal evaluation of this requires a statistical test. The generally accepted statistical test used for this purpose is the evaluation of the confidence interval. This is typically referred to as the "95% confidence interval." The strength of these associations is expressed in epidemiology studies as a point estimate, which may be either an odds ratio or the relative risk, depending on the design of the study. A value of 1 for the point estimate indicates that there are no numerical differences between the exposed and unexposed populations, with regard to the disease in question. If the point estimate is greater than 1, it suggests that there is a trend towards a relationship; however, such a relationship does not by itself imply specific causation. There may be variables that are confusing (confounding) the result in such an observed relationship, such as other exposures, illness, or ascertainment bias. Therefore, additional Hill criteria must be considered before a causal relationship can be concluded.

*Consistency:* A single study is considered to be much weaker evidence of causation than is a group of studies done with differing methodologies that arrive at similar conclusions. As will be discussed in Part II, the weight of the scientific evidence does not support a consistent association between indoor mold and adverse toxicologic health effects.

*Specificity:* Specificity in medical toxicology refers to the general principle that specific diseases are caused by specific exposures. Individual substances are known to cause either one specific disease, or a small group of diseases. Thus, the existing scientific data should consistently show the specific association between the exposure, dose, and the disease in question. For example, if a study showed an association between an exposure and one disease, and another study showed an association between that same substance and a different disease, these studies would not be accepted as representing evidence of disease causation, based on lack of specificity.

*Temporality:* This is an *a priori* criterion. It is necessary that the disease occur after the exposure with an appropriate latency in order for an association between the disease and exposure to be considered causally related.

*Biological Gradient:* This is a fundamental principle of toxicology, which encompasses the concept of the greater the dose of a substance the more likely the response in an individual or in an exposed population. It is also referred to as the dose-response relationship. For example, the more alcohol one consumes, the greater the likelihood of a resulting inebriation.

*Plausibility:* This criterion requires that the association between the exposure and disease be considered plausible. The plausibility criterion is limited only by the almost infinite scientific possibilities. Plausibility does not require scientific proof and is thus considered to be the most minor of the Hill criteria. While it may be a hypothetical possibility that there is a relationship between adverse health effects and indoor mold, it would be biologically plausible only if found under conditions of appropriate dose-response as reflected in the scientific literature.

*Coherence:* There often is a body of information regarding the potential relationship between a disease and an exposure to a substance. This body of knowledge frequently comes from multiple types of experimental and observational data. It is expected that the various types of scientific data should be coherent and consistent with each other for the association to be considered causally related.

*Experimentation:* As described below, there are a variety of scientific approaches to assist in answering a question. For example, controlled studies can be observational or experimental. An observational study is one in which no intentional intervention, such as a substance exposure, has been imposed on a population; rather, an observation has been made. Alternatively, deliberate interventions can be done by exposing a given population, be it humans or animals, to the substance in question and evaluating them for a given effect. This approach is referred to as an experimental study. A causal relationship is suggested by coherency between experimental and observational studies.

*Analogy:* This criterion refers to the increased likelihood of a causal association between an exposure and a disease process if other similar associations are already documented to exist.

## Application of Causation Criteria to Clinical Diagnosis

If an individual has been exposed, has received a quantifiable dose, and the dose is of a sufficient magnitude to cause an injury, then there must be a compatible medical condition. Evaluation of the medical condition is achieved through the traditional approach including a history, a physical examination, and the application of medical laboratory tests. From these, a list of possible diagnoses (differential diagnosis) is assembled. There are entire texts devoted to the differential diagnosis of signs and symptoms. Then the process of differential diagnosis possibilities are included or excluded until the best diagnosis of the person's condition is established. This entity then becomes the subject for a formal toxicologic causation analysis.

Complaints (symptoms) are subjective, and must be supported with objective (physical signs, testing) data. A single medical condition may be caused by more than one substance. Similarly, a chemical may affect several different tissues depending on

the dose and duration at which it was received. Chemicals generally can produce a range of health effects that overlap with, or may be identical to, effects caused by infectious agents, genetic deficiencies, or other factors based on dose-response. Therefore, all causation criteria should be considered in the context of a differential diagnosis, not simply the specific disease or range of conditions found in an individual or group of individuals, before arriving at a final opinion.

A common imprecision in causation investigation is using *post hoc, ergo propter hoc* (after the fact, therefore, because of the fact) reasoning. That is, the suspected symptoms or ailments are used to explain that sufficient exposure has occurred. Then, it may be argued that exposure has now been shown to be sufficient, and this "proof of exposure" becomes a basis for explaining the cause of the symptoms and ailments. In short, the symptoms become the basis for explaining themselves. Circular reasoning is not a medically valid process. Subjective symptoms thus cannot serve as the basis on which to conclude that an individual has in fact been exposed to or received a significant dose of chemicals. Symptoms must be confirmed by objective testing.

## Mycotoxins

Mycotoxins are naturally occurring substances produced by fungi that are not required for growth, but contribute to the mold's survival by interfering with the function of other organisms (Kendrick 1992). When produced, mycotoxins may be found in all parts of the colony, including the hyphae, spores, and mycelium. They may also be found on the media that is supporting the growth of the mold. Over 400 different mycotoxins are known today. Several different types of mycotoxins are shown in Table 5.

The chemistry of mycotoxins suggests that they do not evaporate or "off-gas" into the surrounding environment independent of a particle carrier. Similarly, they do not pass through construction materials. Therefore, in order for an inhalation pathway of exposure to exist, the mycotoxins must become aerosolized as part of spores, mold particles, or colony fragments. There are certain volatile organic compounds released from microbes called MVOCs; when released, they may be the cause of the musty, disagreeable odor associated with molds and "mildew" (Kaminski 1974, Pohland 1993).

Depending on environmental conditions (such as temperature and humidity), the same fungus may produce one or several mycotoxins. Interestingly, different isolates of the same species may or may not produce mycotoxins. This is dependent on several conditions including temperature, humidity, media, available nutrition, and other microbes. The precise interaction of elements required for mycotoxin production is poorly understood. The presence of a fungal species that is capable of producing mycotoxins does not necessarily mean that toxins will be produced (Tobin 1987, Smith 1992, Rao 1996, Tuomi 2000). Therefore, the presence of fungi *per se* does not necessarily imply the presence of mycotoxins in the environment.

Exposure to mycotoxins can occur by ingestion or inhalation (Emanuel 1975). Although, mold fragments will not pass through the skin, large concentrations of mold may cause skin irritation (Drobotko 1945). Fungal toxicity has been reported in animals exposed to mycotoxins (Creasia 1987) or spores (Nikulin 1996, 1997) via inhala-

TABLE 5. Examples of Trichothecene-producing Fungi

Group	Fungi	Major Mycotoxins
A	<i>Fusarium sporotrichioides</i>	T-2 toxin, HT-2 toxin, neosolaniol
A	<i>F. semitectum</i>	Diacetoxyscirpenol, neosolaniol
A	<i>F. equiseti</i>	Diacetoxyscirpenol
A	<i>F. heterosporum</i>	3'-hydroxy T-2 triol, 3'-hydroxy, HT-2 toxin
B	<i>F. graminearum</i>	Nivalenol, deoxynivalenol, fusarenon-X
B	<i>F. culmorum</i>	3-Acetyldeoxynivalenol
C	<i>Cephalosporium crocinigerum</i>	Crotocin
C	<i>Trichothecium roseum</i>	Crotocin
D	<i>Myrothecium roridum</i>	Roridin A, D, E
D	<i>Myrothecium verrucaria</i>	Verrucarins A, B
D	<i>Stachybotrys chartarum</i>	Satratoxins F, G, H
D	<i>Cylindocarpon</i>	Roridin H, 3'-diepoxyroridin
D	<i>Verticillium dactyloides</i>	Vertisproins

tion, ingestion, and injection directly to the peritoneal cavity, in a dose dependent manner.

Mycotoxins may cause certain types of injury when given at sufficient doses under certain circumstances. Most of the reports state that human and animal illness related to mold is caused by ingestion of moldy foods (Drobotko 1945, Pohland 1993, Forgacs 1962, Ciegler 1980).

# 2

## Literature Review

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## Introduction

Because fungi are truly ubiquitous, meaning they are everywhere, we cannot escape exposure to fungal contaminants, whether to their spores, fungal elements, mycotoxins, volatile organic compounds, or other components. For most of us that is not a problem as we have co-existed with fungi from the very origin of mankind; however, fungi can cause health problems for some people. The following discussion will try to put this risk into perspective, based on the most current scientific information on human exposure to environmental molds.

## Background Research

### *Prevalence of Fungal Bioaerosols in the Outdoor Air*

The growth of fungi is pervasive throughout the outdoor environment. Fungal spores, hyphae, and mycotoxins are generated and released directly into the environment by mushrooms and molds growing on and in dead and living plant materials, soil, and water. If undisturbed, the natural flora of an environment controls the types and abundance of living sources of bioaerosols; however, the quantity of these fungal aerosol particles can be intensified by human activity. Many agricultural processes produce concentrated sources of fungi and bacteria, and field crops provide enormous acreage of dead plant material, which becomes colonized with fungi. Similarly, composting concentrates masses of nonliving organic material that provides a substrate for fungi and is therefore a massive source of fungal spores (Burge 1995).

The concentration and mixture of fungal species in the outdoor air varies significantly based on geographic and seasonal changes. As previously discussed, many factors contribute to the variety and concentration of spores, including the availability of substrate, human activities such as mowing grass and harvesting grain, and climatic factors, particularly temperature and rainfall, which have a direct effect on the release of spores into the air. Rain and warmth also promote the development of vegetation on which parasitic and saprobic fungi subsequently develop. Broad correlation between the composition of airborne spores and climatic factors enable predictions to be made that certain spore types will be more abundant in warmer, drier summers whereas others will be more abundant during damp weather (Mullins 2001).

Total outdoor fungal spore concentrations as high as 85,000 spores per cubic meter of air have been reported (Mullins 2001). In a study of outdoor concentrations in the Mid-Atlantic region of the United States, McGuinness, *et.al.*, reported an average, annual count of >7,700 fungal structures per cubic meter (McGuinness 2002). The highest airborne concentrations of fungal spores occurred in the fall and summer and the lowest occurred in the spring and winter. Similar results were obtained in a broader investigation that examined the airborne spore concentrations in six different geographical regions of the United States and across the four seasons of the year (Shelton 2002). These investigators reported a mean outdoor concentration of 930 viable spores per cubic meter (Shelton 2002). They found the highest outdoor spore concentrations in the Southwest, Far West, and Southeast and the lowest concentrations in the Northwest. They concurred that the spore concentrations were highest in the fall and summer and lowest in the spring and winter. The seasonal variability

is logically linked to weather conditions because the summer and fall provide higher temperatures and humidities that trigger increased microbiological activity during these periods. An additional factor that contributes to the lower spore concentrations in the winter is snow-cover, which can prevent fungal spores from being aerosolized by the wind.

*Cladosporium* is typically the most common fungal species in the outdoor air, followed by *Penicillium*, *Aspergillus*, and the *Basidiomycetes* (Shelton 2002, McGuinness 2002). This general prevalence is the same in each season and in each region of the United States (Shelton 2002). In addition, the presence of *Stachybotrys chartarum* was found to be “not highly unusual and this species is present in the outdoor air in similar frequencies across the United States” (Shelton 2002).

### ***Prevalence of Fungal Bioaerosols in the Indoor Air***

Although buildings have become increasingly tight—sealed against penetration of outdoor air—particles present in the outdoor air invariably enter into the indoor environment. These outdoor aerosols contribute both to the ambient aerosols indoors and to reservoirs in which amplification can occur (Burge 1995). The most common indoor molds are *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria* (AAP 1998, Shelton 2002).

Shelton documented the influence of the outdoor air on the indoor air concentration of fungal spores with the finding that the ratio of fungi in the indoor air to the concentration of fungi in the outdoor air was fairly constant in all seasons and locations evaluated. This ratio was essentially 1:1 or lower in 85% of the buildings evaluated (Shelton 2002). Similar results have been documented in numerous studies throughout the world.

Molds readily enter the indoor environment by circulating through doorways, windows, penetrations in the building envelope, and the heating, ventilation, and air-conditioning (HVAC) systems. In addition, spores in the outdoor air can be deposited on people and animals, which then bring these spores into the home environment.

Many factors affect the bioaerosol concentration in homes, but one condition that is totally controlled by the occupant is the use of “natural ventilation” versus air-conditioning. Air-conditioning, a relatively new technology for controlling comfort, is still being investigated for its impact on indoor air quality. One early study of the differences in microbial contamination in naturally ventilated and air-conditioned homes found that there was no significant difference between the bioaerosol make-up and concentration in the outdoor air and the air inside the naturally ventilated residences (Kodoma 1986). Intuitively, this finding makes sense because the indoor environment is simply an “extension” of the outdoor environment in a naturally ventilated building. Kodoma also found that the air within the air-conditioned homes had fewer fungal spores, including significantly fewer *Cladosporium* sp. spores, but there was a significantly greater number of *Aspergillus* sp. spores. These findings suggested that air-conditioning might have both a positive and negative effect on indoor exposures by reducing exposure to certain fungal types while, perhaps, contributing to increased exposure to other fungal species.

## Mold Growth Within the Home

Ideally, the only sources of indoor aerosols should be the outdoor air, the people and pets, and the inevitable dust creating conditions that allow for minimal amplification (Burge 1995). Given the proper conditions, however, molds may also proliferate in the indoor setting. Because Americans spend 75% to 90% of their time indoors, they are exposed to molds that grow indoors (AAP 1998).

Moisture (*i.e.*, water activity) is the limiting factor for growth, and mold growth in buildings is increasingly linked to water problems within the structure, or its "building envelope." Mold growth can occur in any environment that contains excessive moisture, such as from leaks in roofs, walls, plant pots, or pet urine (AAP 1998). Other important sources of moisture in buildings are surface water run-off, windows, and condensation on poorly insulated or cold surfaces.

The potential health consequence of mold growth in buildings has led to numerous guidelines regarding mitigation and remediation, and has spurred a new interest in managing our buildings to prevent mold problems. The EPA provides guidance in its "Mold Remediation in Schools and Commercial Buildings."

## Conclusions

1. The fungi are a complex and diverse group of organisms. They are everywhere in the natural environment, and it is essentially impossible to avoid exposure to fungal elements and their by-products. While there are generally no untoward consequences related to exposures to fungi, some fungi are toxigenic, allergenic, and/or pathogenic and cause diseases in certain individuals. Fungi are a natural contaminant within all buildings as a consequence of infiltration from the outdoors.
2. Under certain circumstances, primarily water problems, fungal contaminants can begin growing within the building, which can have a negative impact on the indoor air quality and potentially affect the occupants of that building. Mold growth within the home can create an "unnatural" environment that may no longer be a reflection of the outdoor environment, and thus, present a unique exposure potential for the building occupants. Nonetheless, this unique exposure does not necessarily translate into negative health effects. The current science does not support a cause-and-effect formula for health risks as a result of exposure to indoor fungal contaminants.



In the case of indoor investigations of the presence of fungi, much of the literature focuses on culturable sampling techniques. Sampling for fungi in the indoor environment is an emerging science. This section, which will critique the literature on sampling, will present both the scientifically valid and controversial issues.

### Viable and Non-Viable Air Sampling

The primary strength of culturable sampling is the ability to speciate the fungi (Dillon 1999). However, culturable sampling identifies only the spores of fungi that can survive the capture process and therefore tends to underestimate total counts, while overestimating tolerant species that reproduce on the capture medium (Pasanen 2001, Rao 1996, Dillon 1999, Jantunen 1997). That culturable sampling does not indicate the presence of non-viable spores, and limits its ability to represent accurately fungi that are capable of producing allergies or irritation. Another weakness is that it requires extended periods of time for incubation after the sampling has taken place.

The selection of growth media can be very involved and difficult, depending upon the potential fungi to be investigated. Malt Extract Agar is generally recognized as a good wide-spectrum growth medium that gives reasonably uniform results (Dillon 1999, Pasanen 2001, Burge 1995, Mishra 1992). It is generally accepted that shorter sampling times (*i.e.*, less than 3 minutes) result in greater variability in samples collected (Pasanen 2001). This is true partially because sampling times must be controlled to a point at which the medium is not overloaded. Long sampling times can produce unreadable or skewed results due to excessive amounts of collected material being present on the sampling medium (Chao 2002). Loading the medium can produce skewed results because the medium may be a better environment for one particular species as compared to another, so the preferred species could prevent the other species from growing. (Dillon 1999).

While many authors have commented that some fungal species have greater survivability rates during the capture process than others, there are no reliable data on the percentage of each species or genera of spores that survive capture. Claims that there are varying capture efficiencies for the different sampling devices that are commercially available are not substantiated by scientific evidence. Most studies use only one type of capture device in an attempt to limit this uncontrolled factor in their investigations, making it virtually impossible to compare data from different studies. To date, the lack of standardization of sampling methods and the resulting inability to compare results from different studies is probably the single largest factor limiting our ability to establish standards for airborne fungal levels. Reliable data cannot be accumulated to begin to test hypotheses about the relative efficiencies of different sampling devices until standardization of sampling methods takes place. Even then, standard procedures for measuring airborne fungal components may have no bearing on whether it is possible to assess acceptable spore levels that are relevant to health issues.

Spore trap sampling suffers from many of the same difficulties as culturable sampling in regard to survivability of the spore structure during the capture process and capture efficiency in regard to the sizes of different spores (Dillon 1999, Pasanen 2001, Burge 1995, Mishra 1992). Spore traps also do not yield the detailed information about speciation that culturable sampling can yield. At this time, research into total

spore counts (non-viable sample) in regard to indoor fungal issues is not well developed, and the method is not considered reliable.

### **Surface and Bulk Sampling**

Surface sampling of the indoor environment has received the least attention in regard to the research reviewed. This is probably due to the fact that there is no reliable evidence that relates the results of surface sampling to exposure/dose levels for the occupants of the structure.

Surface and bulk sampling can increase the likelihood that sources of fungal growth will be identified (Dillon 1999), although dilution plate culturing of any of these samples may lead to skewing of the sample population. It is an established fact that dilution plating does not give reliable data about the species that are active in the ecosystem (Dillon 1999). The usefulness of direct measurement and plating of bulk samples (soil crumb method) is limited to measuring fungal activities in samples (Dillon 1999).

### **Microbial Volatile Organic Compounds**

The study of Microbial Volatile Organic Compounds (MVOCs) is an emerging area, and research efforts claim conflicting results (Fischer 2000, Fidler 2001, Elke 1999). The main emphasis of sampling for MVOCs is the supposition that some fungi have been identified to produce a limited number of MVOCs (Elke 1999). Some researchers feel that a great advantage is gained by using long sampling times that are difficult to achieve with active sampling but are very easy to achieve with passive sampling (Elke 1999).

The theory has been expounded that detection of MVOCs is a more reliable method of detection than estimating spore counts, because MVOCs are produced throughout the growth cycle of fungi whereas sporulation is only intermittent. While this theory may have some merit, there are assumptions made that are unsupported and in some cases directly contradicted by other more extensive and reliable research (Fidler 2001). For example, research has shown that not a single compound is common to all fungal species, even though MVOCs are often described as indicator substances for mold growth. So far, 1-octen-3-ol, 3-octanone, 2-methyl-1-butanol, and 3-methyl-1-butanol have been detected in the large majority of fungal growth situations (Fidler 2001).

### **Fungal Cell Wall Compounds and Biological Particles**

Sampling for detection of growth cycle components of fungi takes many forms. For example, (1-3)- $\beta$ -D-Glucan, a polyglucose compound present in the cell walls of fungal cells, is one of the agents that has been identified as a possible marker agent (Rylander 1999). With emerging research, some of these agents could become definite indicators of value in locating fungal growths and measuring exposure concentrations, although additional research is needed before they can be confirmed as reliable markers and the methods for their effective use are validated.

To better understand the reported allergic reactions to indoor molds, a critical review of the scientific literature is necessary. This section focuses on selected key studies that highlight this review. First presented are two separate case reports that describe hypersensitivity pneumonitis. These reports are based on anecdotal evidence; that is, they describe one particular incidence of an event—in this case, the incidence of hypersensitivity pneumonitis in two different people—without necessarily drawing causal conclusions relating the incidence of illness (*e.g.*, hypersensitivity pneumonitis) to a given exposure (*e.g.*, indoor mold).

For a critique of the literature on the association between molds and asthma and other respiratory illnesses, two main types of studies were examined: epidemiological studies and literature reviews. Epidemiologic studies offer more information than case studies about a possible association between two events, because they are based on data from a population of people and include a comparison (*i.e.*, control) group. Although these studies give some understanding of the prevalence of reports on mold-related illness, they also contain several shortcomings that must be kept in mind:

- Lack of medical records on affected persons;
- Lack of either skin or blood tests to document allergic sensitivity to molds;
- Reliance on reporting from child or parent (self-reporting) of health effects that may lead to inflating the degree of symptoms;
- Reliance on patient detection of the presence of molds that may or may not be accurate;
- Lack of a standardized questionnaire used in various studies;
- Lack of a standardized method to determine if mold is present in a home, either by a trained inspector or through consistent mold sampling; and
- Inability to differentiate the source of allergic reaction, whether the source was mold, chemicals, dust mites, tobacco smoke, or other allergens.

The review of the literature by the NAS (2000) also noted these shortcomings in the available literature, citing both the limited quality and quantity of the research data of mold exposure and asthma.

### Case Studies: Hypersensitivity Pneumonitis

Although hypersensitivity pneumonitis (HP) is primarily an illness that occurs in occupational and agricultural settings, two case studies have also documented this illness in home settings. The first documented case of HP occurring in a home setting was reported in a 17-year old male who developed cough, fever, and dyspnea after working in his basement. Clinical tests confirmed exposure to the fungus *Fusarium napiforme* (Lee 2000). In another study of a 50-year old woman who developed progressive cough and shortness of breath (dyspnea), clinical tests confirmed exposure to the fungi *Aureobasidium pullulans* and *Saccharopolyspora rectivirgula*, which were found in her basement (Apostolakos 2001). It was not clear, however, if the source of her HP was exposure to fungi or exposure to an insecticide containing the chemicals pyrethrins and permethrins. More than 30 aerosol cans containing this insecticide had been sprayed in her home several weeks prior to the onset of her symptoms.

## Epidemiological Studies

Studies that look at populations of people (epidemiologic studies) can provide strong data on determining the prevalence of a condition or disease among people regionally, nationally, or worldwide. These studies do not necessarily determine if a condition is caused by something (*i.e.*, is asthma caused by indoor mold exposure?), but they can provide informative data on populations of people who report health effects and specific exposures to indoor molds. There are several studies that propose a link between molds and respiratory problems in both children and adults (Zock 2002, Huang 1997, Haverinen 1999, Garret 1998, Dales 1991, Walinder 2001, Koskinen 1999).

The strength of the results of an epidemiology study depends, in part, on whether a proper control group is used to compare the relationship between exposure and disease in different populations of people (*i.e.*, exposed and unexposed). See the Medical Toxicology section in Part II of this report for a more detailed discussion of the strengths and weaknesses of epidemiologic study designs in determining a relationship between indoor mold and human illness.

### Children

Most of the studies that focused on children looked at allergic reactions in children to indoor molds in their homes (Garret 1998, Huang 1997, Dales 1991). One case series study conducted in Florida of 44 children found a strong association between indoor molds and a severe form of allergic rhinitis in 25 of the children who had persistent cold-like symptoms during the previous two winters (Huang 1997). A larger study that looked at the effect of home dampness and molds on young children in 30 communities in Canada found that children who lived in damp homes reported 50% more lower respiratory symptoms, including coughing, wheezing, asthma, bronchitis, and chest illness, and 20-25% more upper respiratory and nonrespiratory symptoms (Dales 1991). This study also found that the likelihood of reporting an adverse health effect was associated with the presence of two or more identified mold sites in the home.

Another study reported only vague or unclear findings of children having allergic reactions to indoor molds (Garret 1998). This study of 80 households, 92% of which were found to contain dampness (as measured by air sampling of mold and condensation), used quantitative measures (skin prick test) to determine the health effects of dampness in 148 children, 36% of whom were diagnosed with asthma prior to the study (Garret 1998). For both the asthmatic and nonasthmatic patients, the most common positive reaction, based on the skin prick test (which tests for an allergic reaction), was to house dust mites. The asthmatic children also tended to have a positive reaction to at least one fungal extract (most commonly *Cladosporium* sp.), and had more respiratory symptoms including cough, wheezing, chest tightness, and cough than the nonasthmatic patients. The authors concluded, however, that there were no significant health effects associated with average concentrations of viable or total fungal spores.

One study looked at health effects in children associated with indoor molds in school buildings (Haverinen 1999). Quantitative measures of the presence of molds were obtained in an elementary school, a school for upper secondary students, and a high school building. All health effects were self-reported. The study found moisture damage was noted in the buildings used by both upper secondary students and high

school students, but not in the elementary school building. Reported adverse health effects were significantly increased in the upper secondary and high school students, who more commonly reported nasal congestion, rhinitis, phlegm, hoarseness, and fatigue than did elementary school children. The prevalence of asthma symptoms in the upper secondary students in this study was twice as high as the prevalence that was reported in a previous study of the general population. Without a matched control group to compare the rate of these symptoms in unexposed children of the same age groups, it is difficult to conclude that mold exposure alone caused the health complaints reported in this study.

### **Adults**

Several studies also indicate that an association exists between mold exposure and ill health in adults. A recent population-based study published in 2002 found a strong association between mold exposure in homes and asthma and increased bronchial symptoms in adults (Zock 2002). Conducted by the European Community of Respiratory Health Survey, the review included data obtained on adults from 38 study centers in Europe, Australia, India, New Zealand, and the United States. All 38 centers, regardless of country, reported similar findings. The findings were supportive of indoor mold growth and adult asthma. The reported mold growth was most significant in older houses with recent water damage.

Another study that included 700 adults (*i.e.*, people over age 16) looked at the association between respiratory infections and moisture or molds (Koskinen 1999). Measurements of indoor moisture were obtained from a surveyor, and the presence of indoor mold was reported by home occupants. For people exposed to indoor moisture, as determined by an objective measure, an increased risk of respiratory symptoms such as nighttime cough and dyspnea (*i.e.*, shortness of breath) was found. People exposed to molds, as determined by self-reports, had an increased risk of rhinitis (*i.e.*, inflammation of the nose membranes), sore throat, and daytime and nighttime cough. Overall, the incidence of respiratory infections for people exposed to moisture or mold was significantly higher than for people not exposed. Increased moisture was observed by the surveyor in 52% of the homes. Notably, significantly ( $p < 0.001$ ) more of these homes were built commercially by Scandinavian construction companies than by individual homebuilders.

Other studies, however, do not report a link between indoor mold and respiratory problems (Bjornsson 1995). One well-designed study conducted in Sweden looked at the effect of exposure to indoor molds, bacteria, household dust mites, and dampness on 88 people with asthma (Bjornsson 1995). Objective measures were used to determine levels of dampness, mold/bacteria, and dust mites, as well as the presence of asthma. Although the authors concluded that dampness in homes may cause people to report more respiratory symptoms, they did not find that asthma was directly correlated with the presence of either molds/bacteria or dust mites.

### **Literature Reviews**

Literature reviews, in which investigators compile a number of selected key studies on a topic, often provide some of the most useful information upon which to draw conclu-

sions. There are several good literature reviews on studies that look at the association of molds and indoor health, most of which found an association among dampness, dust mite antigen, indoor mold exposure, and asthma and other respiratory illnesses (Bornehag 2001, Verhoeff 1997, Husman 1996, NAS 2000), with one review reporting ambiguous results (Burr 2001).

In a review of the scientific evidence published in 2001, investigators found that, with few exceptions, most of the studies reveal that cough, wheezing, and asthma are associated with indoor dampness (Bornehag 2001), based on data that included over 100,000 people, both adults and children. The authors also separated the studies into separate categories to see if this association differed among studies that relied on self-reporting measurements of indoor dampness and health effects and studies that used objective measures.

The authors divided the studies into the following four categories: 1) studies in which participants self-reported both the level of dampness in their homes and the status of their health; 2) studies in which participants reported on the level of dampness in their homes but health effects were determined by clinical examination; 3) studies in which home dampness was objectively measured and health effects were self-reported; and 4) studies in which home dampness was objectively measured and health effects were clinically examined.

Overall, an association was found between indoor dampness and respiratory illness regardless of the type of evidence; however, some differences were found among the types of evidence:

- Clinically diagnosed asthma and self-reported asthma were found to be similarly associated with self-reported indoor dampness, although clinically diagnosed abnormal pulmonary function was only weakly associated with dampness, if at all.
- For people who self-reported health effects, a stronger association of health effect was found when the measure of indoor dampness was objectively measured.
- A stronger association was found between clinically detected asthma and objectively measured indoor dampness than between clinically assessed pulmonary function and objectively measured indoor dampness.

Based on this overview, the authors concluded that the evidence for an association between respiratory symptoms and dampness is strong and that people who live or work in damp buildings are at increased risk of developing a number of respiratory symptoms.

Another review, published in 1997, also reported an association between mold exposure and asthma or asthma-like symptoms. Based on nine population-based studies, which used quantitative measurements to test for the presence of fungus or dust in indoor air, the review found that seven of the nine studies indicated that fungi contribute to allergic disease (Verhoeff 1997).

Similar findings were reported by the NAS in their 2000 report undertaken for the EPA. In their review of the literature, they found that exposure to molds exacerbates asthma in people already sensitized to molds, although this was not the case for individuals who were not sensitized to molds. The report also found that the presence of symptoms indicating asthma was associated with damp conditions. Overall, the report concluded:

- Damp conditions are associated with the existence of doctor-diagnosed asthma and with the presence of symptoms considered to reflect asthma (i.e., dampness may lead to the development of asthma);
- Symptom prevalence among asthmatics is also related to home dampness indicators (i.e., dampness may exacerbate existing asthma); and
- The factors related to dampness that actually lead to the development and exacerbation of disease are not yet confirmed, but probably relate to dust mite and fungal allergens (see following section).

Somewhat more ambiguous results were reported in two other reviews (Burr 2001, Husman 1996). The most recent, published in 2001, found that an association between indoor molds and ill health is likely, but emphasized that the evidence remains inconclusive (Burr 2001). The other review, published in 1996, which reported a strong association between mold exposure and symptoms (*e.g.*, irritation of the respiratory tract and eyes), also reported that the evidence for an association between mold exposure and bacterial or viral respiratory infections remains vague. The authors suggested that differences among people, such as sensitivity, age, and smoking, may account for the different responses that people reported.

### Molds, Mites, or Other Pollutants?

It is not easy to distinguish the role that molds play in causing respiratory and allergic problems from the role of other indoor air pollutants that cohabitate many environments (*e.g.*, dampness, pets, mites, tobacco smoke). Several studies that have addressed this issue are reviewed here.

In the large review by Bornehag that found a strong association between indoor dampness and respiratory symptoms, the authors also cautioned that they did not know which agents in the indoor air caused the health effects. They suggest that mite sensitization, which also may occur because of dampness, may explain some of the association, but not all (Bornehag 2001). Thus, the mechanisms behind the association between dampness and health remain unknown.

The difficulty of differentiating the source of allergy, whether molds or mites, was also highlighted in the literature by Burr and colleagues. In addition, the Burr study suggested that different sources of bias may affect both the assessment of mold and the measurement of health. One such bias may stem from a tendency to publish only positive findings that show a correlation between molds and health, but not studies that find no correlation (Burr 2001).

To better isolate the effect of molds alone on human health, it will be necessary to establish criteria to measure the presence of molds in buildings and to determine levels that are associated with toxic effects on health. Newer techniques to measure exposure of indoor molds focus on the use of biochemical and immunochemical markers that indicate the presence of molds, such as ergosterol, which has a high correlation with total spore counts (Bush 2001). Several current approaches to objectively measure the presence and level of molds indoors will be discussed in another chapter. (See the Industrial Hygiene section.)

Also needed are widely available, affordable, and reliable methods to objectively assess the health effects of home dwellers who report symptoms related to their indoor

environment. Dales (1999) reported on studies that used a nighttime cough recorder in homes of 400 Ontario school children and measured ergosterol levels in these homes. Although parents reported increased respiratory symptoms in children when the parents observed mold present in their home, there was no association between measured cough and airborne ergosterol levels. The authors concluded that proof of an association between indoor fungal exposures and ill health requires objective confirmation because of the discrepancies between findings based on self-reports and objective health measures.

## Summary

Overall, the majority of studies indicate an association between indoor mold exposure and ill health, particularly respiratory problems. The strength of that association, however, varies from study to study, with some studies showing a strong association and others only a vague or ambiguous one. These variant results point to an area of much concern in trying to provide solid data on the link between indoor mold and ill health—the lack of a good way to measure objectively the health effects of mold exposure. Much of the data currently used to measure health effects are based on studies that use questionnaires, which ask people to report their symptoms. These “self-report” types of measurement are the least objective and raise questions about bias and other factors that can interfere with a solid analysis. Also lacking is a standardized, reliable, widely available method that can objectively measure mold exposures, as well as an understanding of the mold levels that cause reactions in humans.

To date, a causal relationship is highly suspected for indoor molds resulting in certain respiratory complaints, although this association cannot be distinguished from the involvement of indoor dampness, dust mites, and other factors. Much of the literature points to a strong association between indoor dampness and asthma and other health effects, both in the development of respiratory disease as well as exacerbation of existing problems. Additional well-designed studies are needed to examine this association before a definitive answer can be made on whether mold associated with indoor dampness causes respiratory illnesses.



This section will evaluate the scientific literature on the potential toxicological effects of fungal components. There are several well-described mycological illnesses associated with fungal components, which will be outlined. For others the scientific data is limited and their limitations will be discussed. For example, the effects of some mycotoxins on the hematological (blood) system are well described in both animals and humans. An initial increase in the white blood cells occurs, followed by a large decrease in acute exposures. However, in more chronic exposures, only a decline in the white blood cell count is observed. (Rukmini 1980) The red blood cells and platelets are not affected. Further details are found in the discussion of alimentary toxic aleukia found below.

## **Mold in Indoor Environments and Its Role in Indoor-related Diseases**

A number of studies and presentations (Third International Conference on Fungi, Mycotoxins, and Bio aerosols, Saratoga Springs, N.Y.; 1998) have addressed the issue of mycotoxin-related disease in the indoor environment. Some of these efforts, however, are steeped in controversy among the medical community regarding the scientific validity of the actual studies.

Croft and colleagues (1986) described an uncontrolled case series of six patients, who resided in the same house with complaints of sore throat, diarrhea, headache, fatigue, skin inflammation, and intermittent hair loss. The male adult also complained of severe leg pain. Repeated physical examinations, and blood and urine tests (these were not specified by the authors) did not display any abnormalities. The illness finally disappeared after the house was remediated.

Air samples from the living room, dining room, and upstairs bedroom revealed the presence of *S. atra* spores. Samples from the ceiling material and air duct (where the mold was visible) were extracted and the resulting ethanol-based solution was administered by mouth to five mice (a control group received ethanol only). It is unclear whether the mice developed any signs of toxicity, but a necropsy examination showed signs of tissue necrosis (death) and bleeding within the brain, thymus, spleen, intestine, lung, heart, lymph nodes, liver, and kidneys. The authors were able to isolate several trichothecene mycotoxins from a ceiling sample covered by *S. atra*, but these were not the same compounds that had been administered to the mice.

Several important points need to be mentioned regarding this study:

- The diagnosis of trichothecene poisoning in the affected patients was based solely on clinical descriptions done by others and not from objective findings found by the authors (in contrast to the well-described mycotoxicoses).
- The symptoms presented by these patients may also appear in other medical conditions such as the flu. The authors did not state whether this was ruled out.
- The mycotoxins were isolated from scrapped materials and not from airborne spores.
- The authors established a highly questionable causal link between the mold detected and the symptoms exhibited by the patients.
- The small animal study possesses major methodological flaws. First, the authors did not describe all the components included in the solution administered to the mice and inaccurately assumed that this contained only mycotoxins; second, assuming

that the administered substance represented a mixture of mycotoxins (as the authors assumed), the dose given was never specified. Thus, the mice might have received a very large and lethal dose that does not have biological relevance to inhalation exposure.

Croft's study was the first one to try to establish a cause-effect relationship between indoor molds and illness. This report is frequently cited in investigations and public health statements describing the human health hazards of exposure to *Stachybotrys*. This study was followed by many others that attempted to prove the same conclusions.

Platt and colleagues (1989) found a statistically significant association between house dampness and mold growth and the presence of a variety of symptoms as reported by the tenants. This investigation also found that the economic and smoking status of the responders were associated with these symptoms. The results of this study should be warily interpreted. First, the entire study was based on self-reported symptoms without having supporting objective findings (e.g., physical examination). Self-reporting eventually leads to responder bias whereby the responder is already influenced by the physical condition of the house. Second, it also appears that the authors chose to dismiss the effect of smoking on the incidence of the symptoms.

Dale and colleagues (1991) also reported a similar association between respiratory symptoms such as cough, wheezing, shortness of breath, nose irritation, sneezing, stuffy nose, and throat irritation and the presence of mold and moisture in the house. The Dale study also was based only on self-reported symptoms and by self-evaluation of the house condition by the tenants themselves, leading to an obvious reported bias. Moreover, the evaluated group was never compared to a similar but unexposed group and without comparison it is difficult to make conclusions on causation. An investigation performed by Cooley and colleagues (1998), suffering from the same methodological limitations as the Platt, Dale, and Croft studies, also claimed to find an association between respiratory complaints and mold exposure.

Studies that attempted to relate airborne and surface spore counts (in colony forming units/  $m^3$  – CFU/ $m^3$ ) to ill health in building residents (Li 1997, Van Netten 1997, Su 2001, Scheel 2001), found a relationship between the numbers of CFU/ $m^3$  of different molds to the presence of symptoms. Although the authors concluded that a cause-effect relationship is plausible, this could not possibly be derived from their results for the following reasons: 1) the existence of airborne spores does not equate to the presence of airborne mycotoxins because, as previously stated, their production and release depend on specific environmental conditions; therefore, isolation of a potentially toxigenic fungi from an indoor environment does not necessarily indicate that the subjects were exposed to mycotoxins; 2) these studies did not consider the presence of non-viable spores which may also contain mycotoxins; 3) according to the other studies, their results were based solely on answers from a self-reported questionnaire; and 4) no control group for comparison was used.

In the past 5 years, case-control studies of occupational exposure to *Stachybotrys* in water-damaged buildings have generated more controversy (Hodgson 1998). Case-control studies are studies where the “case” or tested group is compared with an unexposed, control group.

Johanning and co-workers (1998) reported significant differences in self-reported symptoms between “exposed” cases ( $n = 53$ ) and unexposed control subjects ( $n = 21$ ).

These symptoms appeared in the lower respiratory tract (*i.e.*, recurrent shortness of breath, cough, chest tightness, wheezing), eyes (*i.e.*, burning, irritation, blurred vision), skin (*i.e.*, burning, rash, hair loss), and constitutional (*i.e.*, defined by the authors as unexplained low-grade fever, tender and /or enlarged lymph nodes, flu-like symptoms, and muscle pain). The authors did not try to find either alternative diagnoses or to perform a physical examination to confirm the complaints objectively.

The “immunology” assessment performed by the authors did not reveal any differences or abnormalities between the case and control groups except for the number of T-lymphocytes, a subset of white blood cells involved in the body immune system, which was slightly lower (1345 cells/ $\mu$ L vs. 1431.5 cells/ $\mu$ L) in the case group. The clinical significance of this difference is difficult to interpret, and could have been affected by laboratory readings, by intra-individual variation, and by statistical chance. Therefore, the authors failed to demonstrate immunological abnormalities among the case group.

The authors attributed the symptoms to *Stachybotrys* exposure, its mycotoxin satratoxin H (which was not isolated from airborne spores), and other molds (such as *Aspergillus* and *Penicillium*). The study design did not include a mycologic evaluation of the buildings, which served as controls. The authors failed to address the finding that 19% of the “exposed population” were either active smokers or to provide information on the extent of exposure to cigarette smoking. Additionally, the interpretation of these results was limited by the fact that the data were based only on self-reported symptoms without being supported by objective findings.

In another case-control study (Hodgson, 1998) exposure to *Stachybotrys*, *Penicillium*, and *Aspergillus* was implicated in causing self-reported symptoms of interstitial lung disease (ILD) and sick building syndrome among office workers in a water-damaged building. Interstitial lung disease is a lung disease diagnosed by history, physical exam, and ancillary tests such as pulmonary function tests. The present study completely disregarded this fact and also did not find reliable biomarkers of exposure to these molds.

## General Human Mycotoxicosis

The following list depicts the important human mycotoxicoses for which reasonable data exist that associate them with mold exposure and possible mycotoxins:

1. Ergotism
2. Alimentary toxic aleukia (ATA)
3. Aflatoxin related liver disease and cancer
4. Balkan endemic nephropathy
5. Stachybotryotoxicosis

### **Ergotism**

Ergotism is considered the oldest known human mycotoxicosis. It is a condition whereby ergot substances (*i.e.*, alkaloids) may cause narrowing of the blood vessels. Historic outbreaks of this disease reached epidemic proportions in the Middle Ages. The disease was characterized by a cold sensation in the extremities progressing to an

intense burning sensation due to decreased circulation. Severe cases advanced to gangrene (death) of the involved limb(s). The characteristic burning feeling gave the disease the name of St Anthony's fire because these symptoms were relieved by a visit to St Anthony's shrine, located a distance away from the area of exposure.

The disease became associated with the consumption of contaminated rye bread with the molds *Claviceps purpurea* and *C. paspali*, with ergot alkaloids identified as the mycotoxins. Ergotamines are specific products of these particular *Claviceps* species and are not necessarily produced by the molds typically described in moisture-damaged homes. The same ergot substances have a beneficial health effect as a treatment for migraine headaches.

### **Alimentary Toxic Aleukia**

Alimentary Toxic Aleukia (ATA) is a disease that affects the blood-forming system (*i.e.*, bone marrow) by causing a decrease in the number of blood cells. The syndrome is characterized by four clinical stages, which consist of 1) fever, 2) bleeding, 3) decreased white blood cells, and 4) suppression of the bone marrow. Patients have survived but death can be as high as 60% (Shank 1977).

The disease occurred in the Soviet Union during World War II. Famine conditions forced the population to collect and consume grain that had been left under snow during the winter. These grains were contaminated with fungi from the *Fusarium* family (*Fusarium poae* and *F. sporotrichioides*) and the mycotoxin was identified later as a trichothecene T-2 toxin (Joffe, 1986 see Ueno 84). In one research study, monkeys were fed T-2 toxin by stomach tubes for 15 days and developed a syndrome similar to ATA in humans (Rukmini 1980).

### **Human Aflatoxicosis**

Aflatoxins are mycotoxins produced by two fungi from the *Aspergillus* family, *Aspergillus flavus* and *A. parasiticus*. Several types of aflatoxins exist but only aflatoxin B1 is linked to a specific human disease. Aflatoxins are common contaminants in peanuts, soybeans, grains, and dried corn (Shank 1977).

Chronic ingestion of low doses of dietary aflatoxins has been associated with liver cancer in some parts of Africa and Asia, whereas, acute ingestion of high concentrations of these mycotoxins has been associated with irreversible liver damage and death. There is no evidence in the scientific literature to support the position that inhalation of aflatoxins causes liver disease.

### **Balkan Endemic Nephropathy**

Balkan endemic nephropathy, discovered in the late 1970s in clusters throughout rural areas of the Balkan region of Europe (Romania, Bulgaria, and Yugoslavia), consists of kidney injury and possibly urinary tract tumors (WHO 1990). The cause was found to be consumption of cereal grains contaminated with the ochratoxin A, a mycotoxin typically derived from *Aspergillus* and *Penicillium* species. This conclusion is challenged, however, by a more recent study that implicated a virus, rather than mycotoxins, as the cause (Riquelme 2002).

### ***Stachybotryotoxicosis***

Stachybotryotoxicosis refers to the illness caused by fungi from the *Stachybotrys* family.

Drobotko and others initially diagnosed the disease in the Soviet Union in the early 1930s in horses and subsequently in humans. Drobotko (1946) was the first to provide an excellent description of the equine (horse) disease (for details, please refer to reference 24). This was characterized by three clinical stages:

1. The initial stage where horses developed mouth, throat, nose, and lip irritation;
2. A second stage delineated by a gradual decrease in white blood cells; and
3. The final stage with fever, ulcers, bleeding, and marked reduction in the number of white blood cells.

A reduced number of white blood cells may lead to impairment and failure of the animal's immune defense system, making it more susceptible to infections.

A similar disease named "septic angina" was described in Siberia and Bashkieria in 1934. This illness was also characterized by bleeding, ulcers, and a decreased number of white blood cells. The common denominator of both diseases was *Stachybotrys alternans*; however, other *Stachybotrys* species (*S. atra*) were also identified. The horses acquired the disease by eating mold-contaminated hay.

Drobotko was also able to reproduce "septic angina" by feeding healthy horses the same contaminated straw as well as pure cultures of *S. alternans*. This fact is extremely important because it provides the evidence that *S. alternans* mold is temporally and directly associated with the equine illness. It is crucial to recognize that exposure was from ingestion, not from inhalation. There was no description of illness in horses from inhalation.

### ***Human Stachybotryotoxicosis***

According to Drobotko (1942), farmers, from the geographic areas where equine stachybotryotoxicosis occurred, who had handled contaminated fodder, frequently developed a disease consisting of dermatitis (skin inflammation), pharyngitis (throat inflammation), and rhinitis (runny nose), accompanied with bloody secretions, cough, and chest tightness. Rarely, some cases presented with a decreased number of white blood cells and fever (as with horses). Drobotko reported that the same mold, *Stachybotrys*, was associated with the disease.

### ***Pulmonary Mycotoxicosis***

Pulmonary mycotoxicosis refers to the toxicity of mold to the lungs. Lung injury by mold can be classified in three types: allergic, infectious, and toxicologic. The allergic or immune type is discussed in a separate section of this monograph. There is significant overlap in this group of pulmonary diseases with organic toxic dust syndrome (OTDS), which is the feverish reaction to the inhalation of organic material (*e.g.*, grain dust, insect particles, mold, bacteria, and endotoxin).

Pulmonary mycotoxicosis was first described by Soviet scientists in the early 1960s (Forgacs, 1972). The disease was observed in occupations such as cottonseed oil processing plants, grain elevators, food grain processing plants, breweries, and textile

mills. The disease consisted of irritation of the eyes, throat, and nose, shortness of breath, cough, chest tightness, and fatigue. No specific fungi were identified, but the authors speculated that *S. alternans*, *A. fumigatus*, *Dendrochium toxicum*, and *A. niger* might be involved in causing this syndrome.

Emanuel and colleagues (1975) described three cases of lung and upper respiratory tract irritation from mold inhalation in farmers who cleaned silage inside a closed silo. The clinical picture they presented was of fever and shortness of breath, which persisted from 7 to 30 days (mean 16.4 days). Chest X-rays showed a picture compatible with lung inflammation. Fungi from the genus *Penicillium* and *Fusarium* were cultured from lung tissue samples. The authors concluded that this was a non-allergic type lung disease related to mold debris inhalation.

A similar lung disease allegedly related to mold inhalation was reported by Brinton and colleagues (1987). Eleven patients manifested symptoms comparable to the flu accompanied with shortness of breath and cough. These started after the patients had inhaled dust derived from hay that had been dispersed on the floor of a poorly ventilated basement. The authors did not provide any objective evidence of a mold-associated disease, but they theorized that the potential cause might have included inhaled mycotoxins, bacterial toxins, or heavy dust derived from the hay.

Nikulin (1996, 1997) observed that intranasal instillation of an extract of a highly toxic strain of *S. atra* in mice induced severe inflammatory lesions in the lungs, whereas administration of a low toxic strain administered by the same route did not cause any injury. Trichothecene mycotoxins satratoxin G and H, stachybotrylactone, and stachybotrylactam were isolated from the highly toxic strain and only small amounts of stachybotrylactone and stachybotrylactam from the low-toxic strain.

Interestingly, Creasia and colleagues (1987) studied the effects of acute inhalation of pure trichothecene T-2 toxin on the lungs of mice and found no significant pathologic changes in this organ. This study raises the possibility that intact mycotoxin-bearing spores are toxic, but not the pure mycotoxins. The studies by Nikulin and Creasia are not directly comparable, however, because the authors used different types of mycotoxins.

## Neurological Effects and Mycotoxins

### *Animal Studies*

A number of mycotoxins from a variety of fungi have been shown to cause a wide variety of effects in animals, including immune suppression, decreased appetite and weight loss, vomiting, and apoptosis (*i.e.*, individual cell death). Animal experiments have demonstrated an effect on certain chicken brain neuro-transmitters. This included an elevation of dopamine and a decrease in norepinephrine (Chi 1981).

In many such experiments, there were some methodologic factors that limit the interpretation of the findings. For example, the mycotoxins were extracted from fungal spores (which alters their bioavailability), were administered by injection or ingestion (which differs from the route purported in indoor mold-related disease in humans), or were delivered in extremely high doses. Additionally, it must be remembered that animals may react differently or have dissimilar susceptibilities than humans, which makes extrapolating animal data to humans difficult and, possibly, inappropriate.

### **Human Studies**

Various papers have linked mycotoxins to vague, subjective symptoms, including various neurobehavioral symptoms such as fatigue, weakness, nausea, vomiting, headache, concentration difficulty, and depression. Overall, these reports have focused on the presence of *Stachybotrys* in the indoor environment as the potential cause of the patients' complaints. A look at the individual papers, however, reveals serious flaws or limitations with their conclusions. Such flaws include:

- Lack of rigorous and standardized sampling methods or a reliance on subjective measures of moisture infiltration or mold contamination;
- Belated sampling for molds;
- Failure to identify or to quantify mycotoxin levels;
- Neglect of the presence of other more common fungi;
- Failure to document or to quantify both patient exposure and dose, or a reliance on subjective patient reports to do so; failure to consider other environmental etiologies;
- Failure to look at other potential medical causes for the patients' symptoms;
- Reliance on subjective complaints rather than on objective measures of disease;
- Lack of an adequate case definition for the purported disease;
- Lack of adequate controls including failure to control for mitigating or confounding variables such as smoking, socioeconomic status, stress, or overcrowding; and
- An inability to eliminate potential patient biases.

In some cases, the authors' conclusions were based simply on finding the mold present in the indoor environment. In no study were all of the commonly accepted criteria for documenting causality after environmental exposure met. At this time, there remains insufficient evidence to suggest a causal relationship between exposure to indoor molds and any verifiable toxicologic condition.

A few papers have claimed that fungal glucans (specific components of their cell walls) might be the cause of human illnesses such as sick building syndrome, and there are scant data to suggest that, at high doses, glucans might be irritative or inflammatory. The evidence associating glucans with human disease is weak, however, and far from proving a causal relationship.

Similarly, there is no evidence to suggest that MVOCs cause human illness, probably because they are elaborated in such minute amounts.

### **Mold in Indoor Environments and Its Role in Idiopathic Pulmonary Hemorrhage-Hemosiderosis**

Perhaps the most important publications implicating molds (specifically *Stachybotrys*) in human disease came from a cluster of idiopathic pulmonary hemorrhage-hemosiderosis (IPHH) among infants living in water-damaged dwellings in Cleveland, Ohio, followed by isolated cases reports from other geographic regions of the United States (Montaña 1997, Etzel 1998, Jarvis 1998, Elidemir 1999, Flappan 1999, Novotny 2000). IPHH is a rare medical condition of spontaneous bleeding in the lungs. The causes of this condition are varied and, when these are not identified, the disease is called idiopathic or "of unidentified source."

The Cleveland cluster included 10 cases of idiopathic PHH, which were compared to 30 age-matched control infants with no known medical problems. Medical information was retrieved from their medical records and their home assessed for water damage, mold growth (airborne spores), cigarette smoking, and the presence of other household chemicals.

Airborne spores of different types of fungi were identified. *S. atra* spores constituted 0.15% and 0.6% of the total spore count in the case- and control-houses respectively. The cases were 7.9, 16.3, 0.2, and only 1.6 times more likely to reside in households with smokers, to live in water-damaged homes, to be breastfed, and to have *S. atra* in their residences, respectively. These results provide strong evidence that water-damaged houses and smoking are strongly associated with IPHH, whereas the presence of *S. atra* spores is not.

Following these studies, Jarvis and colleagues (1998) showed that *Stachybotrys* strains, isolated from samples taken from both case- and control-houses, under controlled laboratory conditions, were able to produce and release cytotoxic (*i.e.*, a substance that is toxic to cells) and non-cytotoxic trichothecene mycotoxins.

Elidemir and colleagues (1999) were the first to culture *Stachybotrys* from bronchoalveolar lavage fluids (*i.e.*, fluids retrieved from the lung after it is washed) in a child with IPHH, who lived in a water-damaged house. Based on the findings, the authors concluded that the mold was the causative agent of the child's disease; however, they neither had a control group with which to compare the observations nor were *Stachybotrys*-related mycotoxins measured in the home.

The CDC formed a panel of experts to evaluate the claims that IHPP was linked to *Stachybotrys* or other indoor molds. The CDC found serious flaws in the studies, such that a causal relationship between mycotoxins and pulmonary hemorrhage in humans cannot be concluded. The flaws are similar to those described for the other papers trying to link indoor mold with toxicologic symptoms. Limited animal data looking at this issue suggest the possibility that direct insufflation of *Stachybotrys* into the lungs or respiratory mucosa of animals can lead to inflammation, but they remain inconclusive regarding human conditions. All things considered, mycotoxins have been associated with pulmonary hemorrhage, but causality remains unproven.

## Other Systemic Illnesses

Finally, at least one author has reported a case of kidney failure purportedly related to mycotoxins. Again, many of the same problems exist with this case report: specific toxins were not identified or quantified; other, more common causes were not necessarily ruled out; and the authors failed to provide the data necessary to prove causation.

## Conclusions

To summarize, the current association with mycotoxins and sick-building syndrome or other multiple, subjective ill-defined complaints, is weak, as are other systemic diseases such as renal failure and neurologic ailments. The evidence for an association between pulmonary hemorrhage and mycotoxins varies in validity, but there are inadequate data at this time to prove causation. There is no scientific evidence to suggest that mold in



the indoor environment will cause abnormalities of the nervous system. To date, the aggregate data from all of these studies has not provided compelling evidence that the inhalation of mycotoxins causes health effects in humans at levels expected in most mold-contaminated indoor environments.

There are several contemporary reviews addressing the potential association of microorganisms with illness in humans in the indoor environment (Tobin 1987, Menzies 1997, Fung 1998, Robbins 2000, Terr 2001). These studies have only suggested a weak association, and have not been able to causally link *S. chartarum* with toxic illness. Furthermore, the government's CDC found no association between *S. chartarum* and idiopathic pulmonary hemorrhage-hemosiderosis, now referred to as acute idiopathic pulmonary hemorrhage in infants (MMWR 2001). Upon reviewing the literature, several points are worth noting regarding the toxicological aspects of mold and illness:

1. Molds are common in both the indoor and outdoor environments.
2. Inhalation of indoor air has not been demonstrated to cause idiopathic pulmonary hemorrhage-hemosiderosis in children.
3. Certain specific illnesses have been associated with mold and possibly mycotoxin exposure, depending on the dose and route of the exposure.
4. The disease described by Drobotko in humans exposed to *Stachybotrys* included skin inflammation, pharyngitis, rhinitis (sometimes accompanied by bloody secretions), cough, chest tightness, and rarely fever with a reduced number of white blood cells. This case description differs from the cluster of non-specific symptoms presented by individuals residing in water-damaged buildings.
5. In adult humans and animals, inhalation of *Stachybotrys* spores might cause a lung inflammation without pulmonary bleeding as part of the well-described Organic Toxic Dust syndrome, which is a different illness from what has been described in acute idiopathic pulmonary hemorrhage in infants.

## 3

# Individual Article Summaries by Discipline

MYCOLOGY	56
INDUSTRIAL HYGIENE	81
ALLERGY AND IMMUNOLOGY	100
MEDICAL TOXICOLOGY	125

## 1 Toxic effects of indoor molds

AUTHORS: American Academy of Pediatrics, Committee on Environmental Health

JOURNAL: *Pediatrics*

CITATION: 1998; 34:712-714

**STUDY TYPE AND OBJECTIVES:** Literature review in support of a position statement. The statement described molds, their toxic properties, and their potential for causing toxic respiratory problems in infants. The position statement also provided guidelines for pediatricians to help reduce exposures to molds in homes of infants.

**RESULTS:** The authors noted that molds are pervasive throughout the outdoor environment. Given the proper conditions, molds may proliferate in the indoor setting. The assessment of toxic effects was focused on possible pulmonary hemosiderosis (PH) associated with exposure to *Stachybotrys chartarum* spores, with the associated mycotoxins, in water-damaged buildings. The authors concluded that very little is currently known about the cause of this disease. Based on the severity of PH, however, the authors concluded that environmental controls to eliminate water problems and to reduce the growth of indoor molds are wise. Specific recommendations included:

1. In areas where flooding has occurred, prompt cleaning of walls and other flood-damaged items with water mixed with chlorine bleach, diluted four parts water to one part bleach, is necessary to prevent mold growth. Moldy items should be discarded.
2. Pediatricians should ask about mold and water damage in the home when they treat infants with PH. If mold is in the home, pediatricians should encourage parents to try to find and eliminate sources of moisture. Testing the environment for specific molds is usually not necessary.
3. Infants with PH must not be exposed to environments in which smoking occurs.
4. Pediatricians should report cases of PH to state health departments.
5. Pediatricians should be aware that there is currently no method to test humans for toxigenic molds such as *Stachybotrys* or mycotoxins.
6. Infants who die suddenly without known cause should have an autopsy done to look for the presence of hemosiderin.

**COMMENTS:** The authors pointed out the limits of the scientific knowledge related to the causation of PH. Their recommendations both emphasized the importance of minimizing exposures to mold growing indoors and encouraged the reporting and investigation of potential PH cases to add to the medical understanding of this serious disease.

## 2 Aerobiology of the indoor environment

AUTHOR: Burge HA

JOURNAL: *Occupational Medicine*

CITATION: 1995; 10:27-40

**STUDY TYPE AND OBJECTIVES:** This is a state-of-the-art review article focusing on the science of aerobiology. The paper provided information on the nature, prevalence, and distribution of airborne biologic-source particles. The paper also provided an update (as of 1995) on the effects of airborne agents on people, animals, and plants.

**METHODS:** This article was based on the academic course material related to indoor air quality that is presented to students in Harvard School of Public Health's aerobiology program.

**RESULTS:** The article detailed the general physical parameters of aerosol science, namely aerodynamic particle size (related to diameter, shape, and density), the nature of the particle (is it hygroscopic, hydrophilic, soluble, other), electrostatic charge on the particles, and concentrations (instantaneous as well as variability over time). The aerodynamic particle size is the primary factor that controls how particles behave in an aerosol. Aerodynamic diameter relates to the size and shape of the particle and to its density. The aerodynamic size determines how particles are removed from the airstream and affect the choice of samplers for collection of aerosols. The author noted that the concentration of biological aerosols is difficult to predict because the sources are usually discontinuous, the dispersion mechanisms and rates are extremely variable, and activities have a significant impact on the dispersion of particles. This unpredictable characteristic of concentration makes accurate assessment or estimation of exposure levels for bioaerosol very difficult.

The author also related the impact of airborne agents (bioaerosol) on human health through a discussion of the exposure/dose relationship. Airborne exposure was defined by a formula involving the aerosol level, the fraction of aerosol penetrating into the airways and the fraction of aerosol that is deposited at an effective site within the respiratory system. Dose was defined as exposure multiplied by the amount of effector agent released per unit of exposure. Characteristics of indoor aerosols were linked to the infiltration of outdoor air. These outdoor aerosols were defined as contributing both to the ambient aerosol indoors and to reservoirs in which amplification can occur. The primary factors defined as controlling patterns of indoor bioaerosol prevalence were the abundance of sources, concentrations of particles available for release, particle release, and dispersion and decay.

**COMMENTS:** This paper provided an excellent overview of the principles of aerobiology. The discussion of exposure/dose relationship communicated the complexity of accurately assessing the dose of a bioaerosol. In addition, the information on factors controlling patterns of indoor bioaerosols provided a good foundation for understanding the exposure potential.

### **3 Indoor air quality in schools: exposure to fungal allergens**

AUTHORS: Levetin E, Shaughnessy R, Fisher E, Ligman B, Harrison J, and Brennan T

JOURNAL: *Aerobiologia*

CITATION: 1995; 11:27-34

STUDY TYPE AND OBJECTIVES: A limited study to determine some baseline data on the range of bioaerosol levels that exist in schools in four separate areas of the United States.

METHODS: Air sampling was conducted in 13 school buildings in Kansas City, Spokane, Santa Fe, and Orlando during 1991-1992. Sampling methodologies for both viable fungi and total spores were employed. A total of 195 viable fungi samples and 183 total spore samples were collected. Standard analytical procedures were used.

RESULTS: The highest fungal concentrations, both viable and total spores, were found in Kansas City and Orlando, and the lowest in Spokane and Santa Fe. In general, the indoor concentrations and taxa in each city reflected outdoor levels and types. In the majority of the viable and total spore samples, *Cladosporium* was the most abundant genus identified. There were major distinctions in the indoor air spora at the schools because of the geographical and climatic differences and the differences in the ventilation systems employed. Even within each school, variations were found among different classrooms and also within one classroom at different times of the day. Outdoor spore counts as high as 81,172 CFU/m<sup>3</sup> for total spores and 19,968 CFU/m<sup>3</sup> for viable fungi were found.

COMMENTS: This study was somewhat limited in size, but it presented accurate data on the variability of fungal spore concentrations in school buildings. The authors documented regional and seasonal variability in outdoor fungal concentrations, as well as, variability in the indoor concentrations of fungal spores. The magnitude of the outdoor bioaerosol concentration was identified as a factor influencing the indoor levels. In addition, the authors pointed out the importance of the ventilation system in controlling indoor fungal spore concentrations, with a central HVAC system resulting in the lowest indoor/outdoor ratio of fungal spore concentration.

#### 4 The relationship between fungal propagules in indoor air and home characteristics

AUTHORS: Ren P, Jankun TM, Belanger K, Bracken MB, and Leaderer BP

JOURNAL: *Allergy*

CITATION: 2001; 56:419-424

**STUDY TYPE AND OBJECTIVES:** This was a prospective cohort study designed to assess the relationship between residential allergen exposure and development of asthma in infants. The aims of the study were to investigate the applicability of an occupant questionnaire as a measure of potential exposure to fungi, and to investigate the relationship between the presence of fungi in the indoor air and characteristics of houses.

**METHODS:** A questionnaire, designed to characterize those variables that might have an impact on the presence of fungal spores in the indoor air, was administered to an adult occupant of each study home. The questionnaire consisted of 64 items, including design and age of the house; ventilation, heating, and air conditioning; humidifier and dehumidifier; type of flooring; observations of moisture problems, including visible mold growth; the number of occupants; years of occupation; presence of pets and pests; cleaning routines; household income; and education level. A Burkard portable air sampler in combination with DG-18 and malt extract agars (MEA) was used to collect indoor air samples from the infant bedroom and main living areas in 1,000 homes in the Northeast United States, from December 1996 to January 1999. No outdoor air samples were collected.

**RESULTS:** The most frequently isolated molds were *Cladosporium* sp. (84.5%), *Penicillium* sp. (72.6%), *Aspergillus* sp. (50.3%), and *Alternaria* sp. (28.4%). The mean concentration of culturable fungi was 1033 CFU/m<sup>3</sup> for the MEA cultures, as compared to a mean count of 846 CFU/m<sup>3</sup> on DG-18. There were significantly higher concentrations of *Aspergillus* and *Cladosporium* on DG-18 than MEA. Over 70% of the homes studied had indoor fungal concentrations less than 1000 CFU/m<sup>3</sup>. Of the housing characteristics investigated, the room type (e.g., infant bedroom or main living area), season, relative humidity, and temperature were found to be significantly correlated with the number of CFU/m<sup>3</sup> in indoor air. The presence of a cat in the house was also consistently and positively related to the concentration of culturable fungi in the indoor air.

**COMMENTS:** The failure to include an outdoor air sample as a control for the indoor air samples makes evaluation of the true significance of this study difficult. The variability between homes may be associated more with differences in the outdoor conditions than the housing characteristics.

## **5 Review of Quantitative Standards and guidelines for fungi in indoor air**

**AUTHORS:** Rao CY, Burge HA, and Chang JCS

**JOURNAL:** *Journal of the Air and Waste Management Association*

**CITATION:** 1996; 46:899-908

**STUDY TYPE AND OBJECTIVES:** This is a review article. The authors reviewed and compared existing quantitative standards and guidelines for indoor airborne fungi, discussed the limitations, and identified research needs that should contribute to the development of realistic and useful practices regarding these fungal air pollutants.

**METHODS:** The authors critiqued the quantitative standards and guidelines that existed for mold assessment at the time that the paper was published, and provided a summary of research needs.

**RESULTS:** The authors found that, while the focus of their paper was quantitative standards and guidelines, the majority of standards and guidelines available to the public were qualitative in nature. The only official quantitative standard concerning fungi in air was published in 1993 by the Russian Federation and appeared to be targeted to the food processing and pharmaceutical industries. Rao *et al.* also critiqued 18 guidelines from other government agencies/professional organizations and 13 previously published guidelines from individuals/private organizations. The authors noted that the ideal quantitative standard would be based on scientific evidence of fungal concentrations that cause adverse health effects. More information on the levels and patterns of exposure to fungal effluents that cause human disease is necessary to provide a solid basis for quantitative standards. They also concluded that the many different and less-than-ideal microbiological techniques currently available for sampling and analysis of indoor fungal levels and exposures have caused confusion and misconception. They encouraged the development of a new method for fungal aerosol exposure assessment focusing on simple, inexpensive methods for both sample collection and analysis that can be used by relatively untrained personnel, and that provide time-discriminated personal exposure data.

**COMMENTS:** This paper highlighted the lack of a connection between the current standards and guidelines for assessing indoor mold and human health effects. The authors noted that basing standards on health effects is difficult because there is a lack of information on human dose/response relationships for fungi in the air. Developing this information is an essential research need to support health-based standards and guidelines. The dose/response research must address both acute and chronic exposures that are assessed using accurate and sensitive measures of specific fungal allergens and toxin in the air.

## 6 Outdoor allergens

AUTHORS: Burge HA and Rogers CA

JOURNAL: *Environmental Health Perspectives*

CITATION: 2000; 108:653-659

STUDY TYPE AND OBJECTIVES: This review article evaluated the nature and patterns of outdoor allergens and the potential association between outdoor allergen exposure and allergic disease, particularly asthma.

METHODS: The authors interpreted the findings and conclusions of 85 scientific publications.

RESULTS: The authors concluded that much remains to be accomplished in clarifying the role of outdoor allergen exposure and human disease. A clear relationship between exposure, sensitization, and symptoms had not been established for any of the outdoor allergens. The allergenic characteristics of pollen, fungal spores, algae, and lower plants were discussed. The authors noted that these outdoor allergens may infiltrate into the indoor environment and some may colonize indoor substrates and become essentially indoor allergens. Air-conditioning in residential buildings was associated with lower indoor particle levels than naturally ventilated homes, because of the additional barriers to infiltration of these allergens. Changes in weather patterns caused by global warming and changes in agricultural practices were identified as potential contributors to increased levels of outdoor allergens.

COMMENTS: This article provided an accurate evaluation of the current extent of knowledge regarding the impact of outdoor allergens. The authors concluded that outdoor allergens have been the subject of only limited studies with respect to the epidemiology of asthma. In particular they pointed out that fungal allergy remains one of the most frustrating and poorly studied areas in allergic disease. The kinds of fungi and the nature of their allergens that lead to asthma development and exacerbation need intensive study.



## 7 Mycotoxins and building-related illness

AUTHORS: Page E and Trout D

JOURNAL: *Journal of Occupational and Environmental Medicine*

CITATION: 1998; 40:761-762

STUDY TYPE AND OBJECTIVES: The objective of this Letter to the Editor was to express the authors' disagreement with a recently published article on the relationship of exposures to indoor mycotoxins and human health effects. The conclusions that were challenged by the authors were published in an article entitled "Building-associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*, by Hodgson MJ, Morey P, Leung W, *et al.*, *Journal of Occupational and Environmental Medicine*, 1998; 40: 241-249.

METHODS: This Letter to the Editor was based on an additional literature review and interpretation by the authors.

RESULTS: The authors of this letter disagreed with the conclusion reached by Hodgson *et al.* that a "mycotoxin-induced effect is the most likely explanation" of the health problems being experienced by the occupants of the building that had been investigated. Page and Trout cited numerous published accounts of human health effects related to exposures to mycotoxins in the agricultural setting. They stated that these cases of mycotoxicosis, occurring in agricultural or industrial environments, may have involved exposures to fungi and their products in concentrations that can be assumed to be much higher than those experienced by persons in most indoor environments. Alternatively, Page and Trout contended that the studies cited by Hodgson *et al.* did not provide objective evidence that clinical illness was clearly related to mycotoxin exposure. Page and Trout concluded that Hodgson *et al.* were limited in their ability to describe the spectrum of disease among the occupants of the subject buildings. Trout and Page also observed that several undefined case definitions were used and there was a lack of data comparison groups. These deficiencies led Page and Trout to conclude that there was no clear evidence documenting that mycotoxins caused health effects among building occupants.

COMMENTS: The authors' final conclusion was that "the health problems potentially related to the indoor environment, including those potentially associated with exposure to fungi or fungal products, need further evaluation using appropriate environmental, medical, and epidemiologic tools." This conclusion is consistent with an expanding bank of scientific publications acknowledging the lack of an appropriately controlled characterization of a dose/response relationship of fungal growth and human health.

## 8 Environmental mycology and its importance to public health

AUTHORS: Mishra SK, Ajello L, Ahearn DG, Burge HA, Kurup VP, Pierson DL, Price DL, Samson RA, Sandhu RS, Shelton B, Simmons RB, and Switzer KF

JOURNAL: *Journal of Medical and Veterinary Mycology*

CITATION: 1992; 30:287-305

STUDY TYPE AND OBJECTIVES: This is a literature review article examining various aspects of environmental mycology in relation to human health.

METHODS: Ninety-seven scientific papers were reviewed and cited in compiling this summary article. The topics ranged from the relationship between fungi and sick building syndrome to sampling for fungal contaminants.

RESULTS: The authors stated that the relationship of hypersensitivities to fungi or their metabolic products (aldehydes, mycotoxins) is probably the most difficult to define and, in 1992, was the least studied of the potential causes of sick building syndrome. The authors noted that fungal propagules product Type I immediate hypersensitivity. In the discussion on aerobiology, the authors note that the size range of conidia (<2-100 micrometers) affects the densities and distribution of airborne fungi. Therefore, the allergens generally found in indoor air, which is usually lacking in wind currents, represent mostly fungi with small conidia (e.g., *Aspergillus* and *Penicillium*). The authors also cited a paper documenting that the spores of *Cladosporium* from culture (growth) require about twice as much air velocity to be released from cultures (growth) than do the spores of *Aspergillus*. Mishra *et al.* noted that no single sampling method or medium will support the isolation and identification of all fungi. The relationship between flooding incidents and other water problems and increased fungal spore concentrations was well discussed. In the area of health effects, the authors concluded that opportunistic infections are the primary infectious risk, and immediate hypersensitivity to airborne fungal spores (mold allergies) is difficult to diagnose and treat. The authors also discussed health effects associated with high-level agricultural exposures to fungal-contaminated crops. In the discussion of methods for monitoring airborne fungal loads, the authors concluded that pressures from environmental and medical microbiologists may eventually prompt governments to develop laws concerning maximum permissible limits of microbial propagules in indoor air, although the absence of standardized monitoring technologies will make such regulations difficult to enforce.

COMMENTS: This is a very authoritative and comprehensive review of the relationship of fungal contaminants and human health. The authors are well-respected scientists with extensive experience. The statements on the aerobiology of fungal spores are important to assessing and interpreting indoor air quality concerns. The facts that the release of fungal spores from the colony is affected by the velocity of air currents and that small spores, such as *Aspergillus* and *Penicillium*, will predominate in the indoor environment are very important conclusions. These different dynamics within the indoor environment can significantly complicate the interpretation of air sampling results. Care must be taken to address this "environmental" bias as the smaller spore size may result in an artificially elevated concentration of these fungal species.

**9 Effect of environmental molds on risk of death from asthma during pollen season**

AUTHORS: Targonski PV, Persky VW, and Ramekrishnan V

JOURNAL: *Journal of Allergy and Clinical Immunology*

CITATION: 1995; 95:955-961

**STUDY TYPE AND OBJECTIVES:** This was an ecological study based on death certificate records and was designed to examine the relationship of aeroallergen levels and asthma-related mortality in Chicago, IL.

**METHODS:** Data concerning daily tree, grass, and ragweed pollens and combined viable and nonviable mold spores were collected for the period 1985-1989 from Grant Hospital in Chicago. Collection was performed by a rotorod method. These results were related to death certificate records for 124 persons aged 5 to 34 years, with asthma as the cause of death, who were Chicago residents during the period being monitored. Deaths were categorized as pollen-season deaths (March 21 to October 31) or non-pollen season deaths (remainder of year). Univariate and multivariate logistic regression models, with and without interaction terms, were constructed to determine whether the odds of death caused by asthma were significantly higher on days with higher pollen or mold spore levels. These methods were also applied to the data with up to a 4-day lag between mold spore exposure and date of death and for the day-to-day change in mold spore levels.

**RESULTS:** Approximately 57% of the 124 asthma-related deaths among Chicago residents were recorded during pollen season. No clear seasonal pattern was apparent for asthma-related deaths when examined as a 3-week moving average throughout the pollen season. However the mold spore levels were significantly higher for days on which asthma-related deaths occurred. The authors concluded that their study suggests that increased mold spore levels may contribute to deaths caused by asthma. The odds of dying of asthma on days with mold spore counts of 1,000 spores per cubic meter or greater were 2.16 times higher than on days on which spore counts were less than 1,000 per cubic meter. The authors also noted that, in addition to potential confounding by measured variables, this study is subject to limitations inherent in many ecologic studies: confounding by other unknown factors related both to mold levels and death. Use of death certificate data did not permit assessment of other exposures, such as to animals or cigarette smoke, or to those in occupational settings, nor did it allow for the recreation of events leading to the fatal attack. The fact that only one site was available for mold and pollen measurements and that all mold species were combined in a single daily value further limited the ability of this study to distinguish individual exposure levels. Nonetheless, the authors concluded that their study, along with previous reports, suggested that mold may indeed be a risk factor, not only for asthma symptoms but also asthma-related deaths.

**COMMENTS:** Unfortunately, this study lacks accurate exposure data that could help in estimating the actual contribution of fungal spores to asthma-related deaths. As noted by the authors, the fact that the local fungal spore exposures may have varied significantly from the central monitoring site makes it impossible to make an accurate risk assessment. The authors recommended that exposures should be controlled through the reduction of humidity and dampness, adequate ventilation (particularly in bathrooms), and frequent cleaning. Prevention of exposure to outdoor levels is more complicated and generally involves avoidance procedures.

## 10 Profiles of airborne fungi in buildings and outdoor environments in the United States

AUTHORS: Shelton BG, Kirkland KH, Flanders WD, and Morris GK

JOURNAL: *Applied and Environmental Microbiology*

CITATION: 2002; 68:1743-1753

STUDY TYPE AND OBJECTIVES: This was a cross-sectional study designed to quantify and compare both indoor and outdoor fungal concentrations throughout six different geographical regions of the United States.

METHODS: This study involved the aggregation of laboratory results from viable fungal spore air samples that had been collected by numerous different building inspectors as part of indoor air quality investigations in the United States between 1996 and 1998. All samples had been submitted to the same analytical laboratory and had undergone the same analytical procedures. The investigators examined 12,026 fungal air samples (9,619 indoor samples and 2,407 outdoor samples) from 1,717 buildings.

RESULTS: The median indoor fungal concentration was approximately 80 colony-forming units per cubic meter of air (CFU/m<sup>3</sup>), and the values ranged from below the limit of detection (12 CFU/m<sup>3</sup>) to more than 10,000 CFU/m<sup>3</sup>. The median outdoor fungal concentration was approximately 500 CFU/m<sup>3</sup>, with a similar range to the indoor samples. Ninety-five percent of the buildings tested had a median indoor fungal concentration of less than 1,300 CFU/m<sup>3</sup>, and 95% had a median outdoor fungal concentration of less than 3,200 CFU/m<sup>3</sup>. The median outdoor fungal concentrations varies by season and were highest in the fall and summer and lowest in spring and winter. The indoor concentrations varied in a similar manner. The study divided the United States into six geographical regions to assess regional variability and found the highest median outdoor fungal concentration in the Southwest, Far West, and Southeast and the lowest in the Northwest. The indoor concentrations were similar except the lowest indoor concentrations were found in the Northeast. The fungi most commonly recovered from both indoor and outdoor air were *Cladosporium*, *Penicillium*, *Aspergillus*, and the nonsporulating fungi.

COMMENTS: This is a very large study that allows accurate comparison of airborne fungal concentrations from all regions of the United States. This comparison is particularly valid for the outdoor air samples because of the similarity in the sample collection and analysis. The indoor air concentrations are more difficult to compare because of the differences in building types being investigated. Building types were not controlled and included office buildings, schools, hospitals, residences, and industrial facilities; residential construction represented only 4% of the buildings included in the study. In addition, all of the buildings included in this study were being investigated because of an indoor air quality concern; therefore, care must be taken in extrapolating the indoor fungal concentrations from this study to conditions that are likely to exist in typical residences within the United States.

## **11 Introduction and summary: workshop on children's health and indoor mold exposure**

**AUTHORS:** Rylander R and Etzel R

**JOURNAL:** *Environmental Health Perspectives*

**CITATION:** 1999; 107:465-468

**STUDY TYPE AND OBJECTIVES:** This paper is a summary document from a workshop that was organized to develop a basis for risk assessment and formulation of recommendations, particularly for diagnostic purposes.

**METHODS:** Fifteen scientists from eight countries were assembled to evaluate the health consequences of indoor exposure to molds for children. The participants were all active researchers with current experience in child health, molds, and respiratory disease. Three working groups were formed to develop written reports regarding specific problems and questions regarding the different aspects of the relationship between molds and children's health.

**RESULTS:** The authors concluded that all responses to the environment, in terms of both allergy and inflammation, are related to the genetic predisposition of the individual, particularly those with an atopic predisposition. When faced with a child with symptoms possibly related to indoor air, the physician's initial aim needs to be ruling out other diseases such as bronchiectasis and congenital immune deficiency. The authors also concluded that assessment of the conditions of a building and measurements of the presence of molds are important activities in the process of relating a child's symptoms and clinical findings with mold exposure. A questionnaire can support this assessment by soliciting information on musty odors, a history of water damage, visible signs of water damage, symptoms and changes in symptoms, things that aggravate the symptoms, and performance of the heating, ventilation, and air-conditioning system. A site visit was also defined as being important to the assessment process. As to environmental sampling, the authors suggested that sampling in the absence of visible mold may be merited to ensure that there are no further hidden sources of mold; however, they noted that accurate exposure assessment is difficult with currently available sampling and analysis methods. No single measurement technique is entirely suitable, and sampling should never be conducted alone, but in conjunction with an inspection.

**COMMENTS:** The authors' emphasizes the difficulties associated with accurate assessment of exposures to environmental fungi. They noted that a measure of culturable molds (as colony-forming units) in an air sample is of little value because the sampling periods of traditional methods are too short to represent accurately the variability of concentrations over time. Also, the culturable portion only represents a small fraction of the total number of mold spores present in air, dust, or bulk samples. These conclusions have a significant impact on the field of bioaerosol evaluation, since culturable mold sampling is the predominant method used in assessing fungal contaminants in indoor air. Clearly, new methods need to be standardized for appropriate environmental monitoring. In addition, care must be taken when interpreting the possible health consequences associated with exposures that are characterized using culturable methodologies.

## 12 An analysis of outdoor air reference samples collected in the mid-Atlantic region of the United States

AUTHORS: McGuinness M, McGuinness P, Dieda M, Yuran M, Wieller D, and

Warner A

JOURNAL: *Proceedings: Indoor Air*

CITATION: 2002; 329-334

STUDY TYPE AND OBJECTIVES: The goal of the research was to approximate the types of species and measured levels of fungal species present in the outdoor environments in the Mid-Atlantic Region of the United States. The authors tried to define what constitutes "normal" or "usual" microbiological conditions.

METHODS: The data for this study were compiled from outdoor air samples submitted by numerous investigators during the period April 1999 to December 2001. Sampling methods included the Air-O-Cell cassettes for countable fungal material and the Andersen-Graseby N6 size selective impactor for culturable fungal material. All samples were analyzed at the same accredited environmental microbiology laboratory.

RESULTS: Fungal structures persistently identified in the countable outdoor air samples averaged 5,378 *Cladosporium* spp. spores per cubic meter, 1,344 basidiospores, 459 ascospores, 258 *Aspergillus/Penicillium*-like spores, and 183 *Epicoccum* spp. spores. In the countable outdoor air samples, spore from *Cladosporium* spp. predominated in the winter and summer seasons, while basidiospores predominated in the fall and spring. For the culturable spores, the average outdoor air concentrations were 440 colony-forming units (CFU) per cubic meter of *Cladosporium* spp., 88 CFU of *Basidiomycetes*, 65 CFU of *Penicillium* spp., 15 CFU of *Epicoccum* spp., 14 CFU of *Alternaria* spp., and 13 CFU of *Aspergillus* spp. *Cladosporium* spp. were the predominating culturable fungal species isolated during the fall, spring and summer, while *Penicillium* spp. predominated in the winter.

COMMENTS: The "constant" within this study was the fact that all of the air samples were analyzed at the same laboratory. The authors did not make distinctions in the sampling accuracy, however, that might account for individual variability among the numerous people collecting and submitting the samples for analysis. This lack of control over the sampling process may have influenced or skewed the results of the study. The results of this study help in establishing a reference point for the fungal spore burden in the outdoor air throughout the study area, although these reference data should not be used as a substitute for collecting an outdoor air reference sample when conduction and indoor air quality investigation. The authors also noted that *Penicillium*, which is usually considered an indicator of water-damaged building materials in indoor environments when seen at dominant levels, grows naturally in various ecological niches and is considered another typical component of the outdoor aerospora.

### 13 Allergic fungal sinusitis in the southeastern USA: involvement of a new agent *Epicoccum nigrum* Ehrenb. ex Schlecht. 1824

AUTHORS: Noble JA, Crow SA, Ahearn DG, and Kuhn FA

JOURNAL: *Journal of Medical and Veterinary Mycology*

CITATION: 1997; 35:405-409

**STUDY TYPE AND OBJECTIVES:** This is an ecological study that compares fungal species isolated from the mucin collected from the maxillary and ethmoid sinuses of patients with allergic fungal sinusitis (AFS) with the fungal contaminants from indoor air samples of residences and buildings occupied by those patients. The primary objective was to correlate the contribution of indoor fungal contaminants to AFS.

**METHODS:** A total of 215 mucin samples were collected aseptically from the maxillary and ethmoid sinuses of 95 patients in the Augusta, Georgia, area. These samples were processed for isolation of fungal contaminants and plated on Sabouraud dextrose agar, Mycological agar, and Mycobiotic agar. Air samples in the patients' homes and or workplaces were collected with a single-stage Andersen air sampler using malt extract agar.

**RESULTS:** Of the 215 mucin samples collected from 95 patients, 137 of the samples were positive for one or more species of fungi. More than 40 different species of fungi were isolated from these patients, species of *Aspergillus* being the mostly common isolate. Mucin samples from 40 patients yielded the same species for fungi on at least two consecutive samplings. The densities of recoverable fungi from the patients were highly variable, yielding 15-4,500 colony-forming units per sample. Definitive quantitation was not possible. Air samples from the residences from eight of the nine patients with more intense symptoms yielded the same species recovered from the mucin of the corresponding patient. The predominating species isolated from these residences included *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp., *Alternaria alternata*, and *Epicoccum nigrum*. Viable indoor fungal densities ranged from 200 to 3,395 colony-forming units per cubic meter.

**COMMENTS:** The authors highlight that because many of the fungi from AFS patients in their study occurred commonly in the environments studied, they may have been transitory in the sinuses. The authors were thus unable to access the role of mixed species in the syndromes of their AFS patients. However, they also state that the repeated isolation of *Alternaria alternata*, *Aspergillus* spp., *Bipolaris spicifera*, *Curvularia lunata*, *Epicoccum nigrum*, and *Fusarium solani* from mucin samples in conjunction with direct observations of mycelium (selected samples) and the clinical syndromes, including positive skin tests, indicated that paranasal sinuses of AFS patients were colonized with these fungi. They further note that with the exception of *E. nigrum*, these species have been implicated previously as etiological agents of AFS. Finally, the authors conclude that this is the first report that *E. nigrum* can colonize nasal sinuses and cause AFS. One concern with this study is that the authors did not include outdoor air sampling results for comparison with the indoor environments they studied. Therefore, it is difficult to assess whether the nasal sinus colonization and AFS resulted from exposures in the outdoor environment or indoor environment. Clearly, this paper helps with the understanding of a fungal infection; however, it does not provide conclusive evidence on the source of the etiologic agent.

## 14 Clinical experience and results of a sentinel health investigation related to indoor fungal exposure

AUTHORS: Johanning E, Landsbergis P, Gareis M, Yang CS, and Olmsted E

JOURNAL: *Environmental Health Perspectives*

CITATION: 1999; 107:489-494

STUDY TYPE AND OBJECTIVES: This is a descriptive study of patients evaluated in an ambulatory occupational and environmental health clinic, including self- and physician-referred patients primarily from the northeastern United States. This study reviews the exposure conditions, clinical presentation, and morbidity of children and adults with indoor fungal exposure such as *Stachybotrys chartarum*. The study compares the health problems of a group of selected children who consulted an environmental health specialty clinic with adults having similar fungal exposure history.

METHODS: A health symptoms survey, which had been adapted with slight modifications from the Mount Sinai Medical Center Fungal Health Questionnaire, was used to assess health problems in the participants. Possible type I allergic reactivity, allergen-specific IgE, was tested in a panel for *Cheatomium globosum*; *Aspergillus fumigatus*; *Cladosporium*; *Penicillium notatum*; *Stachybotrys chartarum*; mouse, rat, and pigeon serum protein; and American cockroach. A panel of IgG was measured for *Micropolyspora faeni*; *Thermoactinomyces* spp.; *Alternaria alternata*; *Phoma herbarum*; *A. fumigatus*; *Aureobasidium pullulans*; *P. notatum*; *Trichoderma viride*; pigeon dropping; pigeon serum; bovine serum; *Cladosporium*; and *S. chartarum*. Indoor exposure to fungal materials was assessed and verified by generally relying on test methods described by the American Industrial Hygiene Association. No actual air sampling results (exposure data) was presented for the homes of the study participants. Exposure was based on a subjective review of living conditions.

RESULTS: The analysis of clinically recorded health symptoms and organ complaints of adult patients with a history of indoor fungal exposures showed a high prevalence (>50% of all respondents) of symptoms of upper and lower airway, eyes, and central nervous system. Children also showed a high prevalence of symptoms, although the absolute percentage rate was slightly lower than adults. Children appeared to complain slightly more about upper airway problems and slightly less about lower airway problems. Skin, eye, and central nervous system complaints were present in more than 40% of respondents, with similar high proportions for the adults. Five percent of the children had any evidence of specific IgE antibodies to tested fungal allergens (except *S. chartarum*), 9% had IgE reaction (borderline values), and none had an elevated IgG to *S. chartarum*. Based on a review of the available industrial hygiene data, about 90% of the children had verifiable exposure to higher-than-normal indoor levels of primarily *Penicillium* and *Aspergillus* spp., and at least 41% of the children had documented exposure to *S. chartarum*. Only two children and 13 adults had positive (abnormal) laboratory findings for the presence of IgE- and IgG-specific fungal antigens. In this small sample population neither the presence of positive antigen findings nor a fungal exposure history showed a statistically significant association with symptoms.

COMMENTS: The lack of quantitative exposure data complicates the interpretation of the results of this study. The fact that less than 25% of the participants had IgE- and IgG-specific fungal antibodies, used as exposure markers, suggests that exposures were not stimulating a statistically significant response to exposure.



## 15 Airborne *Cladosporium* and other fungi in damp versus reference residences

AUTHORS: Pasanen AL, Niininen M, and Kalliokoski P

JOURNAL: *Atmospheric Environment*

CITATION: 1992; 26:121-124

STUDY TYPE AND OBJECTIVES: This is a descriptive study designed to compare the differences in viable fungal spore concentrations in the air of damp residences and reference homes.

METHODS: The study was conducted during the winter month in Finland to control for the contribution of the outdoor air to the indoor viable spore counts. Spore counts in the outdoor air during winter are very low because of the snow cover and frozen soils. Twenty-five damp residences were included in the study. All 25 residences suffered from dampness problems caused by either improperly weatherproofed outside walls, missing or inadequate drainage, water leaks through the ceiling, or construction errors in insulation. Twenty-one reference residences were studied. Air samples for viable fungal spores were collected using a six-stage Andersen cascade, impaction sampler. The collection medium was modified Hagem agar.

RESULTS: The range of viable fungal spore counts was wide both indoors and outdoors. The total viable spore counts in the indoor air was slightly higher in the damp than in the reference residences. The highest counts of *Aspergillus* spp., *Cladosporium* spp., spores and yeast were also detected in the damp residences, whereas the counts of *Penicillium* spp. spores were somewhat higher in the reference residences. A statistically significant difference was observed only in the counts of *Cladosporium* spp. spores and yeast cells. No statistically significant differences were found in the outdoor data between the residences.

COMMENTS: In this study, the damp and the reference residences were similar with regard to age and location of building. Differences in the ventilation system might confound comparison between the damp and reference residences, because mechanical ventilation was more common in the reference residences. Mechanical ventilation systems have been noted to decrease indoor air fungal spore counts. This study confirms the author's previously reported finding that viable fungal spore counts are not always significantly higher in damp residences than reference ones. While the mean *Aspergillus* spp. spore counts were not significantly different between the damp and reference residences, the three highest counts were observed in the damp residences. These peak spore counts were a few orders of magnitude higher than the reference residences. The authors noted that spore release from a growing fungus at any given moment is strongly affected by humidity, air velocity, and other external factors, and one can therefore expect that even prolific fungal growth is only sporadically reflected in the indoor air spore counts.

## 16 Density and molecular epidemiology of *Aspergillus* in air and relationship to outbreaks of *Aspergillus* infection

AUTHORS: Alexander CA, Leenders CA, Van Belkum A, Behrendt M, Luijenduk AD, and Verbrugh HA

JOURNAL: *Journal of Clinical Microbiology*

CITATION: 1999; 37:1752-1757

STUDY TYPE AND OBJECTIVES: This is an epidemiological study of *Aspergillus fumigatus* and *Aspergillus flavus* infections in immunocompromised hospital patients. The study was designed to address three different issues, namely, to characterize the aerobiology of fungi in the air inside and outside of a hospital in Rotterdam (The Netherlands), to investigate whether the density of the conidia of *Aspergillus* species was higher than usual at the time of an outbreak of aspergillosis, and to investigate the role of genotyping fungal isolates in elucidating the epidemiology of *Aspergillus* species.

METHODS: Over a 62-week period, serial air samples of 1 cubic meter each were taken with a Surface Air System (SAS) sampler containing Sabouraud agar plates. On each occasion, four air samples were taken from the high-efficiency particulate air (HEPA) filtered room (a protected indoor environment), eight samples were taken from other site within the hematology ward, four samples were taken from the generally accessible sites within the hospital, and four samples were taken from locations outside of the hospital.

RESULTS: The median outdoor concentration of colony-forming units per cubic meter of air (CFU/m<sup>3</sup>) was more than 400. The median concentration inside the hospital, but outside the hematology ward, was 32 CFU/m<sup>3</sup>, while the samples from within the hematology ward showed a median of 7 CFU/m<sup>3</sup>. The median concentration within the protective room was <2 CFU/m<sup>3</sup>. The majority (.80%) of the airborne fungi were *Cladosporium* species. *Alternaria* and *Botrytis* species formed the majority of the remainder of isolates. During the surveillance period the concentration of *Aspergillus* species in the outdoor air remained relatively constant, as did the concentrations within the hospital. Within the protective room in the hematology ward, the concentration of *Aspergillus* was <1 CFU/m<sup>3</sup>. Most of the isolates from the outdoor air samples were *A. fumigatus* (>90%) and *Aspergillus niger* (5%).

COMMENTS: The protective benefits of engineering and administrative controls were demonstrated in this study. The fungal concentration showed a gradient reduction moving from the outdoors, into the general hospital, into the hematology ward, and finally into the protective HEPA filtered rooms. The authors conclude that the lower density of fungal spores in the hematology ward may be the result of environmental isolation (closed entrance doors, windows that cannot be opened, no plants or flowers, etc.). An even higher level of isolation exists within the HEPA filtered rooms, which resulted in very low numbers of spores. These findings are consistent with more recent studies in the United States. Increased filtration, direction airflow, minimizing infiltration of outdoor air, and controlling internal sources all contribute to significantly reduce fungal spore concentration in healthcare facilities.

## 17 Allergenic materials in the house dust of allergy clinic patients

AUTHORS: Barnes C, Tuck J, Simon S, Pacheco F, Hu F, and Portnoy J

JOURNAL: *Annals of Allergy, Asthma, and Immunology*

CITATION: 2001; 86:517-523

**STUDY TYPE AND OBJECTIVES:** This is an environmental study to measure the antigen concentration of several common environmental agents in house dust of children seen for asthma and children seen for other reasons. The study was designed to test the hypothesis that there is an association between the presence of fungal allergens in the environment and asthmatic disease in children.

**METHODS:** Dust samples for this study were solicited from patients recruited from a population seen in the allergy/immunology clinic at Children's Mercy Hospital, from staff of the hospital, and from a private allergy office. Individuals were instructed to bring a sample of dust taken from their vacuum cleaner. Samples were approximately 1 cup of dust. Individuals who submitted dust samples were asked to complete a short questionnaire concerning the source of the dust and the general environment. The dust samples were analyzed for fungal antigens specific to *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Candida albicans*. Also, antigens unique to *Dermatophagoides farinae* (dust mite), *Canis familiaris* (dog), *Felis domesticus* (cat), *Quercus alba* (oak), *Festuca eleator* (fescue grass), *Ambrosia artemisiifolia* (ragweed), *Plantago lanceolata* (plantain weed), and *Parapantaria Americana* (American cockroach) were measured. The study subjects were divided into asthmatic and nonasthmatic categories on the basis of the NIH criteria. A physician performed the diagnosis at the time the patient was seen.

**RESULTS:** Sixty-nine dust samples were collected for analysis, and 47 of these samples met the criteria for inclusion in the study. Fewer than 10% of samples tested had detectable levels of *Aspergillus*, American cockroach, plantain, and ragweed. Fewer than 20% of samples tested had detectable levels of *Candida* and fescue. In contrast, 100% of the homes had detectable levels of cat and more than 50% of samples contained measurable levels of *Alternaria*, *Cladosporium*, dog, and oak. There was a significant positive correlation in elevated levels of *Alternaria* and dust mite allergens in the homes of asthmatic patients. There were also several allergens in which the average values for asthmatics were lower, including cat, dog, and oak.

**COMMENTS:** The authors note that although several of the allergens measured were greater in the houses of asthmatic patients on average, the deviation in the measured values is great enough that the differences are not significant. The proposed explanation for the lower concentrations of cat, dog, and oak in asthmatic patient's homes is that asthmatic patients might tend to avoid exposure and thus not own pets. The authors conclude that the data from their studies support the hypothesis that fungal allergen exposure is an important component in the pathogenesis of the clinical condition known as asthma, and these data demonstrate the need for more extensive investigation. One concern with this study is the lack of control associated with the collection of the dust samples submitted by the patients. Collection efficiency almost certainly varied significantly between the vacuum cleaners used and the proficiency of the operator. In addition, quantitation of the allergens may have been impacted from storage, since several of the samples had been in the vacuum for as long as 1 year. All of these factors can impact the true comparability of the samples.

## 18 *Stachybotrys*: relevance to human disease

AUTHOR: Teer AI

JOURNAL: *Annals of Allergy, Asthma, and Immunology*

CITATION: 2001; 87:57-61

**STUDY TYPE AND OBJECTIVES:** This is a review article. The purpose of this review is to examine and critique the published literature on *Stachybotrys* for objective scientific and clinical evidence of disease caused by the presence of this fungal organism in the environment.

**METHODS:** Articles for review were obtained from all published research and reviews of *Stachybotrys* indexed in Medline since 1966. The publications used for this review were those that contained information about human health effects of the organism. The critique of these publications is the author's.

**RESULTS:** The author notes that, although *Stachybotrys* is rarely found in outdoor air (and when found, is present in low quantities), it is potentially an important contaminant of agricultural produce. The fungus has been cultured from soil and substrates rich in cellulose, such as hay and straw, cereal grains, plant debris, rice paddy grains, combine harvester wheat and sorghum dusts, palm debris, and broad bean seeds. *Stachybotrys* may be recovered from indoor air samples, but generally only when there is significant water damage and visible mold growth. Even under these conditions, its concentration is very low compared to the more common *Penicillium* and *Aspergillus* species. *Stachybotrys* is found worldwide in or on a variety of indoor items including urea-formaldehyde foam insulation, fiberboard, gypsum board, carpets, jute, vinyl and paper wall coverings, and other indoor building materials. *Stachybotrys* produces a wide array of mycotoxins with broad biological effects including, cytotoxicity, metabolic effects, hemolysis, plasmin effects, effects of the lung, immunologic effects, cytokine effects, effects on cholesterol, and neurologic effects. Toxic effects in humans can occur through ingestion, inhalation, and skin contact. Ingestion is the best-characterized route of exposure to *Stachybotrys* toxins.

**COMMENTS:** The author concludes that there is a clear discrepancy today between the public perception and the current available scientific and clinical evidence concerning the toxic health effects of *Stachybotrys*, especially as it affects occupants of buildings that have sustained water damage from leakage or groundwater intrusion. *Stachybotrys*, like any other fungus, has the capacity to generate a very large number and variety of chemicals with toxic potential to humans, provided that exposure is sufficient. Based on old reports ingestion of food prepared from *Stachybotrys*-contaminated grains may cause a toxic gastroenteropathy. There is no convincing evidence supporting toxic effects from *Stachybotrys* exposure in the indoor environment. No convincing cases of human allergic disease or infection from this mold have been published. The current public concern for adverse health affects from inhalation of *Stachybotrys* spores in water-damaged buildings is not supported by published reports in the medical literature.

## 19 Evaluation of *Stachybotrys chartarum* in the house of an infant with pulmonary hemorrhage: quantitative assessment before, during, and after remediation

AUTHORS: Vesper S, Dearborn DG, Yike I, Allan T, Sobolewski J, Hinkley SF, Jarvis BB, and Haugland RA

JOURNAL: *Journal of Urban Health: Bulletin of the New York Academy of Medicine*

CITATION: 2000; 77:68-85

STUDY TYPE AND OBJECTIVES: This was a prospective investigation of a home with an infant with pulmonary hemosiderosis. The study assessed the concentration and toxicity of *Stachybotrys chartarum* spores before, during, and after remediation to remove mold-infested wallboard and other building materials. The investigators also performed the initial parallel use of polymerase chain reaction (PCR) for the identification and enumeration of *S. chartarum* and a quantitative protein translation assay for trichothecene toxicity.

METHODS: Air samples were collected using either a cassette filter or BioSampler. Samples were collected for a period between 6 and 90 hours at 10 liters per minute. Quantification of *S. chartarum* spores was performed using an Applied Biosystems 7700 sequence detector. Dust samples were taken from carpet in the dining room (near the infants crib) and from the basement floor (near a water-damaged area). A one-square meter area was vacuumed with a filter apparatus connected to a vacuum pump. Collected spores were counted microscopically. Two direct vacuum samples of water-damaged wallboard were collected for mycotoxin analysis. These samples were subjected to an extraction procedure for analysis. The toxicity of *S. chartarum* was assessed through protein synthesis inhibition and *in vitro* hemolysis tests.

RESULTS: Results of air sampling indicated that the number of airborne *S. chartarum* spores was low before remediation. The number of spores in the air increased by a factor of 14-47 when the furnace blower was activated (typical conditions for the winter months). Airborne concentrations of *S. chartarum* during remediation averaged approximately 2,000 spores per cubic meter of air. Dust samples collected from the carpeting before remediation contained up to 2,000 *S. chartarum* spores per milligram of dust. The trichothecene toxicity of the air samples was low, ranging from 0.3 to 8.2 nanograms of T-2 toxin equivalents per cubic meter. The toxin levels from the direct vacuum samples from the contaminated wallboard were also low.

COMMENTS: The authors noted that, since the route of human exposure to *S. chartarum* has been assumed to be by inhalation, it was surprising that, in a house so heavily contaminated with *S. chartarum*, so few spores were detected in the air before remediation began. This finding was consistent with other investigations in similarly affected homes. Despite this finding, the authors concluded that the very large quantities of *S. chartarum* spores found in the carpet dust samples suggested that surface dust may compose a significant secondary reservoir of spores. The authors suggested that spores in settled dust may be reaerosolized by human activity, resulting in possible inhalation exposures. The significant increase in airborne spore levels during remediation documents the elevated exposure potential associated with disturbance of materials contaminated with visible mold growth, and documents the need to use appropriate remediation protocols to contain the contaminants and protect the building occupants. The recommendations of the New York City Health Department were utilized in this study. The efficacy of the remediation was demonstrated when the follow-up samples, taken 30 days after remediation, were negative for *S. chartarum* spores.

## 20 Airborne microbial contaminants in indoor environments: Naturally ventilated and air-conditioned homes

AUTHORS: Kodama AM and McGee RI

JOURNAL: *Archives of Environmental Health*

CITATION: 1986; 41:306-311

STUDY TYPE AND OBJECTIVES: This is a environmental study designed to compare the atmospheric burden of biologic or viable particles in condominium residences with central air-conditioning to that of more open or "leaky" style (naturally ventilated) of Hawaiian homes.

METHODS: Two-stage models of the Andersen Microbial Air Samplers were used to collect respirable and nonrespirable-size bacteria and fungi in the living room air. Outdoor samples were taken near the intakes of the air conditioning systems for the condominium residences and on the porches or balconies of the naturally ventilated homes. Sabouraud's Dextrose agar was used for the fungal counts. The authors also used a questionnaire, patterned after a survey adopted by the British Medical Research Council, to assess respiratory health status from the occupants of the study buildings.

RESULTS: Some 16 genera of fungi were isolated during this study. Approximately 80% of the fungi collected were of respirable size. *Cladosporium* sp. and *Aspergillus* sp. accounted for about three-fourths of the respirable-size fungi found in the outdoor air. Fungal counts and their distribution in the air of homes with natural ventilation were comparable to those in the outdoors. The numbers of fungi were less in the air-conditioned residences than those in the outdoor air. *Cladosporium* sp. were present in much lower numbers in the air conditioned residences than in the outdoor air. On the other hand, the air-conditioned residences were found to contain more *Aspergillus* sp., even when the total number of fungi were fewer than in the outdoor air. From the health effects questionnaire, when asked to indicate the presence or absence of symptoms commonly attributed to indoor air pollutants, residents of air-conditioned homes responded in the affirmative more often than those occupants of naturally ventilated homes.

COMMENTS: The authors note that it is not surprising that the fungal counts and their distribution were fairly comparable between the outdoors and air in homes with natural ventilation. They also conclude that while considerable variability was obtained in the results, the data suggest that the filters in air conditioning systems effectively screen out many of the fungi found in the outdoor air. They go on to state that, while the filtration units of central air-conditioning systems appear to remove most of the biologic or viable particulates in the outdoor air, conditions somewhere downstream from the filters are sufficiently favorable to support growth of certain microorganisms, which in turn influence the characteristics of air quality in air-conditioned residences. Finally, the authors state that it is not possible to draw any causal relationship between the type of residential ventilation and the health complaints of the occupants on the basis of their study. This study provides data relevant to the residential indoor environment. Specifically, it illustrates that occupant control related to "naturally" ventilating their home could have a significant impact on the indoor environment. In addition, reliance on air-conditioning can change the indoor environment as compared to the "natural" baseline. However, it is not yet possible to assess the full potential impact of this changed environment.

## 21 **Black mold and human illness**

AUTHORS: Texas Medical Association's Council on Scientific Affairs

JOURNAL:

CITATION: 2002; CSA Report 1-1-02

STUDY TYPE AND OBJECTIVES: This is a review paper that is based on a search of the medical and scientific literature and discussions with experts and specialists. The objective was to update the "state of medical science" regarding potential health effects associated with exposure to *Stachybotrys chartarum*.

METHODS: The authors conducted a comprehensive review of the medical and scientific literature.

RESULTS: The authors concluded that the public concern for health effects from inhalation of *Stachybotrys* spores in water-damaged buildings is generally not supported by published reports in the scientific literature. The specific conclusions of this report were:

1. Adverse health effects from inhalation of *Stachybotrys* spores in water-damaged buildings is not supported by available peer-reviewed reports in medical literature.
2. The probability and possibility of causation or exacerbation of a medical condition due to exposure to mold in indoor environments currently exists only for the following:
  - Traditional Type I immune reactions (*i.e.*, allergies, with correlation of symptoms with exposure and *in vitro* demonstration of IgE antibodies by allergy skin testing or RAST test for specific IgE antibodies in blood samples); or
  - Rare Type III immune reactions (*e.g.*, hypersensitivity pneumonitis), pulmonary hemorrhage in infants associated with mycotoxins.
  - For *Stachybotrys* or other molds to be implicated in other disease models, the following must be present:
    - Peer-reviewed medical literature should show clearly that such mold or mold by-product has produced clinical manifestations similar to those displayed by the patient;
    - Evidence of personal causation showing strong links to environmental exposures.

COMMENTS: The authors recommend that remediation of water damage in homes and other buildings should generally be based on non-clinical factors, unless clear medical evidence exists to demonstrate the role of *Stachybotrys* in a particular case of illness. This recommendation is consistent with the current mold remediation guidance documents (*e.g.*, Environmental Protection Agency and New York City Health Department), which focus on removal of visible mold growth regardless of whether there are health complaints from the building occupants.

## 22 Adverse human health effects associated with molds in the indoor environment

AUTHORS: Hardin BD, Kelman BJ, and Saxon A

JOURNAL: *Evidence Based Statements: American College of Occupational and*

*Environmental Medicine*

CITATION: 2002 ACOEM

STUDY TYPE AND OBJECTIVES: A literature review designed to evaluate the current scientific information related to the adverse health effects associated with indoor molds. The primary objective was to support the development of guidelines for addressing these adverse effects.

METHODS: Literature review with the development of a position statement.

RESULTS: The authors concluded that molds are common and important allergens, with about 5% of individuals predicted to have some allergic airway symptoms from molds over their lifetime. The authors noted, however, that molds are not dominant allergens, and that outdoor molds rather than indoor ones are the most important. The reactions for almost all allergic individuals are limited to rhinitis or asthma; sinusitis may occur secondarily due to obstructions. The authors also concluded that fungi are rarely significant pathogens for humans, with only a limited number of pathogenic fungi such as *Blastomyces*, *Coccidioides*, *Cryptococcus*, and *Histoplasma* infecting non-immunocompromised individuals. In contrast, persons with severely impaired immune function, *e.g.*, cancer patients receiving chemotherapy. Organ transplant patients receiving immunosuppressive drugs, AIDS patients, and patients with uncontrolled diabetes, are at significant risk for more severe opportunistic fungal infection. Therefore, except for persons with severely impaired immune systems, indoor mold is not a source of fungal infections. Finally, the authors concluded that the causal association between indoor exposure and adverse health effects remains weak and unproven, even though some molds that propagate indoors may under some conditions produce mycotoxins. The authors stated that the levels of exposure in the indoor environment, dose-response data in animals, and dose-rate considerations suggest that delivery by the inhalation route of a toxic dose of mycotoxins in the indoor environment is highly unlikely at best, even for the hypothetically most vulnerable subpopulations. The authors concluded that the current scientific evidence does not support the proposition that human health has been adversely affected by inhaled mycotoxins in home, school, or office environments.

COMMENTS: The authors provided an excellent, objective critique of the current scientific literature. The authors attempted to bring concerns about the health effects of indoor molds into a proper perspective. Nonetheless, Hardin *et al.* concluded that mold growth in the home, school, or office environment should not be tolerated because mold physically destroys the building materials on which it grows, mold growth is unsightly and may produce offensive odors, and mold is likely to sensitize and produce allergic responses in allergic individuals.



## 23 State of the Science on Mold and Human Health

AUTHORS: Redd SC

JOURNAL: *Statement for the Record before the Subcommittee on Oversight and Investigations and Housing Community Opportunities, Committee on Financial Services, United States House of Representatives*

CITATION: 2002 Centers for Disease Control and Prevention (CDC)

STUDY TYPE AND OBJECTIVES: This is a position statement based on a literature review. The objective was to present the CDC perspective on the state of the science related to mold and health effects in people, efforts to evaluate health problems associated with molds, collaborations related to molds and people's health, and next steps regarding mold and health.

METHODS: This is a literature review methodology, assessing the state of the science.

RESULTS: As discussed below, the authors describe the health consequences that may be associated with exposure to molds. They note that exposure to mold does not always result in health problems. In the review of efforts to evaluate the health problems associated with molds, the CDC reviewers and an external panel of experts determined that, contrary to a previous report, there was insufficient evidence of any association between exposure to *Stachybotrys chartarum* or other toxic fungi and idiopathic pulmonary hemosiderosis in infants. They note that they plan to further evaluate the relationship between pulmonary hemorrhage and *Stachybotrys chartarum* through state-based surveillance, further investigation of identified disease clusters, and focused research studies. In research in "problem" buildings with significant moisture and mold problems the CDC found that there were significant relationships between reports of work-related respiratory disease and visual assessment of water and mold-damage in two studies; there were significant relationships between endotoxin and ultra-fine particles in air and work-related respiratory symptoms; and there were significant relationships between indicators of mold in chair and floor dust and work-related respiratory symptoms.

COMMENTS: The authors conclude that people who are exposed to molds may experience a variety of illnesses. Fungi account for 9% of hospital-acquired infections. Ingestion of food contaminated with certain toxins produced by molds is associated with development of human cancer. Many respiratory illnesses among workers may be attributed to mold exposures.

Uncommon illnesses that collectively can be called hypersensitivity pneumonitis are caused by chronic exposures to high concentrations of mold and are almost exclusively limited to certain agricultural workers in particularly moldy environments. Common illnesses caused by molds include allergic conditions such as hay fever and asthma. Therefore, routine measures should be taken to prevent mold growth indoors because some people are, or may become, allergic to it. The authors note that there are a number of barriers that need to be overcome in investigating the possible effects of mold on health. There are no accepted standards for mold sampling in indoor environments or for analyzing and interpreting the data in terms of human health. It is not known what quantity of mold is acceptable in indoor environments with respect to human health. Because of difficulties related to sampling for mold, most studies have tended to be based primarily on baseline environmental data rather than human dose-response data. For these reasons, and because individuals have different sensitivities to molds, setting standards and guidelines for indoor mold exposure levels is difficult and may be impractical.

## 24 Indoor exposure to molds and allergic sensitization

AUTHORS: Jacob B, Ritz B, Gehring U, Kock A, Bischof W, Wichmann HE, and Heinrich J

JOURNAL: *Environmental Health Perspectives*

CITATION: 202; 101:647-653

STUDY TYPE AND OBJECTIVES: This was an environmental study of effect of ambient air pollutants on respiratory health and atopic diseases in German school children. The study was designed to examine the relationship between viable mold levels indoors and allergic sensitization. The specific objective was to determine whether allergic sensitization in children is associated with higher fungal spore count in settled house dust samples from the living room floors.

METHODS: The study population and selection of homes was based on previous cross-sectional surveys that studied the long-term health effects of ambient air pollutants in German school children. The authors selected affected (case) and unaffected (control) children; cases were defined as children who could be classified as atopic or with a physician diagnosis of asthma. Dust samples were collected from the living room floor (97% were carpeted floors) by vacuuming an area of 1 square meter for 2 minutes in a highly standardized manner using the same vacuum cleaner. Thirty milligrams of sieved house dust was processed for identification and quantification of viable molds. The culture medium was dichloro-18% glycerol agar (DG18). Only dust samples taken in the winter (November-April) were analyzed to minimize the influence of seasonal variation.

RESULTS: There was a large variation in mold levels between households. The geometric mean concentration was 81,367 colony-forming units per gram (CFU/g) in the sensitized case homes and 71,118 CFU/g in the control homes. No samples were free of molds. *Cladosporium*, *Penicillium*, and *Aspergillus* were the prevalent molds isolated from both case and control households. High levels of *Cladosporium* (35,000 CFU/g dust or >90 percentile) in wintertime household dust approximately tripled the risk of allergic sensitization in children. *Aspergillus* spores increased the risk of allergic sensitization at a somewhat lower level, i.e., when the spore count increased above 25,000 CFU/g dust. Sensitization of exposed children was not limited to *Cladosporium*. Rather, children exposed to increased viable mold levels were more likely to be sensitized to other allergens as well, such as pollen, cat, or house dust mites. For *Penicillium* and also for total molds counts, they found slightly increased sensitization risks with exposure at high levels of mold spores in winter, but the estimates were imprecise. In summer, however, *Penicillium* was the most important indoor contributor to overall sensitization.

COMMENTS: The authors found that allergic sensitization was significantly associated with exposure to one or more genera of indoor mold spores. They state that high indoor mold exposure (90th percentile) seems to contribute to allergic symptoms and disease in both sensitized and nonsensitized children; however, because numbers in the nonsensitized group studied were small, effect estimates were imprecise or even nonestablishable in this subgroup. The authors conclude that their results suggest that indoor mold spore exposure, mainly during winter, might increase the risk of sensitization to all allergens in children. Unfortunately, their findings are limited by the difficulties associated with quantifying molds and by the relatively small number of homes that were studied.

## 25 Populations and Determinants of Airborne Fungi in Large Office Buildings

AUTHORS: Chao JH, Schwartz J, Milton DK, and Burge HA

JOURNAL: *Environmental Health Perspectives*

CITATION: 2002; 110:777-782

**STUDY TYPE AND OBJECTIVES:** This was a longitudinal exposure assessment study using repeated bioaerosol sampling protocols. The primary objective was to relate the bioaerosol sampling results to other simultaneously collected environmental measures. This study was part of a larger epidemiologic study to evaluate the role of bioaerosol exposure to building-related symptoms.

**METHODS:** Twenty-one offices, in four buildings, were investigated during a full year of operation. Intensive environmental sampling was conducted every 6 weeks at each location. Bioaerosol samples were collected using single-stage Andersen N-6 samplers employing malt extract agar and dichloran glycerol-18 agar (DG18). Continuous monitoring of temperature, RH, and carbon dioxide levels was accomplished using HOBO-Temp, HOBO-RH, and a GMW21 CO<sub>2</sub> transmitter, respectively.

**RESULTS:** Airborne fungal concentrations, RH, and temperature varied significantly by season. Airborne fungal concentrations and RH were highest in the summer and lowest in the winter. Temperature, and the contrary, was slightly higher in the winter than in summer. There was no significant seasonal variation in CO<sub>2</sub> concentrations. The mean airborne fungal concentration during the full year a testing was 42 colony-forming units per cubic meter. The median concentration was 22 colony-forming units per cubic meter. A total of 32 fungal taxa were observed in the airborne fungal samples. The predominating fungal taxa were *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., and *Penicillium* sp.

**COMMENTS:** The authors note that there are essentially no fungus-free environments in our daily lives. Fungal spores are abundant in the outdoor air, and exposure to fungi and their metabolites occurs commonly in indoor environments. The median concentration of 22 colony-forming units per cubic meter was characterized as very low according to commonly used standards/guidelines. The maximum fungal count, 618 colony-forming units per cubic meter, was not indicative of serious indoor contamination. However, the authors note that a multi-building study reporting similar airborne fungal levels found that BRSs were positively associated with airborne fungi. Total fungal concentrations were negatively related to CO<sub>2</sub> concentrations. Because CO<sub>2</sub> is inversely associated with ventilation, this finding suggests that outdoor air might be a source for indoor fungi in these buildings with no strong internal sources. This study provides important information on airborne fungal concentration over time, in relatively clean office environments. The findings confirm the correlations between airborne fungi and environmental parameters (i.e., CO<sub>2</sub> and RH) longitudinally. These environmental variables have been associated with perceptions of health in office occupants. The use of standardized sampling protocols to allow interstudy comparison with large-scaled cross-sectional U.S. EPA BASE studies should be encouraged.

## 1 Adult-onset asthma is associated with self-reported mold or environmental tobacco smoke exposures in the home

AUTHORS: Thorn J, Brisman J, and Torén K

JOURNAL: *Allergy*

CITATION: 2001; 56:287-292

STUDY TYPE AND OBJECTIVES: This retrospective, nested case-control study evaluated the relationship between self-reported adult-onset asthma and indoor environmental exposures such as smoking, dampness, and mold growth.

METHODS: A sample population (n=15,813), aged 20-50 years, was investigated using a comprehensive questionnaire. The questionnaire addressed 10 types of potential exposure in the home. Complete responses were received from 174 respondents with "physician-diagnosed" (or adult-onset) asthma. There were 870 randomly selected respondents without such a diagnosis that constituted the control group. The researchers determined the risk of having reported a physician-diagnosis of asthma in relation to specific reported exposures in the home environment. The authors controlled for the effects of occupational exposures, age, gender, smoking habits, and atopy.

RESULTS: The study reported statistically significant odds ratios (*i.e.*, increased risk) for physician-diagnosed asthma in individuals with exposure to environmental tobacco smoke (ETS) in "never smokers," presence of a wood stove, and visible mold growth in the home. Visible dampness also was associated with an increased risk for reporting a diagnosis of adult-onset asthma. When the analysis adjusted for the confounding effects of gender, it was found that males were more susceptible to ETS and visible mold growth in the home, while females were more susceptible to the presence of wood stoves. Visible dampness was no longer statistically significant when the analysis adjusted for gender.

COMMENTS: The researchers acknowledged that this type of retrospective, case-control design did not allow for objective exposure analysis and that the time period of exposure may be more important than the actual presence of exposures at the time of diagnosis. The authors addressed the problem of how over-reporting of exposures may affect the outcome of their study by stating: "small amounts of fungi around bathtubs and window sills are easily seen and perhaps frequently reported, but they may not influence the total levels in any important way." This study, while interesting, raised many questions but did not contain enough reliable information to produce definite conclusions about causality. The use of questionnaires alone leaves the reader to wonder how valid the study is due to possible misclassification of data, and the effects of variables that were not evaluated.

## 2 Aerobiology of pollen and fungal allergens

AUTHOR: Bush RK

JOURNAL: *Journal of Allergy Clinical Immunology*

CITATION: 1989; 84:1120-1124

STUDY TYPE AND OBJECTIVES: This review paper, while slightly dated, provided an excellent overview of the pollen and fungal types, morphology, aerodynamic properties, sizes, and limited information on the volumetric air sampling methods available at that time.

METHODS: Literature review.

RESULTS: Some of the topics discussed by the author include the premise that indoor mold/fungi growth is not possible without sufficient heat and humidity. The author reported that "below 30% humidity there is little mold growth." Fungi reproduce through the process of fragmentation or sporulation. Fragmentation involves breakage of the fungal body, while sporulation involves the development of specialized cells on fruiting branches. Due to the wide variety of shapes and sizes, many spores cannot be completely identified without the associated fruiting body. Settling plate-type samplers, while relatively efficient for collecting fungal spores greater than 10 micrometers in diameter (such as *Alternaria*, *Epicoccum*, *Stemphylium* and *Curvularia*), are stated to exhibit losses as high as 90% for smaller spores. These "smaller spores" include important genera such as *Cladosporium*, *Aspergillus*, and *Penicillium*. Therefore, in general, suction sampling is the method of choice for spores. Culturing also has its limitations. The author reported, "many fungus colonies inhibit the growth of other fungi within a distance of several millimeters. Furthermore, some spores are not living at the time of sampling. Finally, other fungi, although theoretically capable of growth, require highly specific substrates."

COMMENTS: It is interesting to note that this paper indicates that even in the late 1980s it was felt that passive air sampling methods were not recommended collection techniques. The researcher recommends the use of suction methods that quantify the volume of air sampled. The researcher also notes that culturable and non-culturable information should be considered, as many fungi grow best on a specific substrate. This paper also provides a good (although relatively short) discussion of how seasonal variations affect outdoor fungal levels and how relative humidity indoors affects fungi growth. The reader should be aware that this paper has few references to support specific claims.

### 3 Aerobiology of the indoor environment

AUTHOR: Burge HA

JOURNAL: *Occupational Medicine: State of the Art Reviews*

CITATION: 1995; 10:27-40

STUDY TYPE AND OBJECTIVES: This review paper discusses the critical elements that affect the dispersion, transport, and collection of biologic aerosols from the air.

METHODS: The critical factors of aerosol science, the biologic aspects of bioaerosols, exposure/dose relationships, characteristics of the indoor aerosol, and principles of representative monitoring are discussed.

RESULTS: The section on particle size gives an excellent discussion on how particle size affects collection efficiency for different collection devices. It also explains that there is very little known about the nature of fungi particle surface properties, electrostatic charges, and aerosol concentrations. Due to these unknowns, the authors of this article feel that concentrations and changes of patterns of concentrations that might occur in an environment cannot be predicted. The next section of this paper discusses the biologic aspects of bioaerosols. Viable cells sampling factors are discussed such as environmental agents in the air that can render the cells nonviable, hydration issues in regard to sampling media, nutrient pH, temperature, and radiation from the surrounding environment. Useful generalities include: 1. Damaged organisms repair themselves best on low-nutrient media such as R2A(bacteria), 2% malt extract agar (fungi). 2. Most airborne fungi that will grow in culture do best at a pH of 6.0 or lower, bacteria favor a pH of about 7.0. 3. Fungi usually grow best between 18-25°C. Environmental bacteria also grow over this range but will also grow at 30°C, a temperature that will limit the growth of fungi. 4. Some fungi require low water media (media with high concentrations of sugar or glycol) for growth, including some important *Aspergillus* species, particularly *A. restrictus* and members of the *A. glaucus* group. 5. Highly selective media underestimate not only the total aerosol but also the desired organisms. For instance, MacConkey's agar underestimates concentrations of gram-negative bacteria. These media should only be used when other organisms are present that will interfere with recovery of the desired organism. 6. Some fungi require light for spore production; bacteria should be incubated in the dark. 7. Fungi should always be incubated in the upright position. If fungal plates are upside down, spores will fall into the lids, and spore-bearing structures may be deformed. Bacterial plates can be incubated upside down if fungi are unlikely to be recovered on the plates. Dilute antigen solutions are known to degrade rapidly unless protective substances are added. Such degradation might become a concern if liquid-based methods such as liquid impingers or electrostatic samplers are used for collection. In general, one can infer the presence of endotoxins by the presence of gram-negative bacteria, since the toxin is an integral part of the cell wall of all these organisms.

COMMENTS: General concepts of exposure levels and dose-response relationships are discussed. But no specific information is provided since little is known of these relationships. The final sections of this paper cover sampling techniques in a very broad method that brings very little new information to light.

#### 4 Airborne endotoxin concentrations in different work conditions

AUTHORS: Liesivuori J, Kotimaa M, Laitinen S, Louhelainen K, Pönni J, Sarantila R, and Husman, K

JOURNAL: *American Journal of Industrial Medicine*

CITATION: 1994; 25:123-124

STUDY TYPE AND OBJECTIVES: This review paper compared the results of previous studies in an attempt to determine the effect of different work environments on endotoxin concentrations.

METHODS: Literature review of studies pertaining to endotoxins, which are thought to be present on the surface of gram-negative bacteria. Airborne endotoxin concentrations were measured in several studies at a regional occupational health institute in Finland from 1986 to 1991 as part of a program to correlate exposure to organic and microbial dust with reported respiratory effects. The program focused on farming, farming-related industries, paper, pulp, sawmills, and wastewater treatment plants.

RESULTS: The authors concluded that the variation in endotoxin concentrations could only partly be explained by differences in work conditions. Other substances, such as peptidoglycan and lipoteichoic acids of bacteria, when present in relatively large concentrations (3-6 orders of magnitude), were thought to influence the specificity of certain toxicity tests (*i.e.*, the LAL test). Exposure to airborne endotoxins may cause acute reactions such as organic dust toxic syndrome (ODTS) and inflammation, whereas long-term health effects depend on the quality, quantity, and duration of the microbial exposure, as well as individual factors. The researchers concluded in this paper that their experience, and the available literature, indicated that the risk of ODTS is great from occupational exposure to emptying grain dryers, where the highest endotoxin concentrations were measured.

## 5 Assessment of exposure to indoor air pollutants

AUTHORS: Jantunen M, Jaakkola JJK, and Krzyzanowski, M

JOURNAL: *World Health Organization Regional Office for Europe*

CITATION: 1997; 78: 1-139

STUDY TYPE AND OBJECTIVES: This monograph was produced by the World Health Organization as an extension of a series of reports published by the Regional Office to address indoor air quality and methods of assessing exposure. Chapters 20, 21, and 22 focused specifically on biological contaminants.

RESULTS AND COMMENTS: Chapter 20 (Pages 99-103) of this document dealt with the sources, health effects, and exposure assessment of bacteria and fungi in the indoor environment. The authors discussed the range of sampling and analytical methods required to assess microorganism exposure in the home, and the need to develop the sampling strategy and methods around the goal of the sampling event (typically the detection of an "intramural source" of bioaerosols). Difficulties in correctly identifying and quantifying the bioaerosol(s) in question also were discussed. This chapter gives a general overview and directives on the covered topics. Chapter 21 (Pages 104-108) of this document focused on dust mites as a common source of indoor allergens. Dust mites can be found in virtually every home, and the authors regarded dust mites as the most important allergen associated with asthma. Factors influencing exposure included humidity and, to a lesser extent, temperature. The altitude was considered a secondary factor as it has a direct effect on ambient humidity and temperature. The authors admitted that little has been done in the area of personal exposure monitoring for dust mites, that quantifying dust mite exposure is extremely difficult, and that indirect measures of mite allergens are typically used in health assessments. Chapter 22 (Pages 109-111) of this document addressed the determinants of microbial growth in the indoor environment. This chapter stressed the fact that, of the essential agents required for microbial growth, moisture is the only one that can be denied to microorganisms in buildings. The authors also stated that high levels of humidity can result in high moisture content in porous building materials, although leaks, condensation, water seepage by capillary action, and water use in kitchens and bathrooms can wet building materials and keep them moist, regardless of the relative humidity of the air. The authors stressed that visual observations are often more useful than direct reading instruments in determining both past and present moisture problems.



## **6 Asthma symptoms and the sick building syndrome—the significance of microorganisms in the indoor environment**

AUTHORS: Norback D, Edling C, and Wieslander G

JOURNAL: *Air Quality Monographs*

CITATION: 1994; 2, 229-239.

**STUDY TYPE AND OBJECTIVES:** The purpose of this study was to examine possible relationships among asthma symptoms, sick building syndrome (SBS) symptoms, and indoor exposures in a random sample of the general population in Sweden. The modifying effects of seasonal variations on the relation between symptoms and indoor exposure also were studied.

**METHODS:** The study was conducted with one thousand subjects answering a standardized self-administered questionnaire that contained questions on different sick building syndrome and asthma symptoms as well as questions on building age, type of ventilation systems, air treatment (e.g., *humidification* or air conditioning), presence of wall-to-wall carpets, presence of newly painted surfaces, and signs of microbial growth, malodors, or building moisture during the past 12 months.

**RESULTS:** Results found a positive relation between asthma symptoms and two workplace exposures, environmental tobacco smoke (ETS) and fresh paint, and a pronounced seasonal modifying effect on these relations. It was found that asthmatics were more often aggravated by ETS during the winter, and that a positive relation between newly painted surfaces at the workplace and asthmatic symptoms occurred during the spring. The authors reported that the significant relationship between self-reported sick building symptoms and signs of building moisture or microbial growth agreed with earlier investigations. The authors suggested that exposure indicators other than signs of dampness or the concentration of airborne viable molds must be identified in future studies in order to obtain a better understanding of the health effects of buildings with indoor mold growth. Some of the indicators that call for further research included microbial volatile organic compounds, endotoxins, glucans, and mycotoxins. The authors suggested that the role of seasonal variation may explain why contradictory results are sometimes published on the effect of particular indoor exposures on asthma or so-called sick building syndrome.

## 7 Biodiversity and concentration of airborne fungi in a hospital environment

AUTHORS: Rainer J, Peimtnner U, and Pöder R

JOURNAL: *Mycopathologia*

CITATION: 2000; 149: 87-89

STUDY TYPE AND OBJECTIVES: This research study evaluated the biodiversity and concentration of fungal populations in a special care unit of a hospital.

METHODS: Air sampling was performed for 6 months in a corridor that was accessible to visitors and in an adjacent bone marrow transplant unit. Sampling was conducted using an air sampler and two isolation media for growth studies.

RESULTS: The study identified 98 fungal species, including *Aspergillus fumigatus* and *Aspergillus terreus*. These fungi were of concern, since cases of invasive aspergillosis had increased with the rising number of transplantations performed at the hospital. The results from monitoring suggested that there might be a possible influence of human activities on diurnal changes of fungal propagule concentrations. There was a wide range of colony-forming units identified on samples grown with DG18 medium, which may have been due to one particular sampling having very high counts of *Wallemia sebi*. This observation points to the variability of sampling that may take place with culturable samples.

COMMENTS: This study did not present comparisons of fungal measurements to "typical" levels of the identified fungi found in the surrounding (outdoor) environment. The authors observed that the methods used in this study were suitable for detecting important pathogens because infection of immunocompromised patients occurs only with living, viable spores.

## 8 Density and molecular epidemiology and *Aspergillus* in air and relationship to outbreaks of *Aspergillus* infection

AUTHORS: Leenders A, Belkum A, Behrendt M, Ludendijk AD, and Verbrugh H

JOURNAL: *Journal of Clinical Microbiology*

CITATION: 1999; 37:1752-1757

STUDY TYPE AND OBJECTIVES: This research paper discussed a 62-week monitoring program conducted to determine the concentrations of pathogenic and nonpathogenic fungal conidia inside and outside the University Hospital of Rotterdam (Netherlands).

METHODS: Sampling methods included the use of serial air samples of 1 cubic meter each, collected with a Surface Air System on agar plates. Four samples were collected in each high-efficiency particulate air (HEPA) filtered room. Additionally, eight samples were collected from other sites within the hematology ward (which was physically separated by closed doors from the rest of the hospital), four samples from other sites within the hospital, and four samples from outside the hospital. Air samples were collected twice a week for the first 2 months of the study, and once a week thereafter.

RESULTS: The program determined that outside levels of the pathogenic fungi *A. flavus* and *A. fumigatus* were higher in summer than winter. Variations in outside levels also were found across the two summers studied, possibly because of different weather conditions. The indoor levels in the air that was filtered by the hospital's general ventilation system were lower than outside levels. The researchers concluded that the levels in the environmentally isolated hematology lab were lower than in the general hospital environment. This was suspected to be because of the closed entrance doors, sealed windows, and absence of plants and flowers. The lowest levels were found in the even more environmentally isolated HEPA-filtered rooms.

## 9 Detection of microbial volatile organic compounds (MVOCs) produced by molds on various materials

AUTHORS: Fiedler K, Schutz E, and Geh S

JOURNAL: *International Journal of Hygiene and Environmental Health*

CITATION: 2001; 204:111-121

STUDY TYPE AND OBJECTIVES: This research study focused on screening 12 fungal species for microbial volatile organic compounds (MVOCs), some of which may emit odors.

METHODS: Fungi were grown on malt extract agar, yeast extract glucose chloramphenicol agar, beech wood sticks, and wood shavings from various conifers. Extraction of the volatile substances was performed by a Solid Phase Micro-Extraction system, and Gas Chromatography/Mass Spectrometry was used to detect MVOCs.

RESULTS: Results show that not a single compound was common to all species, but 1-octen-3-ol, 3-octanone, 2-methyl-1-butanol, and 3-methyl-1-butanol occurred in the large majority of samples. The authors described these compounds as "indicator substances" for mold growth. Every examined species had a defined chromatographic pattern. It was possible to identify MVOCs derived from *Aspergillus versicolor* within a mixed culture due to its chromatographic fingerprint in the range of sesquiterpenes. MVOCs released from two species of one genus may be very similar. The presence and also the concentration of the MVOCs strongly depended on the age of the mold culture. In particular aliphatic and aromatic ether and sulphur compounds were found in older cultures, while geosmin was found to be characteristic of the initial growth stage of *P. expansum*. Under conditions of low air exchange (accumulation of carbon dioxide), the production of secondary metabolites changed and resulted in the formation of aliphatic and aromatic alcohols and numerous esters. Differences in the production of volatile organic compounds occurred when the medium was changed. The authors claimed that the average total MVOC concentration was lower on building materials that are low in nutrients (e.g., wood) than on substrata rich in nutrients (e.g., agar), although there were no quantitative data presented in this paper to support this observation.

COMMENTS: The researchers showed that it is possible to identify fungal species from both single and mixed cultures by monitoring typical MVOCs and MVOC patterns. A more refined method for sampling and analyses may allow detecting the type and intensity of mold contamination in a room with masked fungal growth. One hypothesis is that MVOCs may reveal the stage of infestation at the time of indoor air sampling, since characteristic MVOCs are produced at different times of the growth process. In the case of masked mold growth, MVOC analyses may be helpful to locate the contaminated materials due to the dependence of MVOC emission on nutrient media. A relationship between MVOCs and any known health condition was not presented in this study. The authors also proposed that sesquiterpenes and diterpenes may act as allergens, but did not provide evidence to support this theory.

## 10 Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID: a new feasible approach for the detection of an exposure to indoor mold fungi?

AUTHORS: Elke K, Begerow J, Oppermann H, Krämer U, Jermann E, and Donemann L

JOURNAL: *Environmental Monitor*

CITATION: 1999; 1:445-452

STUDY TYPE AND OBJECTIVES: This paper described a new method of sampling for microbial volatile organic compounds (MVOCs) that uses a passive sampling method.

RESULTS AND COMMENTS: Some fungi have been identified to produce a limited number of VOCs. The authors felt that a great advantage is gained by using long sampling times that are difficult to achieve with active sampling but are very easy to achieve with passive sampling. The theory is expounded that detection of MVOCs is a more reliable method of detection than spore levels because MVOCs are produced throughout the growth cycle of fungi whereas sporulation is intermittent only. This theory has some merit, although it relies on unsupported assumptions that are sometimes directly contradicted by physical evidence. The assumption that MVOCs are produced throughout the growth cycle of fungi was neither supported by any data in the paper nor any existing literature. MVOC release usually depends on the solubility of the particular compounds in the media as well as temperature and atmospheric conditions. The authors claimed that "Dust precipitate samples are thus more suitable to assess the chronic fungal burden, because they are more independent from the time of sampling." While this observation was accurate, the authors failed to make note of the fact that while dust measurements record whether or not contamination exists in the area and how much, normal dust samples do not render any information about the airborne levels of the agents of concern. Without these airborne concentration levels, any prediction of the dose is not possible and thus any health effects cannot be attributed reliably to these agents. A relatively inexpensive tape sample may provide information about the extent of contamination and types of spores and growths that are causing the contamination. The measurement of MVOCs has theoretical merit as an indicator of fungi growth in some cases, but additional research is needed to determine which particular VOCs are MVOCs and how the fate and transport of these MVOCs are affected by other compounds emitted from natural and manufactured building products and environmental factors. The described method of detection of MVOCs has merit as one tool to investigate fungal growth issues, but many of the methodological advantages claimed in this paper are misleading and in some cases even false. Measuring marker substances or agents that are minor contributors may not be as useful as assessing the major agents of concern, which are the airborne biologic particles.

## 11 Environmental mycology and its importance to public health

AUTHORS: Mishra SK, Ajello L, Ahearn DG, Burge HA, Kurup VP, Pierson DL, Price DL, Samson RA, Sandhu RS, Shelton B, Simmons RB, and Switzer KF

JOURNAL: *Journal of Medical and Veterinary Mycology*

CITATION: 1992; 30:287-305

STUDY TYPE AND OBJECTIVES: The purpose of this review paper was to examine the various aspects of environmental mycology in relation to human health.

RESULTS AND COMMENTS: The article addressed the relationship between indoor mold and sick building syndrome (SBS). The authors concluded, "although SBS has been more often related to chemical or 'stale air' problems than to the presence of fungi, adverse allergic and toxic effects of fungal exposure for certain individuals is beyond question." The authors also concluded that "future investigations of fungal associations with SBS should include generic and specific identifications of all the probable causative organisms and of the prevailing ecological conditions, including substrates for fungal growth and production of volatile toxic chemicals as metabolic by-products." Diseases caused by common airborne fungi include systemic fungal diseases such as blastomycosis, coccidioidomycosis, histoplasmosis, and paracoccidioidomycosis. The authors concluded that "relatively few, it is now evident, are capable of causing primary mycotic diseases in individuals with no obvious or discernible defects in their immune system." Mishra *et al.* recommended that we should strive to reduce the number of circulating fungal propagules with the use of proper filtration of the air and lowering moisture levels. The authors emphasized that fungal allergens and exposure patterns had not been adequately studied. The authors acknowledged that "patients with a history of symptoms that would lead one to suspect mold allergy often do not react to skin prick tests using available materials." The lack of correlation between symptoms and objective findings may be related to the paucity of information about exposure patterns to fungal species. The authors suggested that the fungal allergens typically selected for skin testing and immunotherapy may have been based on biased and incomplete exposure studies. This review article documented that airborne fungi present in the work-related, recreational or living environment, are able to cause diseases under certain occupational circumstances. The primary diseases that have been documented are types of hypersensitivity lung diseases, farmers' lung, mushroom-worker's lung, maple bark disease, and sauna-taker's lung. The authors discussed four devices in common use for the measurement of viable airborne fungi: Reuter centrifugal air sampler (RCS); Andersen single or six-stage impactor; surface air sampler (SAS); and slit-to-agar air sampler. The RCS, Andersen, and slit samplers produced similar results on indoor samples but varied greatly when used for outdoor samples. Sampling volumes affected results more in indoor locations than outdoor locations. The use of malt extract, dichloran glycerol, and dichloran rose Bengal agars produced no significant differences in observed samples.

## 12 Exposure to airborne fungi, MVOC, and mycotoxins in biowaste-handling facilities

AUTHOR: Fischer G

JOURNAL: *International Journal of Hygiene and Environmental Health*

CITATION: 2000; 203:97-104

STUDY TYPE AND OBJECTIVES: This study investigated the presence of mycotoxins in bioaerosols in compost facilities.

METHODS: Sampling was conducted in January, April, July, and October during a period of 2 years in a compost facility. Samples were collected at three locations: the loading area, the compost pile hall, and the storage area. The sampling medium was Dichloran Glycerol 18% agar with 100 ppm chloramphenicol. Other media were used to optimize the growth of specific fungal strains. MVOC and mycotoxin production from isolated fungal strains was studied using a variety of pure cultures. Chemical analysis for VOCs and MVOCs was conducted using thermal desorption in combination with Gas Chromatography/Mass Spectrometry. Airborne particulates were collected using a dust sampler and analyzed for secondary metabolites (e.g., mycotoxins) using high-pressure liquid chromatography with diode array detection.

RESULTS: The greatest numbers of mesophilic species (i.e., those fungi that thrive at room temperature) were found in samples from the loading area, followed by the compost pile hall and the storage area. For the thermotolerant species (i.e., those fungi that require high temperatures to grow, such as *Aspergillus fumigatus*, *Paecilomyces variotii*, and *Rhizopus oligosporus*), the values in the loading area were nearly as high as in the compost pile hall during the 2-year sampling period. Seasonal variations found in 1997 were not observed in 1998 due to different annual weather patterns. *A. fumigatus* showed seasonal variations in the samples from the loading area, with the number of colony forming units being several fold greater in the spring than in the summer. A wide range of MVOCs was measured from fungi in pure culture. The authors reported that "MVOC profiles resulted in characteristic fingerprints for most species tested." The spectra of volatiles changed with differing nutritional conditions, but some compounds were produced on all substrata. At each season and on every sampling site, the authors detected the VOCs 2-methylfuran,  $\alpha$ -pinene, camphene, and limonene.  $\alpha$ -pinene and limonene are thought to be derived from plant material in higher amounts, although these compounds also are regularly produced by a number of different fungal species. The authors sought to detect toxic forms of secondary metabolites from *A. fumigatus* but were unable to find any in the samples that were tested. In conclusion, species composition depends on factors such as composition of biowaste, weather conditions, intervals of biowaste collection, and type of process engineering. Species composition may also change throughout the year or during the different phases of composting. The presence of spores of certain species may indicate the degree of decomposition and the hygienic quality of the compost. This study quantified neither MVOCs nor mycotoxins. This study did not investigate whether there was a relationship between secondary metabolites of fungi and human health.

### 13 Indoor air-related effects and airborne (1-3)- $\beta$ -D-Glucan

AUTHOR: Rylander R

JOURNAL: *Environmental Health Perspectives*

CITATION: 1999 Jun; 107(Supplement 3):501-3

STUDY TYPE AND OBJECTIVES: This review article examined the relationship of (1-3)- $\beta$ -D-Glucan to fungal biomass by comparing data from several previous studies.

METHODS: Literature review.

RESULTS AND COMMENTS: The author noted that (1-3)- $\beta$ -D-Glucan is a polyglucose compound present in the cell walls of fungal cells. Rylander indicated that (1-3)- $\beta$ -D-Glucan was a difficult compound to measure, and several different methods were described. None of the analytical methods used has a high degree of reliability or reproducibility. Aggressive air sampling techniques were used in some of the reviewed studies that attempted to relate total biomass to the (1-3)- $\beta$ -D-Glucan levels. According to Rylander, the researchers felt that a more accurate representation of the total fungal mass might be obtained by re-suspending settled materials. This is a somewhat questionable assumption; aggressive air sampling may skew data by artificially elevating bioaerosol particles. An effort also was made to relate the (1-3)- $\beta$ -D-Glucan levels found to the symptoms of building occupants. The general conclusion of Rylander was that there is a relationship between the (1-3)- $\beta$ -D-Glucan levels and the total biomass of fungal material in a structure, and that this relationship was correlated to complaints of respiratory problems, fatigue, and headache. The author even claimed that in some cases a dose-response relationship could be established. The author drew conclusions from a limited amount of data that did not have a high degree of precision.

### 14 Indoor fungal exposure—does it matter, and what can be done about it?

AUTHOR: Burr ML

JOURNAL: *Clinical and Experimental Allergy*

CITATION: 1999; 29:1442-1444

COMMENTS: This paper is a good general summary of the issues involved with measuring exposure concentration, known health effects, and determinants of indoor fungal exposure. It referenced numerous studies involving these topics. It also critically examined the lack of scientific evidence supporting various claims that have been made regarding human health and exposure to indoor molds.

## 15 Outdoor allergens

AUTHOR: Burge HA

JOURNAL: *Environmental Health Perspectives*

CITATION: 2000; 108:653-659

**STUDY TYPE AND OBJECTIVES:** This paper reviewed the nature and patterns of outdoor allergens and the existing evidence regarding an association between outdoor allergens and allergic disease, particular asthma. Burge discussed pollen allergens, pollen prevalence patterns, the nature of fungal spores and their allergens, fungal prevalence patterns, other allergen bearing particles, transport and removal of airborne allergen-bearing particles, predictive modeling, exposure concerns related to particle size, and the relation of asthma to outdoor allergen exposure and its mitigating factors. This paper also provided a thorough overview of the nature and prevalence of pollens with interesting examples of how man has possibly altered the natural pollen levels in many areas. It also gave a general overview of the nature of fungal spores, their allergens, and patterns of prevalence. A discussion of examples of human activities that create allergen clouds was also given in the section on other allergen-bearing particles.

**RESULTS AND COMMENTS:** Burge provided a general overview of the issues involved in transport and removal of spores. The author also described predictive modeling of spore concentrations and emphasized that about 25% of the published models fail and are available for only a few particle types. In the section on exposure, particle size considerations were discussed. Burge pointed out that even though large pollen particles are not considered respirable, they are still inhalable and may penetrate into the distal lower airways. In the discussions on personal exposure and indoor exposure, the observation was made that the short-term peak exposures may be sufficient to induce serious symptoms, which may persist during the non-peak periods. This section also contains a discussion of monitoring for outdoor allergen particles and emphasized that the prevalent methods are to use either arm impactors or suction spore traps. Burge reported that suction spore traps may be more efficient than rotating arm impactors, but they collect fewer than 50% of particles smaller than 5 micrometers, thereby providing little information about many small, potentially important fungal spores. The final section addressed areas for future research. The statement was made that "to date, a clear relationship between exposure, sensitization, and symptoms has not been made for any outdoor allergens." The case was also made for the need for low-cost yet sensitive personal exposure monitoring.



**16 Review of methods applicable to the assessment of mold exposure to children**

**AUTHORS:** Dillon HK, Miller JD, Sorenson WG, Douwes J, and Jacobs RR

**JOURNAL:** *Environmental Health Perspectives*

**CITATION:** 1999; 107:473-480

**STUDY TYPE AND OBJECTIVES:** This paper discussed the assessment of the exposure of children to fungi, substances derived from fungi, and the environmental conditions that may lead to exposure.

**RESULTS AND COMMENTS:** The planning and conducting of exposure assessments section provided a reasonably good explanation of what to look for and the tools needed to do a visual assessment of a site for fungi activities. The discussion of sampling strategies, however, was inadequate. The authors briefly discussed individual components of sampling, such as air sampling, surface sampling, and bulk sampling. The authors did not acknowledge that a thorough sampling plan would contain all of these methods of sampling plus spore trap vacuum samples. The authors also did not discuss that sampling sites should be chosen in such a way that the results of each sample can be correlated together. The authors also failed to highlight that any air sampling locations indoors must be chosen with regard to the building HVAC system and configuration of the interior building space. The article contained a detailed discussion of the available methods of air monitoring for viable fungal spores and the methods used for culturing fungi. The authors pointed out some of the limitations that monitoring and culturing factors pose on the quantitative measurement of viable biologic particles in the air. The authors also discussed the issues associated with surface sampling and bulk sampling for viable biologic particles on these materials. In addition, the collection of non-viable direct microscopic examination samples and the advantages and disadvantages were discussed. The authors failed to address the correlation of data from different procedures, such as concurrent non-viable spore trap samples and surface samples. The authors also introduced the different types of markers such as MVOCs, glucose polymers such as (1-3)- $\beta$ -D-Glucans, mold extracellular polysaccharides, mycotoxins, and fungal allergens. A discussion of exposure measurement methods alluded to the need for personal sampling that has been stressed in many papers. The authors also recognized the validity of using fixed-point sampling to confirm potential exposure and the use of settled dust samples such as tape sampling and surface swabs for providing evidence of exposure integrated over time. The authors also recognized the fact that direct measurement of viable biologic samples was superior to measurement of only marker agents and the importance of these samples to speciation of fungi.

**17 Review of quantitative standards and guidelines for fungi in indoor air**

AUTHORS: Rao C and Burge HA

JOURNAL: *Journal of the Air & Waste Management Association*

CITATION: 1996; 46:899-908

STUDY TYPE AND OBJECTIVES: This 1996 review article summarized the existing standards and guidelines that were available for the interpretation of fungal levels.

RESULTS AND COMMENTS: The authors categorized quantitative standards and guidelines (S/Gs) for fungi as either absolute or relative. On the one hand, "absolute S/Gs specify levels of total or specific fungi in the air that are considered acceptable or not acceptable," whereas "Relative S/Gs are based on the relationship between indoor and outdoor levels as represented by simultaneously collected samples." The authors reported that only Russia had established an official quantitative standard concerning fungi in the air. Many different agencies have issued guidelines for fungi. The article listed guidelines that have been issued by governmental agencies, individuals, and organizations that conducted research sponsored by governmental agencies. Rao and Burge discussed various limitations of existing quantitative S/Gs, such as the lack of information on human dose/response relationships for fungi in air, reliance on short-term grab samples analyzed by culture only, and the absence of standardized protocols for data collection analysis, and interpretation. The article also contained a discussion of allergic reactions and mycotoxins.

**18 The relation between fungal propagules in indoor air and home characteristics**

AUTHORS: Ren P, Jankun TM, Belanger K, Bracken MB, and Leaderer BP

JOURNAL: *Allergy*

CITATION: 2001; 56:419-424

STUDY TYPE AND OBJECTIVES: This prospective cohort study used questionnaires to assess the relationship between home characteristics and household aeroallergen exposure levels. A large sampling base (1,000 homes and 3,998 samples) was issued questionnaires and correlations were assessed for mold growth and factors such as air conditioning, moisture and humidity conditions, and the presence of pets, plants, and humans. Air sampling was followed by culturing of fungi with DG-18 or MEA agars.

RESULTS AND COMMENTS: The authors concluded that "the presence of fungal propagules in indoor air cannot be reliably predicted by home characteristics." The authors recommended that air sampling followed by culturing of fungi were required for proper fungal exposure assessment. The paper established the most likely occurring fungal propagules in homes in the north-east quadrant of the United States and provided a percentage breakdown by genera. Over 100 species of fungi were grown from samples. Various Yeasts, *Cladasporium*, *Penicillium*, and *Aspergillus* species were among the fungi that were detected in the most homes. There was a great deal of variability in the colony-forming units (CFUs) from different homes as well as from different rooms within the same home. Greater numbers of CFUs grew on MEA agar as compared to DG-18. The authors concluded that measurement of fungal levels in homes was more reliable than questionnaires for assessing residential exposure in epidemiologic studies. They further stated that fungal concentrations showed substantial variations or trends among seasons. The use of air conditioning, heating, and humidifiers was not found to be a significant predictor of airborne fungal components.

## 19 Toxic-metabolite-producing bacteria and fungus in an indoor environment

AUTHORS: Peltola J, Andersson MA, Haahtele T, Mussalo-Rachamaa H, Rainey FA, Kroppenstedt RM, Samson RA, and Salkinoja-Salonen M S

JOURNAL: *Applied and Environmental Microbiology*

CITATION: 2001; 67: 3269-3274

STUDY TYPE AND OBJECTIVES: This paper described a case report and follow-up toxicology study of a single home for six toxic bacteria.

METHODS: Indoor air was sampled using a six-stage Andersen sampler. The bacterial isolates were cultured on tryptic soy agar plates, and fungi were grown on cornmeal agar for 10 days. The growths were then harvested, extracted, evaporated, dissolved in a methanol solution, and assayed for inhibition of boar spermatozoa motility, a classic toxicology test. Bacterial isolates were members of the genera *Bacillus*, *Nocardia*, *Gordonia*, *Rhodococcus*, *Dietzia*, *Micrococcus*, *Methylobacterium*, and *Flavobacterium*. A species of *Trichoderma* was the only fungus that was identified and studied.

RESULTS AND COMMENTS: The home was a detached house with natural ventilation, built in the 1950s. The family had lived in the home since 1986 and recently built an extension. Water damage was detected in 1993 and was remediated by installing a subsurface drainage around the house. In 1996, water damage was noticed in the basement bathroom, in the roof, and in the outdoor wall of the extension. One of the occupants complained about health symptoms such as exacerbations of asthma, sinusitis, urticaria, blocked nose, rhinitis, otitis, hoarseness, and ache in joints, myalgia, and tiredness. Damage to the cell structure of boar spermatozoa was observed following administration of extracts from samples containing *Trichoderma* and *Bacillus* species. The authors concluded that these extracts affected the occupant of the house, exposing the person to multiple, differently acting toxins of microbial origin as well as to potential pathogens. There was no direct evidence that any substance extracted from the house caused damage to the occupant.

## 20 Update on airborne mold and mold allergy

AUTHOR: Chapman JA

JOURNAL: *Allergy and Asthma Proceedings*

CITATION: 1999; 20:289-292

STUDY TYPE AND OBJECTIVES: This review article discussed the need to assess exposure potential and perform clinical testing in considering fungal sensitivity in patients.

RESULTS AND COMMENTS: The author expressed the opinion that air sampling is necessary because indoor sources of mold-related allergens may not be visible. The article discussed the differences between the collection efficiencies of rotorod impaction systems that were referenced in the Aeroallergen Monitoring Network of the Aerobiology Committee of the American Academy of Allergy, Asthma and Immunology as well as the standard suction samplers. Chapman supported the universal use of the impaction sampler to improve the uniformity of data collection. The author discussed the problems with proper testing for mold-specific allergic reactions using skin testing. Chapman encouraged the evaluation of outdoor versus indoor contributing factors. Local exposures in the outdoors that need to be recognized by treating physicians were identified. The author also recommended avoidance of fungal sources to reduce health effects of patients proven sensitive to fungal antigens.

## 21 Volatile metabolites from mold growth on building materials and synthetic media

AUTHORS: Wilkins K, Larsen K, and Simkus M

JOURNAL: *Chemosphere*

CITATION: 2000; 41:437-446

STUDY TYPE AND OBJECTIVES: This research study inspected the microbial volatile organic compounds (MVOCs) produced by mold species commonly found in damp buildings.

METHODS: Spores from *Stachybotrys*, *Penicillium*, *Aspergillus*, and *Trichoderma* species originally derived from damp buildings were grown on sterile building materials and some synthetic media. Extractions from successful cultures were analyzed for MVOCs using Gas Chromatography with Mass Spectrometry. The production of MVOCs under wet and dry conditions was studied to mimic weather conditions.

RESULTS AND COMMENTS: The authors found that the patterns of the MVOCs given off were media dependent. Over 20 MVOCs were studied, and the authors observed different MVOC profiles from the individual mold genera studied. The authors acknowledged, however, that it would be difficult to use MVOCs to predict the types of molds present from an unknown source. The authors also noted that there was no reliable relationship between "growth intensity, sporulation intensity, and volatile metabolite excretion."

## 22 The relation between fungal propagules in indoor air and home characteristics

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JOURNAL: *Allergy*

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STUDY TYPE AND OBJECTIVES: This prospective cohort study used questionnaires to assess the relationship between home characteristics and household aeroallergen exposure levels. A large sampling base (1,000 homes and 3,998 samples) was issued questionnaires and correlations were assessed for mold growth and factors such as air conditioning, moisture and humidity conditions, and the presence of pets, plants, and humans. Air sampling was followed by culturing of fungi with DG-18 or MEA agars.

RESULTS AND COMMENTS: The authors concluded that "the presence of fungal propagules in indoor air cannot be reliably predicted by home characteristics." The authors recommended that air sampling followed by culturing of fungi were required for proper fungal exposure assessment. The paper established the most likely occurring fungal propagules in homes in the northeast quadrant of the United States and provided a percentage breakdown by genera. Over 100 species of fungi were grown from samples. Various Yeasts, *Cladasporium*, *Penicillium*, and *Aspergillus* species were among the fungi that were detected in the most homes. There was a great deal of variability in the colony forming units (CFUs) from different homes as well as from different rooms within the same home. Greater numbers of CFUs grew on MEA agar as compared to DG-18. The authors concluded that measurement of fungal levels in homes was more reliable than questionnaires for assessing residential exposure in epidemiologic studies. They further stated that fungal concentrations showed substantial variations or trends among seasons. The use of air conditioning, heating, and humidifiers were not found to be significant predictors of airborne fungal components.

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CITATION: 2001; 67: 3269-3274

STUDY TYPE AND OBJECTIVES: This paper described a case report and follow up toxicology study of a single home for six toxic bacteria.

METHODS: Indoor air was sampled using a six-stage Andersen sampler. The bacterial isolates were cultured on tryptic soy agar plates and fungi were grown on cornmeal agar for 10 days. The growths were then harvested, extracted, evaporated, dissolved in a methanol solution, and assayed for inhibition of boar spermatozoa motility, a classic toxicology test. Bacterial isolates were members of the genera *Bacillus*, *Nocardia*, *Gordonia*, *Rhodococcus*, *Dietzia*, *Micrococcus*, *Methylobacterium*, and *Flavobacterium*. A species of *Trichoderma* was the only fungus that was identified and studied.

RESULTS AND COMMENTS: The home was a detached house with natural ventilation, built in the 1950s. The family had lived in the home since 1986 and recently built an extension. Water damage was detected in 1993 and was remediated by installing a subsurface drainage around the house. In 1996, water damage was noticed in the basement bathroom, in the roof, and in the outdoor wall of the extension. One of the occupants complained about health symptoms such as exacerbations of asthma, sinusitis, urticaria, blocked nose, rhinitis, otitis, hoarseness, and ache in joints, myalgia, and tiredness. Damage to the cell structure of boar spermatozoa was observed following administration of extracts from samples containing *Trichoderma* and *Bacillus* species. The authors concluded that these extracts affected the occupant of the house, exposing the person to multiple, differently acting toxins of microbial origin as well as to potential pathogens. There was no direct evidence that any substance extracted from the house caused damage to the occupant.

## 24 Update on airborne mold and mold allergy

AUTHOR: Chapman, JA

JOURNAL: *Allergy and Asthma Proceedings*

CITATION: 1999; 20:289-292

STUDY TYPE AND OBJECTIVES: This review article discussed the need to assess exposure potential and perform clinical testing in considering fungal sensitivity in patients.

RESULTS AND COMMENTS: The author expressed the opinion that air sampling is necessary because indoor sources of mold-related allergens may not be visible. The article discussed the differences between the collection efficiencies of rotorod impaction systems that were referenced in the Aeroallergen Monitoring Network of the Aerobiology Committee of the American Academy of Allergy, Asthma and Immunology as well as the standard suction samplers. Chapman supported the universal use of the impaction sampler to improve the uniformity of data collection. The author discussed the problems with proper testing for mold-specific allergic reactions using skin testing. Chapman encouraged the evaluation of outdoor versus indoor contributing factors. Local exposures in the outdoors that need to be recognized by treating physicians were identified. The author also recommended avoidance of fungal sources to reduce health effects of patients proven sensitive to fungal antigens.

## 25 Volatile metabolites from mold growth on building materials and synthetic media

AUTHORS: Wilkins K, Larsen K, and Simkus M

JOURNAL: *Chemosphere*

CITATION: 2000; 41:437-446

STUDY TYPE AND OBJECTIVES: This research study inspected the microbial volatile organic compounds (MVOC's) produced by mold species commonly found in damp buildings.

METHODS: Spores from *Stachybotrys*, *Penicillium*, *Aspergillus*, and *Trichoderma* species originally derived from damp buildings were grown on sterile building materials and some synthetic media. Extractions from successful cultures were analyzed for MVOCs using Gas Chromatography with Mass Spectrometry. The production of MVOCs under wet and dry conditions was studied to mimic weather conditions.

RESULTS AND COMMENTS: The authors found that the patterns of the MVOCs given off were media dependent. Over 20 MVOCs were studied and the authors observed different MVOC profiles from the individual mold genera studied. The authors acknowledged, however, that it would be difficult to use MVOCs to predict the types of molds present from an unknown source. The authors also noted that there was no reliable relationship between "growth intensity, sporulation intensity and volatile metabolite excretion."

**1 Hypersensitivity pneumonitis from ordinary residential exposures**

AUTHORS: Apostolakis MJ, Rossmore H, and Beckett WS

JOURNAL: *Environmental Health Perspectives*

CITATION: 2001; 109(9):979-981

STUDY TYPE: Case report.

**BACKGROUND:** The authors described a previously healthy 50-year-old woman who developed progressive cough and dyspnea, which responded to oral corticosteroids.

**METHODS:** The patient's high-resolution CT scan chest revealed ground glass opacities bilaterally and peripherally. Pulmonary function tests were normal, but the patient experienced oxygen desaturation after a brief period (2-minutes) of brisk walking. Her serum contained precipitating antibodies to *Aureobasidium pullulans* and *Saccharopolyspora rectivirgula*.

The unfinished basement of the house had evidence of mold growth on the cement wall and fiberglass insulation mounted on plastic sheeting which covered all four walls. Fiberglass was cultured and produced:

- $3.5 \times 10^5$  CFU fungi/gm of *A. pullulans*;
- $4.4 \times 10^4$  CFU/gm *Humicola* species; and
- $1 \times 10^3$  CFU/gm of *Actinomyces* with morphology similar to *S. rectivirgula*.

**RESULTS:** The patient's symptoms cleared completely on prednisone as did the objective findings, however, symptoms relapsed when prednisone was discontinued. Her symptoms improved rapidly with reinstitution of prednisone, which was continued until the home was sold and the patient moved. In the new residence, the patient remained well while maintaining her previous employment, although not all of her respiratory function tests returned to normal (*i.e.*, diffusing capacity for carbon monoxide "remained abnormally low"). A confounding factor was the indoor exposure to 30-34 cans of insecticide, because of fleas on the pet dog and cat living in the home. Each can contained 71 grams of 1% pyrethrins and permethrins. These cans had been activated "several months" before the diagnosis of hypersensitivity pneumonitis (HP). The patient had left the home for the recommended 30 minutes and did not report any exacerbation of symptoms with the use of these products. The authors acknowledged that these substances have been associated with HP in two previous case reports.

**CONCLUSIONS:** HP is a form of allergic irritation to the alveoli of the lungs that has been reported to be associated with many inhaled biologic materials and occasional industrial chemicals. In North America, home exposures have only occasionally been causal except for those associated with a specific contaminated aerosol from either a humidifier or an air conditioner. This case suggested that home mold exposures due to moisture problems might be associated with HP. This case report did not distinguish between the effect of exposure to indoor molds and exposure to dust mites.

## 2 Asthmatic symptoms and indoor levels of micro-organisms and house dust mites

AUTHORS: Bjornsson E, Norback D, Janson C, Widstrom J, Palmgren U, Strom G, and Boman G

JOURNAL: *Clinical and Experimental Allergy*

CITATION: 1995; 25:423-431

STUDY DESIGN: Cohort with clinical and industrial hygiene endpoints.

OBJECTIVE: This is an investigation of 88 randomly selected residents of Uppsala, Sweden, who were studied to determine if there was a relationship between symptoms and objective signs of asthma and indoor exposure to micro-organisms (*e.g.*, bacteria and mold), house dust mites (HDM), and indoor dampness.

METHODS: The study was performed by experienced investigators. Airborne microorganisms were collected on sterile polycarbonate filters (pore size of 0.4  $\mu\text{m}$ ) and a sampling rate of 2.5-liter/minute over a 1.5-hour period. Airborne microorganism levels were assessed using epifluorescence microscopy. The authors reported that this technique was comparable to other microscopy techniques for estimating the concentration of microorganisms. Viable molds and bacteria were identified after being cultured on two different media. The detection limit of viable microorganisms was 50 colony-forming units (CFU)/ $\text{m}^3$  of air. The following clinical tests were conducted: skin prick to assess allergen-specific reactions; lung function and bronchial challenge testing to assess respiratory function; and blood testing for IgE antibody levels. Questionnaires were issued that addressed both health and indoor environmental topics.

RESULTS: The study found that:

- The relative humidity was above 33% in all houses and above 45% in 85% of all homes.
- HDM levels had statistically significant positive correlation coefficients with both air humidity and age of the house.
- Total levels of mold had a weak but statistically significant positive correlation coefficient with air humidity. The positive correlation coefficient for total mold concentration and total levels of bacteria was stronger (*i.e.*, more reliable) and it, too, was statistically significant.
- Asthma-related symptoms were reported by 47 (53%) subjects.
- HDM level was found to be an independent risk factor for asthma symptoms; however, none of the inhabitants in the mite-infested homes had an immediate positive skin-prick test to HDM.
- The objective asthma measurements (*i.e.*, FEV1, bronchial hyperactivity (BHR) or peak expiratory flow variability) were not positively correlated with indoor measurements of microorganisms.
- No correlations were found between keeping cats or dogs and respiratory symptoms.
- Airborne bacterial levels showed a positive correlation to asthma-related symptoms.
- The total level of mold was NOT an independent risk factor using multivariate analysis, but showed a relationship to asthma symptoms using univariate analysis.

CONCLUSIONS: Home dampness may be correlated with increased reporting of respiratory symptoms. In this study there was no correlation between objective asthma measurements relative to the presence of microorganisms or HDM.



### 3 Dampness in buildings and health

AUTHORS: Bornehag C-G , Blomquist G, Gyntelberg F, Järnholm B, Malmberg P, Nordvall L, Nielsen A, Pershagen G, and Sundell, J

JOURNAL: *Indoor Air*

CITATION: 2001; 11:72-86

STUDY DESIGN: Literature review.

OBJECTIVE: This is an exhaustive, in-depth, critical review of the medical literature published prior to July 1998.

METHODS: A literature search identified 590 articles:

- 477 articles were excluded because they were reviews, case studies, studies on occupational exposure, or studies on mites.
- 113 studies were evaluated by the review group.
- 52 of these articles were excluded because they were judged as “non informative or inconclusive.” Those articles are listed in their table 6.
- Thus, 61 studies provided the foundation for their review.

RESULTS: The authors described four sources of dampness and moisture in buildings:

1. Leakage of rain and snow into the building construction or moisture from the ground (outdoor sources);
2. Moisture from humans and indoor activities, e.g., cooking, bathing, human expiration, humidifiers (indoor sources);
3. Moisture within building materials in constructions from erection time due to materials that have not been protected against rain and snow or have not had sufficient drying out (building sources); and
4. Water leakage from pipes, flooding, etc. (accidents).

The reviewers divided the studies into four categories according to the design of each study. By doing so, studies that were based on self-reporting of symptoms were differentiated from studies based on objective observations and measurements. In the authors' discussion section, they reported that the studies included more than 100,000 humans and showed that, with few exceptions, dampness in buildings was associated with respiratory symptoms such as cough, wheeze, and asthma. The authors also discussed whether the association between dampness and health may be due to causal links or the result of several forms of bias generated by patient selection, information, or confounding.

CONCLUSIONS: The authors' conclusion was that strong evidence supported an association between indoor dampness and respiratory symptoms, although the indoor air agents that cause the health effects had not been determined. Mite sensitization may explain some but probably not all associations. The authors discussed some studies suggesting an increased risk associated with the presence of both airborne molds and bacteria.

- There is a lack of knowledge about the mechanisms behind the association, but this should not preclude intervening when there are moisture problems in buildings.
- To gain more information about mechanisms, future research should test new hypotheses such as:
  1. Effects of specific chemical and microbiologic agents; and
  2. Studies of interventions against dampness.

## 4 Health effects of indoor molds

AUTHOR: Burr ML

JOURNAL: *Reviews on Environmental Health*

CITATION: 2001; 16(2)97-103

STUDY DESIGN: Review.

OBJECTIVE: To review the molds that primarily grow indoors which are health problems, identified as *Penicillium*, *Cladosporium*, and *Aspergillus* in Europe, North America, Australia, and Israel.

METHODS: The author reviewed a variety of topics relating to the indoor factors that function as determinants of mold growth. The author observed that humidity, poor ventilation, structural disrepair that permits the penetration of water from outside, carpets, and pets were associated with higher mold levels. Burr also discussed the different techniques for measuring molds, including the limitations of each method. The author concluded that the limitations of both objective and questionnaire methods contributed to the poor correlation between the different methods. The health effects of indoor molds were another major focus of the article. Burr reviewed 15 articles on the relationship between mold and human illness that had been published in the prior 15 years. The reports were divided into those in which an association was sought by patient-reported symptoms and those investigations in which there were objective health indices.

CONCLUSIONS: The author found that the evidence linking indoor mold with adverse health effects was not wholly conclusive. The author observed that different sources of bias affect both the assessment of mold and the health indices. The author also noted that differentiating between allergy to molds and allergy to mites is difficult because dampness favors both sources of allergens and that most mold-sensitive persons are also sensitive to mites. The author believed it was possible that there is a publication bias, with a tendency for positive but not negative findings to be published. Burr stated that "it seems likely that indoor molds do affect health, taking all the evidence together." The author suggested that a randomized trial of mold eradication in the homes of patients with mold-sensitive asthma would greatly clarify the issue.

COMMENTS: The problem with the author's recommendation for a randomized trial of mold eradication to gain further understanding is that efforts to reduce humidity and eliminate molds also would reduce mite levels, so it would be impossible to discern which biological source caused the health complaints.

## **5 The role and abatement of fungal allergens in allergic diseases**

**AUTHORS:** Bush RK and Portnoy JM

**JOURNAL:** *Journal of Allergy and Clinical Immunology*

**CITATION:** 2001; (107)3:S430-S440

**STUDY DESIGN:** Review.

**OBJECTIVE:** The goal of this article was to review the following:

- Existing methods for measuring exposure to fungi in the indoor environment, including markers that may be measured for the presence of fungi;
- The relationship of indoor mold to either toxic or adverse immunologic effects; and
- The reliability and sufficiency of the evidence addressing a direct cause and effect relationship.

**METHODS:** The authors addressed the following aspects of assessment of fungal exposure:

- Volumetric air samplers: Total spore counts or individual genera can be microscopically identified from collections using volumetric samplers, which sample a known volume of air.
- Questionnaires.
- Biochemical and immunochemical markers for fungal presence: These tests may offer an indication of the extent of contamination of an indoor environment when specific assays potentially relevant fungal species may not be available. Although these markers do not measure fungal allergen exposure, they have been reported to be indicators of the presence and relative extent of fungal growth. Markers include ergosterol, extracellular polysaccharides, (1-3)- $\beta$ -D-Glucan, volatile organic compounds, and mycotoxins.
- Fungal allergen-specific immunoassays: Allergen-specific immunoassays were used to measure the concentration of fungal sensitizers. In dust, the brief collections may not accurately represent the integrated exposure over time.
- Indoor fungal exposure studies: The authors summarized studies examining conditions in the indoor environment that encourage fungal growth.

**RESULTS AND CONCLUSIONS:** The authors concluded:

- Fungal allergen exposure was associated with the development and severity of asthma in sensitized individuals.
- The contribution of indoor fungal allergen exposure to allergic diseases has still not been completely clear.
- Methods to assess fungal allergens by using immunoassays were still in the infancy.
- Original methods of exposure assessment with spore counts and quantitative cultures suggest that an indoor fungal exposure contributes to allergic airway disease.

## 6 Correlation between the prevalence of certain fungi and sick building syndrome

AUTHORS: Cooley JD, Wong WC, Jumper CA, and Straus DC

JOURNAL: *Occupational and Environmental Medicine*

CITATION: 1998; 55: 579-584.

STUDY DESIGN: Cohort study with microbial assessment and health questionnaire survey.

OBJECTIVE: The objective of this 22-month study was to determine whether or not indoor fungi played a role in the occurrence of sick building syndrome in 48 schools experiencing indoor air quality (IAQ) problems in states along the United States Gulf of Mexico and the Atlantic seaboard.

METHODS: Health and building-related issues were reported in a questionnaire.

Microbiological identification included taking all samples with a two-stage Andersen Model 2000 sampler at a flow rate of 28.4 l/min for 5 minutes. Swab samples were taken from areas of visible dust or fungal growth as well as HVAC systems, wet areas, and standing water. Specimens were plated onto agar and fungi were identified. Carbon dioxide, formaldehyde, nitrogen dioxide, hydrogen sulphide, sulphur dioxide, and carbon monoxide also were sampled. Samples were taken in both complaint and non-complaint areas. The authors reported that measurements of particulates, temperature, and relative humidity were measured using standard devices.

RESULTS: Over 80% of the 48 surveyed schools were elementary schools. Less than 30% of the total staff reported symptoms or complaints. Students were not included in the analysis. There were no statistically significant differences among the reported health complaint (except nausea) for the various schools, allowing the authors to pool the findings for all of the different sites. The most common complaints were nasal drainage/ congestion and itchy, watery eyes. The authors reported that "[m]ore than half of the occupants that had IAQ complaints also complained of increased respiratory infection (such as tonsillitis, bronchitis, and pneumonia). One-third of the occupants that registered complaints claimed that an increase in the relative humidity resulted in an increase in their symptom severity." The precise numbers for these estimates were not provided in the tabulation of raw data. The CO<sub>2</sub> concentrations were higher indoors than outdoors, but the authors noted that these levels did not differ statistically and there were no correlations between the indoor concentration and complaints or symptoms. All chemicals were within the normal acceptable range. The same was true for particulate measurements. No raw data were shown for carbon monoxide, chemical or particulate measures. *Stachybotrys* was not measured in any indoor air samples, but was found in swab samples from 11 schools. Again, the data were not shown.

CONCLUSIONS: The authors commented that the validity of results from questionnaire studies may be altered by biases introduced by the observer or by the respondents, and believed that their results show that *Penicillium* and *Stachybotrys* species are strongly associated with symptoms of sick building syndrome. The findings showed that *Cladosporium* was not associated with the indoor air sample complaint areas, but its presence indicated that the conditions favored fungal growth and could allow other fungal to be a dominant organism.

COMMENTS: The authors acknowledged that "a causal relationship is rarely discernible even with strong statistical significance. Thus, at best, associations can be drawn only between the exposure and the effect." The authors appeared to rely on differences between groups that were not statistically significant. For example, in 11 schools from which *Stachybotrys* could be measured from swab samples, there were no statistically significant differences in the indoor air samples from complaint areas as compared to non-complaint areas. Nonetheless, the authors interpreted their findings as providing strong evidence that *Stachybotrys* is implicated in sick building syndrome.

## 7 Respiratory health effects of home dampness and molds among Canadian children

AUTHORS: Dales RE, Zwanenburg H, Burnett R, and Franklin CA

JOURNAL: *American Journal of Epidemiology*

CITATION: 1991; 134(2):196-203

STUDY DESIGN: Cohort study.

**OBJECTIVE:** The authors conducted in 1988 a large questionnaire-based study on the respiratory health of young children and the presence of home dampness and molds in Canadian homes in 30 different communities in five different Canadian provinces.

**METHODS:** Questionnaires were distributed to the parents of children in kindergarten through grade two. Children between 5 and 8 years old were studied. The questionnaire included a series of questions that applied to "primary exposure variables" and were used to identify indoor dampness/mold. These questions addressed mold sites with visible mold or mildew in the past year, moisture (*e.g.*, the appearance of wet or damp spots excluding the basement), and flooding (*e.g.*, the appearance of water damage or leaks in the basement). Questions focused on whether exposure to these primary variables occurred within the past year. The number of mold sites observed in the home was recorded. "Primary health outcomes" concentrated on respiratory and sinus complaints and symptoms. Potentially confounding variables (*e.g.*, age, gender, socioeconomic status, other environmental exposures) also were assessed using adjusted (stratified) risk analyses.

**RESULTS:** The parental questionnaire responses yielded prevalence estimates for molds of 32.4%, moisture 14.1%, and flooding 24.1%. Household smoking was reported more frequently in damp homes (55% vs. 51%); although the difference in percentage was slight, the difference between the two values was statistically significant. The reported prevalence rates of mold allergy and dust allergy were not significantly different for houses with dampness/mold. When all symptoms of lower respiratory symptoms/disorders were grouped together (*e.g.*, cough, wheeze, asthma, bronchitis and chest illness), the prevalence rates reported by parents were approximately 50% higher in damp homes. Upper respiratory and nonrespiratory symptoms were increased by 20-25% in damp homes. Based on the unadjusted (crude) odds ratios for the specific lower respiratory symptoms, there was a statistically significant increased risk for every individual symptom in homes with dampness/mold, flooding and moisture (*i.e.*, odds ratios ranged from 1.32 to 1.89). Some of these odds ratios (*e.g.*, for wheezing in flooded homes) were no longer statistically significant after adjusting for confounding variables; it would have been helpful if these data had been presented in tabular form. The presence of two mold sites appeared to be the exposure with the greatest odd ratios for all health indicators, suggesting a dose response relationship, although no analysis was conducted to demonstrate that there was a statistically significant increase in symptoms associated with the number of mold sites. The associations between dampness/mold and symptoms were not as strong if a child was reported to have either mold or dust allergy.

**CONCLUSIONS:** The reported presence of indoor molds and dampness may cause adverse respiratory and sinus symptoms in Canadian children. The authors noted that stratifying for confounding variables did not erase the observed relationship between reporting indoor dampness and most health complaints, although reporting bias could not be excluded. The authors also acknowledged a major limitation of their study was the use of reporting by study participants. The authors also conceded that they did not have objective health or industrial hygiene measurements to support the subjective questionnaire findings.

## 8 Indoor air quality and health: validity and determinants of reported home dampness and mold

AUTHORS: Dales RE, Miller D, and McCullen E.

JOURNAL: *International Journal of Epidemiology*

CITATION: 1997; 26(1):120-125

STUDY TYPE: Cohort study.

**OBJECTIVE:** The study goal was to test the validity of questionnaire reports completed by occupants as an indicator of the burden indoor fungi as measured by home fungal biomass. The study also sought to relate the levels of ergosterol, a chemical byproduct of many fungi, with questionnaire responses as well as the presence of visible mold.

**METHODS:** Questionnaire responses from 403 families (from an original list of 1,438 families) in Ontario homes were collected during the winter of 1993-94. The study population was identified from elementary school lists. The self-administered questionnaire had been tested previously by the authors and found to have moderate to good reproducibility for accurately identifying visible mold growth and damp interior surfaces. Ten-minute dust samples were collected by vacuuming and cultures were grown on Martin's Rose Bengal Agar. The authors attempted to measure ergosterol as a fungal marker in air samples that were collected over a 14-20 hour period from bedrooms and living rooms. Ergosterol measurements were assessed using high pressure using liquid chromatography and gas chromatography mass spectrometry, but most of the samples (79% of bedroom and 90% of living room values) were below the detection limit of 500 picograms (representing approximately 200 spores). Accordingly, the ergosterol measurements were reported as either detectable or not detectable.

**RESULTS:** There were 362 homes sampled for dust and 373 sampled for ergosterol. "Allergies" were reported by one-fourth of all respondents, regardless of whether they also reported mold dampness or water damage in their homes. The authors reported that the mean concentration of total viable fungi was 50% greater when visible mold, water damage or moldy odors were reported. The authors also claimed that "the sum of the predominant indoor air fungi, *Aspergillus* plus *Penicillium*, were [sic] best indicated by visible mold growth;" although the levels of *Aspergillus* plus *Penicillium* were higher for the homes in which visible mold was reported, this value did not differ significantly from the value reported for homes where visible mold was not reported. Mean airborne ergosterol was greater when mold moisture or water damage was reported, but the percentage of homes with detectable ergosterol was unrelated to the questionnaire reports.

**CONCLUSIONS:** The authors suggested that reporting bias was evident (*i.e.*, respondents reporting allergies were more likely to report visible mold growth, despite the objective detection of low levels of viable fungi), although this was not a statistically significant finding. From the results of this study, the authors concluded that the lack of a stronger association between measured fungal biomass and reported visible molds can theoretically be explained by problems both with fungal measures and respondent reports. Future research should concentrate on developing accurate objective measures of exposure to fungi, which could be used to develop valid questionnaires. Currently, objective measures and not questionnaires are recommended to clarify the health effect of indoor fungi. The authors recommended that "[r]elying on questionnaire information alone for studying the health effects of indoor fungi should be abandoned because of inaccuracy and possible systematic biases which [sic] would not be corrected by large sample sizes."

## 9 Testing the association between residential fungus and health using ergosterol measures and cough recordings

AUTHORS: Dales RE, Miller R, and White J

JOURNAL: *Mycopathologia*

CITATION: 1999;147:21-27.

STUDY DESIGN: Cohort study.

**OBJECTIVE:** This study was undertaken to examine the relationship among several factors thought to be causally associated with indoor mold, including subjective reporting of health complaints in questionnaires, objective measurement of nighttime coughing, and detection of indoor fungi, ergosterol (a byproduct of molds), and dust mite antigen.

**METHODS:** The study group consisted of 400 schoolchildren around 10 years of age who were recruited from an Ontario, Canada community. The study was done during the winter of 1993-94. A questionnaire was used to ascertain whether or not mold growth had ever been observed in the home for more than 30 days. Parents also responded to health questions regarding the child. The questionnaire was essentially the same as that used in other studies by Dales and colleagues. Qualitative microbial measurements were based on viable fungi cultured from dust samples. The authors used ergosterol levels from air samples collected from both the main living area and the children's bedrooms. Dust mite antigens Der p1 and Der f1 were measured by vacuuming up samples from mattresses and bedding. Overnight coughing was recorded for 10 hours.

**RESULTS:** The authors found that there was a 25-50% increase in the prevalence of various symptoms (*e.g.*, irritation, coughing, asthma, chest illness) when mold was reported by parents to be either currently present or present in the past year. Detectable levels of airborne ergosterol were found in 26% of bedrooms, but symptoms were not related to airborne ergosterol either in the child's bedroom or in the main living room of the house. There was no association between symptoms and ergosterol when the analysis was adjusted for the child's age, sex, history of parental illness, any smoking in the household, or presence of dust mite antigens. There was no observable association between cough and any of the following factors: reported mold growth, detectable ergosterol, and fungal species.

**CONCLUSIONS:** The authors stated, "one should not uncritically accept a causal relationship between common indoor fungi and health effects." The authors found that self-reporting of symptoms, indoor mold, and indoor dampness did not necessarily correspond with objective health or ergosterol measurements. The authors noted that unlike their current study, there are other publications that found an association between indoor mold and health complaints, based on questionnaire responses. The authors emphasized that dust mite antigens need to be considered as a causal factor for some symptoms that have been blamed on indoor mold. The authors emphasized that proof of a causal relationship between indoor mold exposure and ill health was not available at the time this article was published. The authors indicated that future efforts should identify accurate markers of chronic exposure to indoor fungal biomass as well as objective measures of health responses to mold.

## 10 Indoor airborne fungal spores, house dampness, and associations with environmental factors and respiratory health in children

AUTHORS: Garrett MH, Rayment PR, Hooper MA, Abramson M, and Hooper BM

JOURNAL: *Clinical and Experimental Allergy*

CITATION: 1998; 28:249-467.

STUDY DESIGN: Cohort study with clinical and environmental testing.

OBJECTIVE: This study was conducted to determine whether indoor dampness was associated with airborne fungal spore concentrations and whether these factors bore any associated with respiratory illness in Australian children.

METHODS: Children from 80 households in one geographic location were recruited to participate in this study. Of the 80 households, 43 had at least one asthmatic child between the ages of 7 and 14 (mean age at study onset was 10.2 years); the remaining 37 households did not have a child that had not been diagnosed as asthmatic. A total of 148 children from the 80 households was included in the study. Six bimonthly testing sessions were made at each house over a 12-month period. During each of the six testing visits, air samples were collected for both culturable and total fungal spore levels after some activity in the room. In addition, viable and total fungal spore samples were collected from children's bedrooms, living rooms, kitchens, and outside each house. Health outcomes were determined a respiratory questionnaire that was completed for each child during an interview with a parent to each household. Objective immune responses were assessed using skin prick tests for most of the children (three not tested) using 12 common inhalant allergens.

RESULTS: The authors found that the percentage of asthmatic children with a skin reaction to either *Penicillium* or *Cladosporium* species was significantly higher than that for non-asthmatic children. The authors also reported that the frequency of asthma was "significantly associated" with exposure to *Penicillium* in winter, although the association was no longer significant if three children were excluded from the analysis. The authors suggested that this association could be a chance finding. No other measures of spore exposure or dampness (*e.g.*, musty odor, damp house, visible mold growth, condensation evidence) exhibited a statistically significant association with asthma. There was a statistically significant association between atopy (as defined by as a single positive skin to any antigen) and exposure to *Aspergillus* spores. No other statistically significant associations between atopy and fungal exposure measures of dampness were seen. *Cladosporium* concentrations in the indoor air were significantly reduced in houses that kept the windows open 10 months of the year as compared to 2 to 6 months of the year. Indoor *Penicillium* levels were significantly lower in houses with relative humidity less than 60%.

CONCLUSIONS: The authors concluded that their findings showed a large overall effect of fungal exposure on child health (especially in winter). Average concentrations of viable or total fungal spores, however, were not significantly associated with health outcomes.



## **11 An approach to management of critical indoor air problems in school buildings**

**AUTHORS:** Haverinen U, Husman T, Toivola M, Suonekt J, Pentti M, Lindberg R, Leinonen J, Hyvarinen A, Meklin T, and Nevalaine A

**JOURNAL:** *Environmental Health Perspectives*

**CITATION:** 1999; 107(3)509-514

**STUDY DESIGN:** Questionnaire health survey with structural and microbial assessments.

**OBJECTIVE:** This study assessed the moisture and microbial status of a complex of three school buildings in a small town in southern Finland. The health complaints of the school occupants also were assessed and the authors attempted to establish a strategy for remediating the problems that were identified.

**METHODS:** Three school buildings, A, B, and C, were assessed. The upper secondary and high schools were located mainly in building A, but used some facilities in building C. Elementary students were in building B, which the authors considered a "non-damaged, reference building." An analysis of moisture status of the structures and the causes of moisture accumulation was undertaken by extensive onsite investigations. Microbial samples were taken from the air, surfaces, and bulk materials. A questionnaire was delivered to the occupants of the buildings before any investigations began. Older children responded to the questionnaires themselves whereas the responses for the younger children were acquired from their parents. The questionnaire asked about respiratory disorders and other irritative and general symptoms.

**RESULTS:** The study found that significantly more irritative and nonspecific symptoms, such as nasal congestion, rhinitis, phlegm, dyspnea, eye irritation, hoarseness, and fatigue, were commonly reported among the upper secondary and high school students as compared to the elementary school children. After adjusting for age, sex, smoking, and atopy (not defined in this study), the observed differences among the study groups remained statistically significant for nasal congestion, rhinitis, phlegm, hoarseness, and fatigue. The exposed students also had more long-term and repetitive flu, cough, and eye symptoms. Self-reports of asthma were common among upper secondary students, with a prevalence of 13%. The authors compared this value to an average prevalence of asthma of 4 to 7% among this age group in different regions in Finland, although no statistical analysis was provided. During the 4 years preceding the study (1993-1996), 18 children reported a new diagnosis of asthma whereas only four to six children reported a new diagnosis of asthma in earlier 4-year periods (1980-1984, 1985-1988; 1989-1992). There were no differences in the number of asthma-related doctor visits or medications for the upper secondary students as compared to the other groups.

**CONCLUSIONS:** The authors commented that incorrect methods of construction, maintenance, or repair were the primary problems in building C. Damage in the stone school (building A) was caused by moisture penetration from the ground. Although some moisture faults were also found in the elementary school (B), significant differences were found among the health status of the occupants of buildings A and C compared to the occupants of building B. Although the reporting of symptoms may have increased as a result of common awareness and publicity in the community, information collected in self-administered questionnaires is not always as valid as that collected in clinical studies. The self-reported prevalence of asthma among the upper secondary school students was twice that of the general population prevalence among teenagers. The authors reported that the diagnoses of asthma in this study were "made by a physician and can be regarded as objective findings." This statement is not entirely accurate, however, since there were no clinical studies conducted for this article and the reports of physician-diagnosed asthma were from the children or their parents, not from the actual physician.

## 12 Mold-allergy is a risk factor for persistent cold-like symptoms in children

AUTHORS: Huang S-W and Kimbrough JW

JOURNAL: *Clinical Pediatrics*

CITATION: 1997; 36: 695-700

STUDY DESIGN: Case control.

**OBJECTIVE:** This 2-year study assessed whether there was a relationship between indoor mold and symptoms related to perennial allergic rhinitis seen in children during the winter months. The authors described persistent cold-like symptoms (PCLS) that occurred during the winter. The symptom cluster included nasal congestion, cough, red eyes in the morning, nighttime postnasal drip, frequent headache, and fatigue.

**METHODS:** The study was based on a total of 44 children, ages 4 to 14, with perennial allergic rhinitis that were diagnosed in the allergy clinic in the winter months. Skin tests in all of the patients showed sensitivity to at least three mold antigens but not to house dust mites, animal dander, or cockroaches. The cases consisted of 25 children diagnosed with PCLS for at least the last 2 years, and the controls were 19 children without a diagnosis of PCLS. Evidence of respiratory infection had been ruled out, and sinusitis excluded by abnormal sinus X-rays. Parents filled out symptom score sheets on two separate office visits in January and February and the average represented the final score on the following symptoms: red eyes, mouth breathing, rhinorrhea, heavy nasal voice, postnasal drip, and headache. A mold survey included sampling of air inside the children's bedrooms and outside all 44 homes within 3 weeks after clinic visit during the winter. Molds were identified microscopically and expressed as CFU/m<sup>3</sup> of air. Nasal discharge was collected from all patients through nasal blowing and the number of eosinophils was expressed as percentage of the total white blood cells.

**RESULTS:** Symptom scores of children with and without PCLS in the winter months differed significantly for all of the symptoms on the symptom score sheets. The mean nasal eosinophils was 32% in the PCLS group (range 15-43%) and did not differ significantly from the value for the control group (range 15-35%). Five fungal strains isolated in the homes accounted for 80% of the molds isolated: *Cladosporium*, *Penicillium*, *Curvularia*, *Epicoccum*, and *Alternaria*. The mold counts from the homes in the winter months were significantly higher for the PCLS group (1130 ± 109 CFU/m<sup>3</sup>) and the non-PCLS group (424 ± 28 CFU/m<sup>3</sup>). The outdoor mold counts for the two groups did not differ in either the winter or the summer months. The symptom scores for all 44 of the children entered in the study correlated positively with mold counts in the homes during the winter months.

**CONCLUSIONS:** The authors believed that they had defined a group of children with a severe form of allergic rhinitis caused by mold allergy. The authors reminded clinicians that mold-related PCLS may be distinguished from viral respiratory infections, even though both are common in the winter months. The authors were unable to determine whether or not "a critical threshold level of mold counts that would cause the development of symptoms" in patients with PCLS can be identified. The authors proposed that the lack of a standardized microbial threshold may be related to the fact that the clinical threshold is individually determined.

## 13 Health effects of indoor-air microorganisms

AUTHOR: Husman T

JOURNAL: *Scandinavian Journal of Work and Environmental Health*

CITATION: 1996; 22(1):5-13

STUDY DESIGN: Review.

**OBJECTIVE:** This mid-1990s review from Finland summarized current knowledge and health effects of indoor-air microorganisms, and briefly reviewed the pathophysiological processes causing these health effects. The effects of mites, bacterial endotoxins, and algae were not addressed in this paper.

**METHODS:** In buildings with moisture and microbiological problems, the human exposure is to a complex mixture of microorganisms, organic and inorganic dust, and volatile chemicals. In epidemiology research, it is practically impossible to distinguish between the effects of various exposures. The authors observed that a number of epidemiological studies on indoor air problems have been published without proper evidence of exposure. The most prevalent indoor molds in "healthy" houses are *Penicillium*, *Aspergillus*, and *Cladosporium*. Outdoor air was identified as the most important source of fungi in normal indoor air. The authors explained that water incursion may contribute to a change in the profile of indoor microorganisms such that mold and other moisture-dependent microorganisms will emerge if a building is damaged by water. The presence of water in a building is not necessarily a determining factor as to whether or not the airborne concentration of mold spores will be high. The author suggested that even low indoor concentrations of mold species that are not typically found indoors "may provide a hint of hidden mold growth in the building." The author identified five categories of health effects of indoor air microorganisms and mold: irritative symptoms, respiratory infections, allergic diseases, alveolitis and organic dust toxic syndrome, and chronic pulmonary diseases (chronic bronchitis).

**RESULTS:** Husman observed that "much confusion has been caused by the fact that there is no linear correlation between the airborne mold spore counts and the occurrence of symptoms." The various symptoms that are expressed by exposed individuals may be determined by both evident and hidden factors, such as individual sensitivity, age, smoking, atopic predisposition, prior exposure to similar microorganisms, and other irritants and chemicals. The author identified literature supporting the theory that the association between mold exposure and irritative symptoms of the respiratory tract and eyes is strong, although the exact mechanisms behind these inflammatory processes remain obscure. The author reported that an association between elevated indoor humidity and lower respiratory symptoms was more controversial, with some studies supporting an association and others not proving that a relationship existed. The author also noted that some studies reported a relationship between indoor mold and fatigue, headaches, and other vague symptoms whereas other studies could not find such an association. Husman also discussed the fact that "[t]he specificity, sensitivity, and repeatability of skin tests have been rather poorly documented," leading to a great degree of variability in the reporting of the prevalence of mold-related allergy in different studies.

**CONCLUSIONS:** The author concluded that 1) the evidence for an association between mold exposure and respiratory infections was not well established, 2) the allergenicity of some molds in humans was not disputed, 3) patients exposed to indoor air microorganisms may describe a variety of nonspecific symptoms referred to as chronic fatigue syndrome or fibromyalgia, and 4) useful diagnostic criteria and reliable disease markers for the syndromes thought to be related to indoor mold had not been developed at the time this article was written, making it difficult to properly conduct a differential diagnosis.

## 14 The relationship between moisture or mold observations in houses and the state of health of their components

AUTHORS: Koskinen OM, Husman TM, Meklin TM, and Nevalainen AI

JOURNAL: *European Respiratory Journal*

CITATION: 1999; 14:1363-1367

STUDY DESIGN: Cohort study.

**OBJECTIVE:** This study identified health problems in adults that were associated with reports of either indoor moisture made by an independent surveyor or indoor mold growth by occupants.

**METHODS:** A randomly selected sampling of homes in four different cities in Finland led to the identification of almost 700 adults over age 16. The occupants' health data were collected by a self-administered postal questionnaire before the site visit of the surveyor. The occupants were interviewed by the surveyor after the site evaluation was conducted. There were two phases to the study, the first having exposure defined by the surveyor's observance of moisture and the second with exposure being defined by the occupants' observance of mold.

**RESULTS:** For phase one, the surveyor identified whether the occupants were exposed depending on the presence or absence of moisture. The surveyor observed moisture in 52% of the houses, of which a large proportion (86%) had water damage according to the owners. There was a statistically significant greater number of "moisture present houses" that were built commercially by a construction company as compared to homes that were built privately by the owner, according to the surveyor. More than half (52%) of the adults were living in houses in which they were exposed to moisture. There were no statistically significant differences in atopic predisposition or doctor-diagnosed allergies between the exposed and non-exposed groups. A total of 22 symptoms was reported. There was a statistically significant increase in the risk of respiratory infections (sinusitis or acute bronchitis), nocturnal cough, nocturnal dyspnea, and sore throat for the exposed group as compared to the unexposed group. In the second phase, the occupant's exposure was defined as the visual observance of mold by the occupants. Mold was reported by the occupants of 27% of the houses. The percentages of occupants reporting mold in homes built by commercial companies and by owners did not differ significantly from each other (mold absent = 53%, mold present = 46%). There was a statistically significant difference in the frequency of water damage in homes with mold reported by occupants (62%) as compared to homes in which mold was absent (81%). The presence of moisture stains showed a similar statistically significant pattern (31% for mold-absent and 53% for mold-present homes). The authors reported a statistically significant increased risk for nine of the 22 symptoms on the questionnaire for mold-exposed respondents as compared to individuals that did not report mold in their homes. Two or three of these risk factors had confidence intervals that included 1.0, however, raising a question as to whether the values were truly different statistically.

**CONCLUSIONS:** The authors concluded the following: respiratory infections were significantly increased with exposure to moisture, episodes of respiratory infection were significantly higher in the exposed group, nonrespiratory symptoms were increased in adults, there were no significant differences in doctor visits or sick leave between the exposed and non-exposed groups, and indoor mold was a consequence of water condensation.

**15 Serum IgE specific to indoor molds, measured by basophil histamine release, is associated with building-related symptoms in damp buildings**

AUTHORS: Lander F, Meyer HW, and Norn S

JOURNAL: *Inflammation Research*

CITATION: 2001; 227-23

STUDY DESIGN: Cross-sectional survey and *in vitro* immunology testing.

OBJECTIVE: The authors described application of the basophil histamine release test for the detection of specific IgE to molds when commercial extracts were not available for skin prick test or RAST method. A growth of *Penicillium chrysogenum* was collected from two schools where employees had a high frequency of complaints over indoor air quality. The authors developed this test using the relevant strain of *P. chrysogenum* from the building because specific IgE reactions were not produced in response to five molds from the commercial standard extracts.

METHODS: A questionnaire was used to solicit complaints of building-related symptoms from 86 employees of two municipal schools that had repeated reports of water problems. Ten individuals were reported in the past or presently suffering from asthma and 23 from hay fever. Blood was drawn and frozen for future IgE testing. Fungal material also was preserved by freezing. Laboratory staff were blind regarding the symptoms associated with the blood samples being tested. Passive sensitization was used to transfer serum IgE to the cultured cells that were used for the histamine release test. Release of histamine by basophils was assayed using a fluorometric system.

RESULTS: The authors found a statistically significant association between a positive *in vitro* histamine release to *P. chrysogenum* and nasal irritation, nasal congestion, and fatigue after adjustment for confounding variables such as smoking, employment history, and sex. There was no increased odds ratio for symptoms of eye irritation, pharyngeal irritation, facial skin irritation, headache, and lack of concentration. More importantly, the authors reported, "the prevalence of self-reported asthma and hay fever did not differ between individuals with and without histamine response to the molds."

CONCLUSIONS: The authors concluded that a positive histamine release test to indoor molds was another method of demonstrating serum IgE reactions in patients that were specific to the fungus under evaluation. The relationship between the histamine release test and specific allergic symptoms of rhinitis and asthma was not demonstrated.

**16** Hypersensitivity pneumonitis caused by *Fusarium napiforme* in a home environment authors: Lee S-K, Kim S-S, Nahm D-H, Park H-S, Ph Y-J, Park K-J, Kim S-O, and Kim S-J

JOURNAL: *Allergy*

CITATION: 2000; 55:1190-1193.

STUDY DESIGN: Case report.

OBJECTIVE: To describe a case of hypersensitivity pneumonitis that the authors attributed to indoor mold exposure.

METHODS AND RESULTS: A 17-year-old previously healthy male developed dyspnea, cough, and fever 2 days after repairing the basement in his home. Physical exam showed inspiratory crackles in both lung bases. Chest CT demonstrated multiple tiny nodules in ground-glass opacities bilaterally. Spirometry showed a severe restrictive defect and DLCO with 46% of predicted. Skin prick tests to 80 common inhalant and food allergens were positive to *Trichophyton spp.* Total IgE was normal. Transbronchial lung biopsy demonstrated lymphocytic infiltration within the alveolar walls. Two days after hospital admission, the patient improved progressively and was discharged with medication for tuberculosis and steroids on day 8. After a few hours at home, his symptoms recurred and progressed to the point of requiring emergency room admission. Within 24 hours post second hospital admission, he improved. The student remained well after moving to the home of his uncle. One month later pulmonary function tests and CT scan were normal. Cultures (Sabouraud glucose agar plates left open for 2 hours) from the patient's bedroom, living room, and bathroom yielded predominantly *F. napiforme*. Antigens were prepared from these cultures. Immunoblot analysis using the patient's serum showed binding of specific IgG in patient's serum and control serum.

CONCLUSIONS: The authors believed this was the first case of HP occurring in response to home exposure to *F. napiforme*.

## 17 Aeroallergens and work-related respiratory symptoms among office workers

AUTHORS: Menzies D, Comtois P, Pasztor J, Nunes F, and Hanley JA

JOURNAL: *Journal of Allergy and Clinical Immunology*

CITATION: 1998; 101(1): 38-44

STUDY DESIGN: Cohort survey with nested case control.

OBJECTIVE: This study examined the association between respiratory tract symptoms and immediate skin test reactions of Canadian office workers with exposure to fungal and house dust mite aeroallergens at their work sites.

METHODS: An initial survey of all occupants of six mechanically ventilated office buildings distinguished workers with the respiratory symptoms of interest from workers without these symptoms. Target symptoms included nasal or lower respiratory complaints. Of the 1,415 eligible workers, 107 cases with symptoms were matched with an equal number of control persons without symptoms (total of 214 workers). Each participant in the second phase of the study underwent skin prick testing using traditional allergens present in that community. Each participant's office environment was inspected and a sample of floor dust was collected for measurement of house dust mite antigens and fungal colony-forming units (CFUs). Fungal parameters were also measured in the HVAC systems of each building on two occasions 6 months apart. On each day sampling for fungal spores and CFUs was performed in outdoor air and in the supply air systems downstream from the HVAC filters. Total airborne particulates (dust) were collected over a 24-hour period with volumetric air samplers onto preweighed filters.

RESULTS: The authors reported, "the two major findings of this study were the association of work-related respiratory tract symptoms with low-level aeroallergen exposures in some of the workers and the failure to identify any environmental cause for more than 80% of workers with symptoms." Eleven percent of workers complained of respiratory symptoms. The office levels of *Aspergillus*, *Cladosporium*, and *Penicillium* did not differ between the case and control groups. The levels of *Alternaria* species in the air supply or at the office locations were generally around the limit of detection. The odds ratio for exposure to *Alternaria* species measured in the HVAC supply air showed a statistically significant elevation for the cases as compared to the controls. The risk of exposure for the cases to dust mites, elevated moisture content, and smoking were all statistically elevated after adjusting for confounding variables. Most of the skin tests did not show an association between symptoms and reactions to microbial allergens, except *Alternaria* species.

CONCLUSIONS: The authors believed that work-related symptoms were not caused by exposure in other environments such as outdoor or exposures at home. This survey was distinguished from others by the measurement of exposure directly at the workstations of symptomatic participants. Limitations to estimating the exposure of individuals in this study included the possibility of temporal variation. In this study, symptoms and skin test results were not associated with total fungal levels, but only with exposure to a single species, *Alternaria*. In other epidemiologic surveys of office workers, total fungal spores of viable fungi were measured and failed to detect any relationship with symptoms. The authors suggested that measuring total fungus level gave only a crude measure of exposure. The authors noted that there is evidence in the literature indicating that *Alternaria* species contain potent allergens that may contribute to asthma. Only 2% of the total number of workers demonstrated a positive skin reaction to *Alternaria* species as well as respiratory symptoms that were consistent with an allergic reaction. The authors recommended that additional research should focus on a relationship between *Alternaria* species and allergic symptoms.

## 18 Fungus spores, air pollutants, and other determinants of peak expiratory flow rate in children

AUTHORS: Neas LM, Dockery DW, Burge H, Koutrakis P, and Speizer FE

JOURNAL: *American Journal of Epidemiology*

CITATION: 1996; 143(8):797-807

STUDY DESIGN: Cohort study with extensive environmental exposure assessment.

OBJECTIVE: The authors studied the impact of summertime daily variation in environmental factors in 108 children living in State College, Penn.

METHODS: Environmental measures included daily 12- and 24-hour averages for meteorologic and air pollutant variables and 24-hour average fungus spore concentrations. Precipitation, wind direction, and solar radiation were also measured daily by the Environmental Protection Agency.

The study excluded children who used any asthma medication during the previous year. The authors identified symptomatic cases "as those who were reported as ever wheezing or as usually having a cough for as much as three months during the year. Asymptomatic children had no history of wheeze or chronic cough and no prior diagnosis of asthma." Pulmonary function tests were done at baseline and repeated up to four times during the summer. Peak flow measures were recorded on arising in the morning and again in the evening before bedtime. Values were expressed as deviation in peak expiratory flow rate (PEFR). Respiratory symptom diaries were recorded twice daily. The findings were based on a final cohort of 62 symptomatic children and 46 asymptomatic children.

RESULTS: The authors found that decreases in the morning peak flow rates were associated with airborne presence of *Cladosporium*, *Epicoccum*, and *Coprinus*. There was a weak association between decrease in evening peak flow rates and various measures of air pollution. Pollen counts were generally low during the study whereas airborne fungal spore counts varied considerably, depending on wind and rain.

CONCLUSIONS: The authors found that precipitation affected the deviation in PEFR and concentrations of airborne contaminants. They concluded that 1) "airborne spore concentrations of *Cladosporium*, *Epicoccum* and *Coprinus* were associated with decreases in the mean morning PEFR for the entire cohort," 2) the effect of particle-strong acidity on the mean deviation and evening peak expiratory flow rate was modest, and 3) "summer episodes of excessive aerosol acidity and particulate pollution are acutely associated with declines in peak expiratory flow rates" and increased respiratory symptoms in children studied.



## 19 Health effects of mycotoxins in indoor air: a critical review

AUTHORS: Robbins CA, Swenson LJ, Nealle ML, Gots RE, and Kelman BJ

JOURNAL: *Applied Occupational and Environmental Hygiene*

CITATION: 2000; 15(10):773-784

STUDY DESIGN: Literature review.

OBJECTIVE: This was an extensive review with over 90 references (through 1999) addressing the role of mycotoxins in the alleged toxic health effects of molds in the indoor environment. A lack of exposure standards for measuring molds or mycotoxins makes it difficult to determine the role that these bioaerosols may play in causing disease in any particular case.

METHODS: The paper reviewed several types of studies for relevant findings on exposure to mold in indoor, occupational, agricultural, and nonagricultural environments. The types of articles that were reviewed included animal exposure studies, case reports, epidemiologic studies, and primary literature concerning inhalation of mycotoxins or potentially toxin-producing molds.

RESULTS AND CONCLUSIONS: The authors concluded that the current literature does not provide compelling evidence that exposure to mycotoxins at levels expected in most mold-contaminated indoor environments is likely to result in measurable health effects. The authors acknowledged that evidence exists of a relationship between high levels of inhalation exposure or direct contact to mycotoxin-containing molds and health effects in animals and health effects in humans. The authors observed that "there is a general need to develop better methods of estimating exposure to molds that are relevant to the health effects of interest. Improved methods are needed to accurately quantify exposures to the irritant, allergic and toxic components (for inhalation and dermal exposures) of molds." The authors emphasized that exposure standards for the toxic effects of indoor molds and mycotoxins did not yet exist when this review was written. The authors proposed that standards may help in determining when indoor mold crosses the line between being an unsanitary nuisance and a health problem. The authors also discussed the fact that a particular focus on indoor exposure to *Stachybotrys* species by health care professionals may not be supported by the scientific literature.

## 20 Fungal spores: hazardous to health?

AUTHOR: Sorenson WG

JOURNAL: *Environmental Health Perspectives*

CITATION: 1999; 107(3):469-472

STUDY DESIGN: Literature review.

OBJECTIVE: The author reviewed the *in vitro* and animal literature regarding health effects of mycotoxins.

METHODS: Individual articles were reviewed for relevance to the topic of human health effects of inhalation of mycotoxins. The effects of mold byproducts on pulmonary and immune function were analyzed. This publication extensively summarized studies reported through 1998.

RESULTS: The author reported that few studies exist to demonstrate a direct association between exposure to mycotoxins and respiratory or immune problems in humans. The bulk of the human literature relating mycotoxins to disease have focused on aflatoxin-associated cancer. The author identified evidence that inhalation of purified components of mold, such as T-2 toxin, caused alterations in immune function in mice. Tissue damage was observed in the lungs of animals when aflatoxin was infused directly into the lungs of animals.

CONCLUSIONS: The author's conclusion stated: "Because of technical limitations, it is difficult to accurately assess the role of mycotoxins in human health effects associated with damp environments. For example, demonstration of mycotoxin production in the laboratory does not prove their presence in the environment; we are generally unable to measure mycotoxins in the field at the site of exposure and, with the exception of aflatoxin, there is a general lack of biomarkers of either exposure or effect. In addition, it appears that isolates of many toxigenic fungal species vary in their ability to produce mycotoxins, but it is uncertain to what extent this is due to actual genotypic differences between the isolates or to the effect of the stress of isolation in artificial culture."

## 21 Health risk assessment of fungi in home environments

AUTHORS: Verhoeff AP and Burge HA

JOURNAL: *Annals of Allergy*

CITATION: 1997; 544-556

STUDY DESIGN: Literature review.

OBJECTIVE: This study reviewed "the status of the data on the relationship between exposure to fungi in the home environment and allergic health effects" in an effort to determine whether or not it was plausible to establish guidelines for permissible fungal levels.

METHODS: After identifying 10 years of literature as a potential pool of information, the authors based this review on "[n]ine population-based studies were identified that examined the relationship between allergy and the presence of fungi in the home environment. These studies included quantitative measurements of fungal presence in either air or dust." Each study was evaluated for its strengths and weaknesses in design, methodology, and data analysis. The authors reviewed the literature addressing the potential health effects of fungi, including allergic rhinitis, allergic asthma, and extrinsic allergic alveolitis/hypersensitivity pneumonitis. They also presented background information regarding components of fungi that may contribute to health effects, including 1) (1-3)- $\beta$ -D-Glucan, which may have inflammatory or adjuvant effects; 2) toxic metabolites such as mycotoxins, which were under investigation for causing non-allergic respiratory symptoms; and 3) volatiles (*e.g.*, alcohols, aldehydes, ketones), which are best known as odorous irritants, "which may produce symptoms such as headache, eye, nose and throat irritation or fatigue."

RESULTS: Evaluation of the nine studies evaluated showed that six studies used randomly selected populations and health effects assessed by self-administered questionnaires. Only three studies validated findings of respiratory symptoms using pulmonary function measurements. Overall, one or more positive associations were found between fungal levels and health outcomes in seven of the nine cross-sectional studies.

CONCLUSIONS: The authors concluded that fungi contribute to allergic disease and the extent of their involvement may be greater than had been identified in previous studies. The authors stated, "[t]he review of epidemiologic studies where fungal exposure was quantitatively assessed did not reveal data that might allow the setting of guidelines for fungi in home environments based on health risk assessment." The authors' discussion of the limitations of the literature provided a useful overview of the studies that existed in the mid 1990s. The authors suggested that future studies should attempt to standardize testing and analytical methods for assessing indoor mold and its relation to human health.

## 22 Nasal lavage biomarkers: effects of water damage and microbial growth in an office building

AUTHORS: Walinder R, Wieslander G, Norback D, Wessen B, and Venge P

JOURNAL: *Archives of Environmental Health*

CITATION: 2001; 56(1):30-36

STUDY DESIGN: Cross-sectional study.

**OBJECTIVE:** The authors studied the relationships between residents in a damp office building with objectively verified microbial growth in its construction, as well as nasal functional measurements using nasal peak flow measurements and nasal lavage. The nasal lavage fluid was studied for signs of inflammation using the following: cell counts of leukocytes, erythrocytes, and epithelial cells; measurements of eosinophil cationic protein (ECP), myeloperoxidase (MPO), lysozyme, tryptase, and albumin.

**METHODS:** The study, conducted in Uppsala, Sweden, over 3 days during the non-pollen season, Twelve of the 19 workers from the damp building that volunteered to participate ended up being eligible for the study. Eight of 15 workers from an adjacent building without moisture problems qualified to served as controls. The control building had natural ventilation whereas the damp building had "a mixed type mechanical supply and exhaust ventilation" system. There was no difference between exposed and unexposed subjects in regard to smoking status, presence of atopy (15%), or history of asthma. A questionnaire was administered inquiring about nasal symptoms, and a physician questioned the participants about health complaints. Air and bulk sampling was conducted as part of the overall evaluation of the damp building, but it was not clear as to whether uniform sampling was done in the control building.

**RESULTS:** Microbial growth was found in different parts of the water-damaged building, mainly on the mineral fiber insulation and gypsum board. Many samples from mineral fiber insulation contained elevated concentrations of both bacteria and molds. An increased microbial biomass was measured on 35% of all samples that were taken. Viable microorganisms identified after cultivation showed a dominance of *Streptomyces* sp. (> 50% of the samples), whereas *Stachybotrys* sp. and *Chaetomium* sp. were present in 14% and 8% of samples. The average total VOC concentrations were low in both the water-damaged (46 mcg/m<sup>3</sup>) and the control building (22 mcg/m<sup>3</sup>). There was a statistically significant elevation in the values of ECP, MPO, and albumin in the nasal lavage fluid of workers from the water-damaged building than the controls. There were no statistically significant differences in the concentration of tryptase or lysozyme between the two groups. All cell counts were not statistically significant between the exposed group and the control group. The number of cases (63%) with eosinophils present in the nasal lavage differed significantly from the number of controls with detectable eosinophils (0%). Nasal expiratory flow rates and self-reported nasal symptoms did not differ significantly between the two groups.

**CONCLUSIONS:** The authors concluded that there was "a significant relationship between exposure to indoor air of a building with dampness and microbial and signs of inflammatory reactions in the nasal epithelium" as demonstrated by measures by nasal lavage. The nasal lavage fluid from the exposed group showed increases in some but not all biomarkers. The authors observed that the pattern of biomarkers that was suggestive of an inflammatory rather than allergic response among the exposed individuals. The authors believed that measurement of specific biomarkers in nasal lavage fluid can be used to study inflammatory nasal mucosa response to exposures in the indoor environment.

## 23 Housing characteristics, reported mold exposure, and asthma in the European Community Respiratory Health Survey

AUTHORS: Zock J-P, Jarvis D, Luczynska C, Sunyer J, and Burney P

JOURNAL: *Journal of Allergy and Clinical Immunology*

CITATION: 2002; 110:285-292.

STUDY DESIGN: Cohort study.

**BACKGROUND:** A number of studies in different countries have shown adverse effects of dampness and mold exposure in homes on adult respiratory health in regard to symptoms of wheezing and cough. The effect of dampness and mold exposure on adult asthma was less clear, and the association with bronchial responsiveness has not been studied. Published data consisted of separate studies with different instruments to assess exposure to dampness and mold. Different climatic conditions and housing characteristics between countries may cause important differences in exposure to dampness and molds. Thus, there was a need for information from an international study on adult asthma using a common protocol for assessment of exposures to dampness and mold in asthma outcomes including bronchial responsiveness.

**OBJECTIVE:** The European Community Respiratory Health Survey, an international multicenter epidemiologic study on asthma, collected standardized information on asthma prevalence and known or suspected risk factors for atopy and asthma. The objective of this study was to investigate the associations between housing characteristics related to dampness, mold exposure, and house dust mite levels in adult areas in all areas of ECRHS.

**METHODS:** The study included 38 study centers located in Europe and outside Europe (Australia, India, New Zealand, and the United States). An interviewer-led questionnaire collected information on respiratory symptoms, self-reported asthma, allergic disorders, environmental, and lifestyle factors. Patients were asked whether they had experienced certain respiratory symptoms in the past 12 months. Information on housing characteristics was also obtained, and bronchial reactivity and allergy testing was assessed using standardized methods.

**RESULTS:** The study found that reported mold exposure in the past year was associated with asthma symptoms and bronchial responsiveness (OR range 1.4-1.44). This effect was homogeneous among centers and stronger in subjects sensitized to *Cladosporium* species. In centers with a higher prevalence of asthma, the prevalence of reported indoor mold exposure was also high. This association was observed for reported mold exposure by asthmatic subjects as well as reported mold exposure by nonasthmatic subjects. Reported mold exposure was highest in older houses with recent water damage.

**CONCLUSIONS:** The authors concluded that mold exposure in homes had an adverse on adult asthma symptoms and bronchial hyperresponsiveness. This effect was consistent across different countries, and they believed it was unlikely to be caused by bias.

## 24 The Effect of Outdoor Fungal Spore Concentrations on Daily Asthma Severity

AUTHORS: Delfino RJ, Zeiger RS, Seltzer JM, Street DH, Matteucci RM, Anderson PR, and Koutrakis P

JOURNAL: *Environmental Health Perspectives*

CITATION: 1997; 105(6)622-635

STUDY DESIGN: Longitudinal Cohort.

BACKGROUND: Fungal spores are believed to adversely affect pulmonary function tests. Weather conditions may also be a factor in addressing abnormal pulmonary function tests.

OBJECTIVE: This study was conducted in a small southern California community to determine if spore counts adversely affected pulmonary function

METHODS: The study used a panel study design, longitudinal analysis of daily data for a panel of each asthmatic, with subject acting as his or her own control. Patients were eligible to participate in the study if they had physician-diagnosed asthma with a minimum history of 1 year, asthma exacerbations during several weeks of the warm months March-October requiring the use of prescribed asthma medication, age between 9 and 49 years, home and school or work address in the Alpine area, and absence of patient/parental or indoor household smoking. After excluding two of noncompliant subjects, the study population included 22 symptomatic asthmatics, 9 adults age 24-46, and 13 children age 9-14. Classification of allergy among subjects was based on the presence or absence of positive allergen reactivity as assessed using skin prick tests for common pollens or molds common to the study area.

RESULTS: The study found that, controlling for the weather, total fungal spore concentrations were associated with all outcomes. Asthma symptom scores increased (inhaler use increased, PEFr decreased, and there were no significant associations to low levels of pollen or O<sub>3</sub>).

CONCLUSIONS: The study showed that outdoor fungal spore concentrations have an adverse effect on daily asthma severity, particularly in regard to reported asthma symptom levels. The finding was supported by adverse effects on evening PEFr and as-needed beta agonist inhaler use. No significant effect from air pollution was between as-needed inhaler use and PM 10 (particulate matter of aerodynamic diameter less than 10 micron). There is no association with O<sub>3</sub> exposure, although O<sub>3</sub> levels were low. There were several limitations to the study:

1) Indoor sampling was not done, 2) the degree to which outdoor fungal concentrations represented personal individual exposures is unknown, 3) outcome misclassification in diary studies is a potential source of error due to a lack of truly verifiable patient data, and 4) effects of fungal spores were not entirely consistent across the three asthma outcomes.

## 25 Domestic fungal viable propagules and sensitization in children with IgE mediated allergic disease.

AUTHORS: Dill I and Niggemann B

JOURNAL: *Pediatric Allergy Immunol*

CITATION: 1996;7; 151-155

STUDY DESIGN: Prospective Case series.

### BACKGROUND:

**OBJECTIVE:** This study was conducted to investigate the extent of viable fungal propagules in the homes of children with allergic disease and to compare these results with sensitization to fungi in terms of presence of specific IgE directed against molds

**METHODS:** The study included 19 patients age 5-16 from a German university hospital outpatient clinic suffering from mild to moderate asthma as defined by ATS and one child with atopic dermatitis only. A positive proof of specific IgE PCS class  $\geq 2$  using a KAP-systemic. Six common fungi known as important allergens in Germany were investigated: *Cladosporium herbarum*, *Alternaria tenuis*, *Aspergillus fumigatus*, *Penicillium notatum*, *Mucor racemosus*, and *Fusarium moniliforme*. Besides these fungi, a panel of six common inhalant allergens representing tree pollen, weeds, house dust mite, cat and dog, and six food antigens were investigating using the same assay system.

**RESULTS:** The study found no visible fungal grown in the homes of eight patients, and the maximum concentration of viable fungal propagules was below those of corresponding outdoor levels. These homes were then classified as "not fungally contaminated." The homes of 12 patients were classified as "contaminated" due to elevated CFU values and/or visible fungal growth: homes of three patients showed visible fungal growth but a relatively low concentration of airborne spores. Predominant outdoor fungi were *Cladosporium* and *Mycelia sterilia*, and in fungally contaminated homes, *Penicillium*, *Cladosporium*, *Aspergillus*, and *Mycelia sterilia* and yeasts. The highest risk of fungal growth was the bathroom. Results of RAST testing showed that all children except three showed a polyvalent sensitization to several fungi. With the exception of one patient, all were also sensitized to common inhalant allergens such as dust mites, pollens, or animal dander. A close correlation between fungal exposure and specific IgE to fungi was not found. There was an association suggested for *Penicillium* with six of seven exposed patients sensitized and 10 of 13 not exposed were not sensitized. A similar association was also evident for *Mucor* with 15 of 20 not exposed patients not sensitized.

**CONCLUSIONS:** The authors found no remarkable correlation between sensitization and domestic exposure. Sensitization to fungi in children who do not show sensitization to other inhalant allergens was rare. Most of the children were broadly sensitized and also suffered typical clinical symptoms of allergic disease.

**1 Possible sources of sick building syndrome in a Tennessee middle school**

AUTHORS: Scheel CM, Rosing WC, and Farone AL

JOURNAL: *Archives of Environmental Health*

CITATION: 2001; 56:413-7

**STUDY TYPE:** Observational laboratory study. Students and staff at Central Middle School experienced symptoms allegedly related to *Stachybotrys* contamination. This led the students to study the environmental health of the school. During this project they identified a black fungal growth on cellulose ceiling tiles throughout the building. The areas of growth were located under areas where the pipes and roof had water leaks.

**OBJECTIVES:** This study was a school project designed to identify the black fungus and to propose remediation measures for the fungal growth.

**METHODS:** The study was conducted at Central Middle School in Murfreesboro, Tenn. during January and February of 1999. Specimens were collected by students from hallways, restrooms, and classrooms. Samples were inspected for visible fungal growth and slides were prepared for examination under a light microscope. Samples identified as *Stachybotrys* were cultured in a cornmeal/ malt/yeast extract agar. Bulk samples were taken from nine mold-contaminated ceiling tiles and from one ceiling dust sample.

**RESULTS:** One-third of the tile samples and the single dust sample were positive for viable spores of the genus *Stachybotrys*. The presence of viable *Stachybotrys* spores was not reported for the other six bulk samples that had been suspected of being contaminated with black mold. Other fungi identified by light microscope were *Aspergillus*, *Penicillium*, and *Ulocladium*.

**AUTHORS' CONCLUSIONS:** Symptoms reported by students were thought to be related to *Stachybotrys* contamination. The authors implicated the fungi found as "the possible cause" of their illness. The authors recommended a remediation of the contamination.

**COMMENTS:** Symptoms, which were not defined or objectively verified in this article, were not shown to be causally linked to *Stachybotrys* contamination. The *Stachybotrys* spores were suspected to be of the *S. chartarum* species; however, this was never confirmed. Unfounded speculations were raised regarding the route by which the mold might have been dispersed in the school. *Stachybotrys* mycotoxins were never measured.



## 2 Infant pulmonary hemorrhage in a suburban home with water damage and mold (*Stachybotrys atra*)

AUTHORS: Flappan SM, Portnoy J, Jones P, and Barnes C

JOURNAL: *Environmental Health Perspectives*

CITATION: 1999; 107:927

STUDY TYPE: Case report.

OBJECTIVES: The authors sought to associate indoor mold exposure with the occurrence of pulmonary hemorrhage in one infant.

CASE DESCRIPTION: A 1-month-old, previously healthy infant presented to the emergency department with acute respiratory distress and shock (*e.g.*, low blood pressure with signs of impaired blood flow to the tissues). Due to progressive respiratory distress, he was placed on a breathing machine. Diagnostic tests indicated lung bleeding. Laboratory tests showed an increased white blood cell count (which may occur in acute insults such as bleeding, but also in infections), decreased hemoglobin (indicating blood loss), impaired coagulation, and increased platelet count. Blood, urine, cerebrospinal, and tracheal aspirate cultures were performed to rule out an infectious process. All test results were negative. Chest X-ray showed diffuse involvement of both lungs. The attending physician knew that the American Academy of Pediatrics had published a statement urging physicians to follow up on a possible association between any cases of childhood pulmonary bleeding and mold exposure. For this reason, an environmental evaluation was conducted by an industrial hygienist in the child's house, specifically looking for *Stachybotrys*. The family completed a questionnaire. Air and surface samples were collected. The collection process was done during the winter. Using a light microscope, spores were counted and classified into their respective genera based on shape, size, and color.

RESULTS: Water damaged was identified in the house, purportedly due to a leak from roof shingles damaged during a storm. Fungal growth was observed on the attic ceiling and in the closet of the child's bedroom under the damaged attic site. *Stachybotrys* airborne spores (3% of the total spores count) were identified only in the air sample collected from the child's bedroom, in surface samples from water-damaged materials (*i.e.*, from closet ceiling, closet door, and from black stains on the attic ceiling), and in surface samples of dust in the infant's bedroom (*i.e.*, from the supply vent, crib, and mattress). Samples from visible fungi were also collected for cultures: two types of *Penicillium*, gram-negative rods, and *Rhodotorula* yeast were identified. The authors did not mention whether they tested for these other growths from the same areas where *Stachybotrys* was identified. The mycotoxins Roridin L-2, Roridin E, and Satratoxin E were measured at levels of 0.5, 0.7, and 3.2 ng/cm<sup>2</sup> respectively.

COMMENTS: In this case, the authors tried to relate the child's health condition to fungi or specifically to *Stachybotrys* exposure. Only 3% of the total airborne spores counted in the child's bedroom were of the *Stachybotrys* genus. The ability to detect molds as well as *Stachybotrys* mycotoxins does not imply any causal relationship between *Stachybotrys* and the child's disease. Moreover, no attempt was made to isolate spores or mycotoxins from this child's lungs.

### 3 Bioaerosol lung damage in a worker with repeated exposure to fungi in a water-damaged building

AUTHORS: Trout D, Bernstein J, Martinez K, Biagini R, and Wallingford K

JOURNAL: *Environmental Health Perspectives*

CITATION: 2001; 109:641-644

STUDY TYPE: Case report.

**CASE DESCRIPTION:** A 48-year-old previously healthy white male, presented to his primary care doctor with a 2-month history of a dry cough and a 1-week history of fever and shortness of breath. Initial chest X-ray was normal. A pulmonary function test showed a restrictive lung disease pattern. He served as a hotel manager for 14 years. Two months prior to the initial presentation, he and a co-worker assessed water damage in several hotel rooms where fungal growth was visible. The manager did not wear protective gear while conducting the evaluation, which included active disruption of fungal growth on walls. Shortly thereafter he reported the onset of a dry cough, which was accompanied by shortness of breath while he was in the hotel. An environmental evaluation was conducted, including collecting air and bulk samples from 19 rooms damaged by water. Bulk samples were taken from materials that appeared to be contaminated with fungi. Samples were cultured and assayed for mycotoxins. Cultured and uncultured airborne fungi were identified for 14 interior buildings. One outdoor location served as a control.

**RESULTS:** Seven months after his initial presentation, a serum total IgE (*i.e.*, the antibody that increases in concentration during allergic reactions) was 3.5 times greater than the normal concentration. Specific serum IgE and IgG (*i.e.*, another type of antibody that indicates prior exposure) to *Stachybotrys* were negative. Antibodies were positive only for *Thermoactinomyces vulgaris*. An effort to was made to develop an antibody assay to Roridin-A, a mycotoxin produced by some *Stachybotrys* species. Chest X-ray and high-resolution chest CT (HRCT) were normal. Several weeks later, a second HRCT showed a pattern consistent with bronchiolitis. A bronchial fluid examination and a transbronchial biopsy or a lung biopsy done with the aid of a bronchoscope were unremarkable. He was diagnosed as having hypersensitivity pneumonitis and because he was clinically not improving, treatment with immunosuppressant drugs was started. The patient's condition gradually improved although his pulmonary function tests and HRCT remained unchanged.

**AUTHORS' CONCLUSIONS:** Serologic testing was not useful in differentiating workers who were probably occupationally exposed to mycotoxins from those who were not; however, it did yield evidence that individuals may make specific IgG antibodies to macrocyclic tricothecene mycotoxins.

**COMMENTS:** The authors stated that the patient's cough appeared shortly after he worked in the water-damaged hotel rooms. It was not clear how the authors reached this conclusion, although it is possible that this was based on what the patient reported to them. On the laboratory evaluation, the authors divided the employees into two groups, based on a complete questionnaire. A physical exam was not performed. The fact that group assignment was based on self-reporting again might have led to bias in the selection of the group. The numbers tested in each group were too small to provide useful information regarding causation. The authors concluded that the patient had hypersensitivity pneumonitis, which allegedly was due to extensive exposure to indoor fungi. If the diagnosis was correct, the authors had not ruled out the fact that the presented case had positive antibodies to *Thermoactinomyces vulgaris*, which is a known cause for this condition. The authors made a common causation error. The fact that airborne spores were detected does not mean that they were the direct cause of this patient's disease.

#### 4 Pulmonary mycotoxicosis

AUTHORS: Emanuel DA, Wenzel FJ, and Lawton BR

JOURNAL: *Chest*

CITATION: 1975; 67:293-7

STUDY TYPE: Case series describing exposure to fungi by inhalation inside silos.

DESCRIPTION: This study reported on 10 patients (nine males and one female) ages 16 to 56 years old. The exposure occurred while the workers cleaned off a mold layer. While removing the moldy silage, they inhaled large quantities of dusty material. The cases reported fever, chills, and shortness of breath. It lasted between 7 and 30 days (mean 16.4 days). All patients recovered and returned to work without developing a recurrence. Case 1: 40-year-old male developed cough, shortness of breath, burning sensation of the eyes and throat, fever, and chills after he finished cleaning the moldy silage. His physical exam showed infected conjunctiva (inflamed eyes), nose, and throat. A chest X ray revealed an interstitial pneumonitis (lung inflammation). A lung biopsy performed supported the clinical diagnosis of lung inflammation. Both *Fusarium* and *Penicillium* growths were cultured from the lung tissue, although no bacteria were cultured. Allergy tests to thermophilic actinomycetes and to moldy hay were negative. The patient gradually improved, returned to work and the illness did not return. Case 2: As in case 1, the patient developed a self-limited disease, which included cough, fever, chills and irritation of his eyes and throat, after cleaning the moldy silage inside the silo. His chest X ray revealed mild interstitial lung disease (similar to case 1). Serology tests to thermophilic actinomycetes, a crude extract of moldy hay, and five *Aspergillus* organisms were negative (these tests check for specific antibodies in serum to these components). Case 3 was the daughter of Case 2.

AUTHORS CONCLUSION: The authors concluded that this disease differed from Farmer's Lung Disease, which is a hypersensitivity-immune reaction. The authors based their conclusion on the fact that the silo workers recovered and never developed a similar disease after they returned to work in the farm, as opposed to Farmer's Lung Disease, in which patients may develop a similar disease after they are re-exposed. Moreover, the authors stated that the lung biopsy of patient 1 was histologically different from the lung findings that correlate with Farmer's Lung Disease.

COMMENTS: These cases exhibit an illness that might have been a reaction to dust. Other authors have described an allergic reaction to the non-tested fungi. In the article by Brinton *et al.*, pulmonary mycotoxicosis was defined as organic dust toxic syndrome, because the organic dust consists largely of microbial hyphae and spores. The cases described by Emanuel, Wenzel, and Lawton were consistent with organic dust inhalation.

**5 An outbreak of organic dust toxic syndrome in a college fraternity**

**AUTHORS:** Brinton WT, Vastbinder EE, Greene JW, Marx JJ, Hutcheson RH, and Schaffner W

**JOURNAL:** *Journal of the American Medical Association*

**CITATION:** 1987; 258:1210-2

**STUDY TYPE:** Case series.

**DESCRIPTION:** Organic dust toxic syndrome (ODTS) was defined as a non-allergic, noninfectious, respiratory illness caused by inhalation of organic dust from moldy silage, grain, hay, or other agriculture products. The authors equated ODTS with pulmonary mycotoxicosis and silo-unloader's syndrome.

**METHODS:** A party was held in the basement of a fraternity house that had poor ventilation and hay was dispersed on the floor. The basement became dusty, to a point where it was impossible to see across the room. Members of the fraternity sought medical attention the next day, complaining of an acute respiratory illness. A review of medical records and an evaluation of a questionnaire completed by the fraternity members were performed. A case was defined as at least two of the following symptom groups: (1) shaking chills, or sweat, (2) cough or shortness of breath, and (3) muscle aches. Acute serum samples from 16 ill and seven unaffected individuals were tested for antibodies to several respiratory viruses, as well as to 13 fungal (including *Aspergillus*, *Penicillium*, and thermophilic actinomycetes) and bacterial antigen preparations (hypersensitive pneumonitis panel—used for the diagnosis of Farmer's Lung Disease). Convalescent serum samples (18 days later) from seven ill and one unaffected individuals were sampled for the same antibodies. Serum antibodies to an extract of the fraternity straw were also tested.

**RESULTS:** Eleven cases sought medical attention. Their symptoms included malaise, chills, cough, chest pain, and back pain. The physical examination revealed low-grade fever (temperature < 38°C), tachycardia, dyspnea, and basilar rales. Four patients had chest X rays, which were normal. The white blood count was elevated in all. Of the 80 fraternity members, 82% met the case definition criteria. Symptoms appeared 9.4 hours (range 1.3-13 hours) from the time the party started. The illness lasted from 4.5 hours to 7 days (median, 60 hours). Attack rates were proportionally related to the time the attendees spent at the party. *Laboratory data:* Four non-ill participants developed a fourfold rise in antibody titer, each to a different virus: influenza A, B, parainfluenza type 3, and RSV. All acute serum samples tested negative for the hypersensitive pneumonitis panel and to the straw extract.

**AUTHORS' CONCLUSIONS:** The patients developed ODTS from inhalation of heavy dust derived from the straw on the basement floor.

**COMMENTS:** The disease described here is the consequence of a non-inflammatory response of the lung to foreign material. In the cases described, the cause was never identified, although the authors theorized that it was related to inhaled dust. This case series is typical of Organic Toxic Dust Syndrome.

## 6 Airborne outbreak of trichothecene toxicosis

AUTHORS: Croft WA, Jarvis, BB, and Yatawara CS

JOURNAL: *Atmospheric Environment*

CITATION: 1986; 20:549-552

STUDY TYPE: Case series and animal toxicology study.

DESCRIPTION: This study was designed to assess the toxicologic effects of airborne material extracted from a home with indoor mold and water damage. The cases resided in a house located in a northern suburb of Chicago; all complained of sore throat, diarrhea, headache, fatigue, generalized weakness, dermatitis (inflammation of the skin), and intermittent focal alopecia (*i.e.*, hair lost). Repeated testing to detect heavy metals including blood, urine, and hair were all normal. The authors proposed that the fungus *Stachybotrys atra* was responsible for the health complaints of the family, since it produces a group of mycotoxins named macrocyclic trichothecene, which may be toxic.

METHODS: Air collected from the living room, dining room, and upstairs bedrooms revealed the presence of *S. atra* spores and trichothecenes. The resulting substance was dissolved in ethanol and administered by mouth to five Sprague-Dawley rats (gender and age unspecified) and five adult mice (gender and age unspecified). A control group of rodents received ethanol only.

RESULTS: Necropsy of the animals showed necrosis and hemorrhage within the brain, thymus, spleen, intestine, lung, heart, lymph node, liver, and kidney. The authors identified the macrocyclic trichothecene Verrucarrol, Verrucarins B and J, Satratoxin H, and Trichoverrins A and B from a sample scraped from the ceiling fiber board, which the authors noted was covered by *S. atra* spores.

AUTHORS' CONCLUSIONS: The authors concluded that the signs and symptoms presented by these patients were suggestive of trichothecene toxicity based on clinical descriptions by others of the illness they produce. The authors claimed to have isolated newly discovered trichothecene, Verrucarins B and trichoverrins A and B, from *S. atra*.

COMMENTS: The signs and symptoms presented by these patients may appear in other health conditions such as viral infections. The authors did not state whether this was ruled out. The animals were fed an unidentified substance dissolved in ethanol, presumably derived from the visible *S. atra*. The exact components of the substance were not specified. Assuming that this substance represented a mixture of several mycotoxins, they were neither specified nor was the dose provided. It is also possible that the pathological findings were not necessarily related to mycotoxins but to other impurities, which probably were present in this mixture. The trichothecene were isolated from scraped material and not from airborne *S. atra* spores. Thus, it is not known whether the patients inhaled airborne mycotoxins. Moreover, isolation of spores from their lungs was never attempted.

## 7 Clinical-epidemiological investigation of health effects caused by *Stachybotrys atra* building contamination

AUTHOR: Johanning E

JOURNAL: *Proceeding of Indoor Air*

CITATION: 1993; 1:225-230

STUDY TYPE: Case series.

**BACKGROUND:** An office building located in New York. The sheet rock walls were found to contain more than 100,000 viable *S. atra* colony counts per meter. Bulk samples were also found to contain Satratoxin H, a macrocyclic trichothecene.

**METHODS:** A survey questionnaire completed by the 49 building occupants collected information regarding health complaints, medical history, work characteristics, and environmental conditions. There was no control group with which to compare. Only the cases with employment duration of more than 3 months were included in the data analysis. Serum IgE (*i.e.*, a type of antibody that usually is elevated in allergic responses) and IgG (*i.e.*, a different type of antibody) antibodies specific against *S. atra* were measured. A subgroup of employees with either positive antibodies to *S. atra* or with known direct contact with *Stachybotrys*-contaminated material and significant symptoms had a more comprehensive immunological testing, which included: (1) lymphocyte counts, (2) T and B lymphocytes flow cytometry, (3) lymphocyte mitogen response assays, (4) IgM, IgA, IgG, and IgE antibody panels, (5) angiotensin converting enzyme, (6) liver function tests, and (7) hepatitis C screening.

**RESULTS:** Forty-three cases responded to the questionnaire. This group consisted of 34 females and 9 males with a mean age  $35 \pm 11$  years-old and mean employment duration of  $2.6 \pm 2.5$  years. Approximately 50% of the cases complained of skin, eye, respiratory, and central nervous system disorders and 44% complained of asthma, hay fever, and sinusitis. Twenty-six percent of the cases complained of chronic fatigue and 37% stated that the symptoms worsened while they stayed in the office. Twelve had elevated total serum IgE (defined as  $> 180$  IU/L). Four were positive to *Stachybotrys*, *Aspergillus*, and *Penicillium*, and five to *Cladosporium*. It is unclear whether these were the same four subjects. All the IgE positive subjects had a medical history of asthma, hay fever, or sinusitis. Eight subjects were positive for specific serum IgG to *S. atra*.

**COMMENTS:** Study results were based on subjective responses, leading to bias. There was no physical examination to confirm the complaints. This very small group was never compared to a control group, which, in this case, should have been occupants of other building(s) without apparent *S. atra* exposure. Hence, all the findings presented here should be interpreted with caution. The authors mentioned that *S. atra* was found in the building without providing information on airborne *S. atra*. The positive serum IgE concentrations mean that these subjects have a propensity to develop allergies or are allergic, possibly to molds or other environmental factors. The significance of positive IgG to *S. atra* indicated that these persons were exposed sometime in their lives to *S. atra*, but not that they were exposed recently. These findings do not infer toxicity whatsoever.

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

AUTHORS: Johanning E, Biagini R, Hull D, Morey P, Jarvis B, and Landsbergis P

JOURNAL: *International Archives of Occupational and Environmental Health*

CITATION: 1996; 68:207-218

STUDY TYPE: Case-control design.

**METHODS:** This study was a follow up to a case series published in 1993 by Johanning. A survey was conducted for medical, occupational, and environmental histories. The case group was compared with a control group, the latter consisting of office workers with similar job activities as the case group, but who did not come in contact with the "contaminated" building. There were 53 cases (39 females and 14 males; mean age 34.8 years old; mean employment time: 3.1 years, 40 h/week) and 21 controls (11 females and 10 males, mean age 37.5 years old). Air (indoors and outdoors) and bulk material samples for viable and nonviable fungi were collected. Air sampling was performed under two types of collection parameters, quiescent (*i.e.*, under normal operating conditions) and aggressive (*i.e.*, abnormally disturbing the interior finishes to aerosolize settled dust simulating indoor activity). The bulk samples were taken from visibly moldy surfaces, from which the mycotoxin, Satratoxin H was isolated.

**RESULTS:** Analysis of the air and bulk samples verified that fungi were present in a number of the building sites. Visible molds included *S. atra*, and unknown species of *Cladosporium*, *Penicillium*, and *Aspergillus*. *Stachybotrys* was identified in samples from walls and carpeting in areas that had been damaged from flooding, such as the sub-basement. There were large differences in the concentration of most fungal genera when quiescent air sampling was compared to aggressive air sampling. For the quiescent air sampling, *Cladosporium* was the dominant species from all sampling sites. *Stachybotrys* was found only in the sub-basement area and only represented 14% of the total mold population. For the aggressive air sampling, *Stachybotrys* was found in greater numbers in the basement and sub-basement samples. *Cladosporium* and *Aspergillus niger* predominated in the outdoor samples. The number of cases complaining of lower respiratory tract, eye, skin and constitutional problems was statistically greater than the number of controls reporting these problems. Significantly more cases also complained of chronic fatigue symptoms as compared to controls. Other physical complaints did not differ between the two groups. The laboratory tests did not reveal any differences between the case group and the control group. There were no health-related abnormalities identified for the cases from the laboratory studies. The only result that was found statistically different was the number of mature T lymphocytes, which was slightly lower (1345 cells/mL) than controls (1431.5 cells/mL). All the other subsets of T lymphocytes were not found to be different from the control group.

**COMMENTS:** Nineteen percent of both groups were active smokers. No information was provided as to whether the other cases were exposed to passive smoking, which might have a confounding influence. The authors concluded that the exposure was high where large numbers of fungi, especially *Stachybotrys*, were culturable. No physical examinations were done to objectively confirm the subjectively reported complaints. Complaints were based only on self-reporting, a well-known source of bias. The authors failed to demonstrate immunological abnormalities in the case group, and although they tried to convince the reader that workers who spent most of the time in the sub-basement had some abnormalities, these were neither statistically significant nor clinically relevant. The authors reported that the cases with a history of upper respiratory tract infection had lower CD3 lymphocytes, but the significance of this observation is unclear because these results were not compared with findings for the control group. The authors' statement that the cases possessed an impaired immune system is scientifically unfounded.

## 9 Building associated pulmonary disease from exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*

AUTHORS: Hodgson MJ, Morey P, Leung WY, Morrow L, Miller D, Jarvis BB, Robbins H, Halsey JF, and Storey E

JOURNAL: *Journal of Occupational and Environmental Medicine*

CITATION: 1998; 40:241-249

STUDY TYPE: Nested case-control study.

**METHODS:** A new courthouse and two interconnected associated buildings were constructed between 1986-1989. Occupants reported mucous membrane irritation (*i.e.*, of the mouth, nose, and eyes), fatigue, headache, and chest tightness occurring within weeks after moving into the buildings. Moisture problems were identified in these buildings and attempts to remediate the moisture problems reportedly failed. Mold found in the ventilation ducts was cleaned; however, the occupants' complaints persisted, ultimately leading to the evacuation of the buildings. Air sampling was performed using both quiescent and aggressive conditions while the building was occupied and also after it was vacated. Two ceiling tiles contaminated with *S. chartarum* were used for mycotoxin extraction and analysis using high-performance liquid chromatography. Medical evaluations were undertaken for 14 occupants selected by the insurance carrier. The evaluation consisted of interviews, questionnaire responses, and review of medical records. Interstitial Lung Disease (ILD) was defined as having fever, muscle pain, shortness of breath, and chest tightness. The control group consisted of occupants of two buildings selected by convenience, without having any air quality evaluation. Both groups underwent full lung function studies and completed a battery of neuropsychological tests. The screening group also underwent full lung tests if their chest symptoms were accompanied by self-reports of fever, muscle pain, and shortness of breath. Laboratory studies included antibodies (IgG and IgE type) to several fungi and *Thermoactinomyces*, although it is unclear whether all antibody tests were performed in both groups.

**RESULTS:** The building revealed an area of 100 m<sup>2</sup> with visible mold. For the 14 indoor samples, the mean concentration of fungi was 368 CFU/m<sup>3</sup> (range, 24-1343 CFU/m<sup>3</sup>) and *Aspergillus versicolor* was the predominant type. No information was provided as to whether other species were present in the same samples. There were seven outdoor samples with a mean total fungal concentration of 700 CFU/m<sup>3</sup> (range, 161-1650 CFU/m<sup>3</sup>). Bulk sampling revealed the presence of *S. chartarum* and *A. versicolor*. The mycotoxins satratoxins G and H were isolated at concentrations of 2-5 ppm for the samples taken from moldy ceiling tiles. Medical examinations found that three cases had "work-related" asthma; three were diagnosed with ILD disease, and six with rhinitis (*i.e.*, runny nose) that improved over the weekend. Forty-seven individuals volunteered to undergo screening examination. The subjects were more likely to participate in the screening examinations if they had symptoms consistent with ILD. Of the 44 subjects undergoing lung function tests, three had declines of greater than 10% in FEV1 across a work shift and four had declines of 5-9%. No statistically significant association was found with respiratory symptoms and FEV1 or FVC decline (both are indexes for the presence of airway obstruction in asthma, or ability of the lungs to expand after each inhalation).

**COMMENTS:** The control buildings were arbitrarily selected without assessing them for mold, raising doubt as to their suitability as controls. Also of note, the outdoor fungal concentration was higher than the indoor concentration, which does not indicate an indoor problem. Questionnaire use for retrieving medical information is an important source of bias that may affect the results. Differences found between the groups in lung function tests were probably related to smoking habits. Physical examinations were not performed in both groups. No attempts were made to find objective findings to support an ILD diagnosis.



## 10 **Toxigenic fungi in a water-damaged building: an intervention study**

AUTHOR: Sudakin DL

JOURNAL: *American Journal of Industrial Medicine*

CITATION: 1998; 34:183-190

**STUDY TYPE AND OBJECTIVES:** This study consisted of a series of case reports. This study evaluated the environmental conditions of a building with moisture problems as well as health symptoms among its occupants. The occupants ultimately were vacated from the building.

**METHODS:** The building survey included an inspection of the ventilation system, collection of bulk and surface samples from sources suspected of fungal and bacterial contamination, and air sampling from 15 indoor locations, for fungal and bacterial bioaerosols. The sampling was performed 1 and 2 months after the building was vacated. The different samples were cultured in different agars, since different molds grow preferentially on different agars. The HVAC system also was sampled. A health hazard evaluation was conducted by NIOSH and consisted of a self-reported questionnaire administered to current and former employees who had occupied the building.

**RESULTS:** Elevated mold counts were observed in several locations inside the building. The predominant molds were from the *Penicillium* and *Phoma* species. *Stachybotrys chartarum* was not cultured from the air samples. The predominant airborne mold was *Penicillium*, representing 58% to 86% of the identified mold genera from different samples. On Czapek agar, *Aspergillus* and *Penicillium* were the predominant molds, but *S. chartarum* was found in only one of the material bulk samples. Thirty-seven occupants responded to the questionnaire. Symptoms reported before relocation appeared to improve after the respondents were relocated. The symptoms that were reported to improve included central nervous system symptoms such as fatigue, headache, anxiety, difficulty concentrating, cough, shortness of breath, wheezing, nasal and sinus congestion, sore throat, bloody nose, diarrhea, skin rash, and flushing. The majority of the participants (70%) described their overall health as "better" after being relocated, although 15% described their health as the same or worse. Almost 90% of the responders had read the report discussing the environmental conditions within the building.

**COMMENTS:** The author pointed out that measurement of airborne *Stachybotrys* spores may inaccurately measure true exposure since nonviable spores, which under dry conditions may become airborne, can contain a high concentration of mycotoxins. Additionally, mycotoxin production is dependent on the environmental conditions of its growth. Isolation of a toxigenic fungus from an indoor environment does not necessarily indicate exposures to mycotoxins have occurred. The health effects were collected from a questionnaire. Many were already exposed to literature regarding health effects of endotoxins and mycotoxins, leading to a substantial bias.

## 11 Indoor pollution and sick building syndrome symptoms among workers in day-care centers

AUTHORS: Li CS, Hsu CW, and Tai ML

JOURNAL: *Archives of Environmental Health*

CITATION: 1997; 52:200-208

STUDY TYPE: Survey and sampling study.

OBJECTIVES: To associate respiratory symptoms among workers in day-care centers and indoor pollution.

METHODS: Twenty-eight Taiwanese day-care centers were randomly selected. Centers were not near any major industrial sources of ambient air pollution. All workers completed a questionnaire, identifying so-called sick building syndrome-related symptoms. Environmental monitoring included carbon dioxide, temperature, and humidity. Airborne fungi and bacteria were collected on malt extract agar (MEA) and malt tryptic agar (TSA), respectively. Dust mite antigens *Der p I* and *Der p V* were quantified from a dust extract.

RESULTS: The indoor fungal count was not statistically significant different from the outdoor count (1212 CFU/m<sup>3</sup> vs. 1032 CFU/m<sup>3</sup>). The most commonly sampled indoor fungus was from the *Penicillium* genus following by *Cladosporium* and *Aspergillus*. The same trend applied to the outdoor samples. Indoor and outdoor carbon dioxide did not differ from each other, the values ranging from 370-1250 ppm indoors and 280-1100 ppm outdoors. Both ranges are well below the acceptable levels determined by American regulatory agencies (e.g., OSHA, NIOSH) and industrial hygiene organizations (e.g., ACGIH). The levels of indoor bacteria were significantly greater than outdoor levels. A total of 264 day-care center workers completed the questionnaire. In general, more females complained of health problems than males, although this increased prevalence of symptoms was statistically significant only for the following: eye irritation, nasal discharge, pharyngeal symptoms, skin rash, headache, lethargy, fatigue, and difficulty in concentration. Respiratory symptoms did not differ significantly between the genders. The authors identified five environmental phenomena that were studied as risk factors: stuffy odor, visible mold, flooding, water damage, and dampness. The authors found the risk of eye irritation and pharyngeal irritation was increased significantly with the presence of all five environmental factors, after adjusting for factors such as socio-demographics, age, gender, and education. These five environmental factors did not affect the risk for the other symptoms in any consistent fashion, although dampness was a statistically significant risk factor for cough, headache, fatigue, difficulty in concentrating, and wheezing.

The authors found an association between the age of the building structures and the presence of bacteria, and the dust mite antigens, *Der p I* and *Der p V*. A number of allergen concentrations were significantly decreased in day-care centers that reported the use of air conditioners, dehumidifiers, and air cleaners. Higher humidity was associated with statistically significant increases in the concentrations of total fungal and *Der p I* allergens but not total bacterial concentrations.

COMMENTS: Health effects were assessed by self-reported symptoms. No physical examination or tests were done. No discussion of other plausible explanations was offered by the authors. Smoking habits of the workers were not assessed. This article did not address a causal relationship between symptoms and risk factors, although some statistically significant associations were identified in the adjusted odds ratio analyses. Statistical adjustments may sometimes amplify small differences between groups, especially when many different comparisons are conducted in the same analysis as was done in this study.

## 12 Investigation and remediation of diesel-converted trolley buses associated with extensive fungal growth and health complaints

AUTHORS: Van Netten C, Brands R, and Dill B

JOURNAL: *American Industrial Hygiene Journal*

CITATION: 1997; 58:726-731

STUDY TYPE: Case series combined with a controlled environmental assessment.

**METHODS:** Fifteen bus drivers complained of itchy, watery eyes, runny nose, and headaches while driving diesel-converted trolley buses. These symptoms, which were thought to be related to the ventilation system of the bus, resolved after driving and reappeared when they started to drive. No mildew was visible, but the buses were remediated anyway. These measures did not eliminate the complaints, so an environmental assessment was conducted on six of the diesel-converted trolley buses (out of 47 "problem" buses), four of which had been cleaned previously with a bleach-based solution. Four buses that were not associated with health complaints were selected as controls. All buses were investigated for volatile organic compounds. Fungal samples were taken using an Andersen sampler and were cultured on dextrose agar plates. Outdoor samples served as controls. Cases were asked to complete a health questionnaire which addressed the following symptoms: runny, itchy nose, watery, itchy eyes, stuffy nose, dry throat, difficulty wearing contact lens (related to low humidity), chest tightness, wheezing, shortness of breath, tiredness, nausea, headaches, fever, and joint pain. They were also asked to refer to symptoms that occurred only for a specific time frame, which corresponded to the time before widespread recognition of complaints and before drivers were reluctant to drive the buses. The drivers completed the questionnaires, however, at least 6 months after remedial measures were implemented.

**RESULTS:** Fungi were found in both the case and control buses, although no statistical analyses were performed to determine whether there were statistically significant differences in mold counts between the case and control buses. Three case buses had high counts of > 71000, >107000, and >1080 CFU/m<sup>3</sup>, respectively. The other case buses had mold counts that overlapped with the counts for the controls. The predominant species were *Penicillium* and *Cladosporium* and occasionally *Aspergillus*. The authors noted that there was greater diversity in the genera of fungi in the outdoor samples. The authors tried to relate self-reported symptoms and fungal contamination. Symptoms were found only in the case buses (including those with similar fungal counts to the control buses) and none in the control buses. About 50% of the responders reported experiencing some of the symptoms while driving diesel buses. Smoking status, allergies, and symptoms were similar and were not statistically significant between the symptomatic and asymptomatic groups. The most frequently reported symptoms were: blocked, runny, itchy nose, itchy, watery, irritated eyes, nausea, and dry irritated nose. There was one case of reported fever and seven drivers reported joint pain. No volatile substances were detected in the air samples from the case buses.

**AUTHORS' CONCLUSIONS:** The authors did not identify health problems among the drivers that were associated with the discovery of mold in the buses.

**COMMENTS:** The questionnaires were completed 6-12 months from the day they drove the diesel-converted buses. This may constitute a source of bias. No physical examination was performed to confirm the presence or absence of objective findings. Smoking as a contributing factor for the presence of symptoms was not assessed.

### 13 Fungal exposure of children at homes and schools: a health perspective

AUTHORS: Su HJ, Wu PC, and Lin CY

JOURNAL: *Archives of Environmental Health*

CITATION: 2001; 56:144-149

STUDY TYPE: Case control.

**METHODS:** One hundred fifty-five (77 asthmatic, as diagnosed by their physician, and 78 matched non-asthmatics for control) children were randomly selected from two schools. The study was performed during both winter and summer (47 asthmatic and 33 non-asthmatic assessed) in their schools and houses were assessed. Airborne fungal concentration was measured in their bedrooms and outside the entrance of their home. The sample was cultured on a malt extract agar plates. The ambient conditions of humidity and temperature were continuously recorded. For every measurement taken at home, an identical sampling inside and outside the child's classroom was performed. Fungi were identified under a dissecting microscope by a trained technician. Fungal exposure was estimated by summing the products of percent of time spent (assessed by a self-administered daily report card for each child) and concentration measured at each environment of the child. Health was estimated by a self-reported questionnaire of symptoms such as: cough, chest pain, wheezing, runny nose, and eye irritation. A health performance scoring system was designed.

**RESULTS:** No statistical differences were found in total mold and type-specific mold count between asthmatics and non-asthmatics at their homes and school. The total mold count was higher in winter than summer. However, the authors did not perform a statistical analysis. *Cladosporium* was the predominant type. In schools the same trend was preserved. However, this time it was statistically significant. The same species were predominant. Symptoms scores between higher- and lower-exposure groups (as described in methods) did not differ with respect to total airborne fungi.

**COMMENTS:** This study shows that molds are ubiquitous and common in the environment. They are found throughout the year with probably some seasonal variations. The authors showed that the mold count was not statistically different between the asthmatic and non-asthmatic groups. However, this applies only to molds that are supported by MEA agar for their growth. There is still a chance that other fungi may contribute to the prevalence of asthma.

## 14 Inhaled mycotoxins lead to acute renal failure

AUTHORS: Di Paolo N, Guarnieri A, Garosi G, Sacchi G, Mangiarotti AM, and Di Paolo M

JOURNAL:

CITATION: 1994; 9(Suppl.4):116-120.

**CASEDESCRIPTION:** A 52-year-old woman presented to the authors' clinic with acute renal failure (malfunction of the kidneys). Five days prior to presentation, she sieved wheat stored for over 2 years in a closed granary. A layer of mold covered some of the wheat. The following evening she started to complain of shortness of breath, chest and abdominal discomfort. Because of her aggravating condition, she was admitted to the intensive care unit with pulmonary effusion (fluids in the lungs), periorbital (around the eyes) and bilateral leg edema (swelling), decreased urine output accompanied by impaired kidney function tests (this suggests kidney malfunctioning), and proteinuria (protein in the urine- an abnormal condition). Laboratory tests to rule out immune causes for this condition were negative. Kidney biopsy was performed and showed a histology pattern of tubular necrosis. No evidence of an immune-based cause was found. The kidneys restored function after 40 days without the need of dialysis. *Environmental assessment:* A sample of the wheat, cultured in Czapek agar, yielded the growth of colonies of *Aspergillus ochraceus*. The authors succeeded in isolating ochratoxins from flour made with the moldy wheat. *Experimental procedure:* 8 kg of the wheat were placed in the bottom of a closed, continuously ventilated container containing 4 guinea pigs and 4 rabbits. After 8 hours the animals were taken back to their cage. A rabbit, guinea pig, and a second rabbit died 16, 24, and 34 hours respectively following their return to their cage. On the 5<sup>th</sup> day the remaining animals were sacrificed. A necropsy done on the expired rabbits showed macroscopic signs of liver and kidney injury. One also had lung edema (fluids in the lungs). Microscopic examination of the rabbit and guinea pig livers showed signs of degeneration. Microscopic kidney tubular cells damage and liver degeneration were identified in two of the five animals sacrificed. The other three did not show any signs of damage.

**COMMENTS:** This article lacks the proof of causation. Although an attempt was made to rule out immunological diseases which cause acute renal failure, I have not seen her past medical history or history of medications used. Both are crucial in trying to elucidate the cause(s) of renal impairment. The authors stated that they have isolated ochratoxin. However, they did not provide any analytical or laboratory evidence to that effect. They were assuming the substance isolated was indeed ochratoxin, based solely on the identification of the fungi as *A. ochraceus*. They have also not stated how experienced the individual was who identified the mold. This might be important because the entire case is based on that. Furthermore, only certain ochratoxins have been associated with nephropathy. Recently viral particles have been found in persons with Balkan Nephropathy. The animal experiment only showed that animals inhaling "moldy" wheat might die or have liver injury. The authors assumed that whatever the animals inhaled was homogeneously distributed in the container, yet no measurements or air monitoring was attempted to verify what substance(s) were inhaled by the animals during the 8-hour period. There was also no information on the inhaled dose.

## 15 Adverse health effects among adults exposed to home dampness and molds

AUTHORS: Dales RE, Burnett R, and Zwanenburg H

JOURNAL: *American Review of Respiratory Diseases*

CITATION: 1991; 134:196-203

STUDY TYPE: Cohort study.

OBJECTIVES: To investigate the association between dampness and symptoms in adults.

METHODS: Adults (at least 21 years old) from 30 Canadian communities of similar socioeconomic and geographic locations were asked to complete a health and environmental questionnaire. The health questions included symptoms of the respiratory system (lower respiratory symptoms: persistent cough and phlegm, wheezing, shortness of breath; chronic respiratory disease: doctor's diagnosed asthma, emphysema, and chronic bronchitis; upper respiratory symptoms: nose irritation, stuffy nose, sneezing, throat irritation) and other symptoms such as headaches, muscle aches, fever and chills, vomiting, and diarrhea. The environmental questions included topics regarding home dampness, moisture, and mold.

RESULTS: A total of 14,799 questionnaires were completed. The responders were divided into two groups based on the presence of reported mold/ dampness in their house. Both groups appeared (no statistical analysis was performed) similar regarding age, ethnicity, gender, and smoking habits (current and past). The percentage of respondents reporting dehumidifier use, passive smoking, pets, and hobbies with potential sources of dusts and fumes (*e.g.*, oil painting, furniture stripping, and wood working) was higher in the mold/ dampness-present houses, but no statistical analysis was done. Improved insulation (undefined by the authors) was higher in the houses that were absent of mold (60%) as compared to the houses containing mold (50%). Lower respiratory symptoms and chronic respiratory disease were approximately twice as prevalent among current as compared to ex-smokers as well as those who never smoked. When stratified by smoking status, the prevalence of all symptoms was approximately 150% greater in damp houses. The authors used a multivariate analysis to control for variables such as age, gender, ethnicity, maximum parental education, household crowding, region, occupation, cigarette smoking, secondary smoke exposure, use of woodstoves and gas stoves for cooking, hobbies, pets, use of kerosene and gas heaters, and the use of a fireplace to heat. Odds ratios suggesting that there was an increased risk (*i.e.*, an odds ratio greater than 1.0 suggests an increase) for the following respiratory symptoms in houses with dampness and/or mold: upper respiratory symptoms 1.5, lower respiratory symptoms 1.62, chronic respiratory disease 1.45, asthma 1.56, eye irritation 1.63.

COMMENTS: The major limitation of this study is that all responses were self-reported and were never objectively verified by a medical examination. For example, the authors classified someone as asthmatic if they answered "yes" to the question "has a doctor ever said that you have asthma?" The results of this study may be affected by significant biases and should be interpreted very cautiously. Also the presence or absence of molds/ dampness was determined by the respondent and not by an environmental assessment. The respondents, as with other studies, might have exaggerated their health complaints and the extent of dampness in their houses.

## 16 Damp housing, mold growth, and symptomatic health state

AUTHORS: Platt SD, Martin CJ, Hunt SM, and Lewis CW

JOURNAL: *British Medical Journal*

CITATION: 1989; 198:1673-1678

STUDY TYPE: Cross-sectional study of random household samples containing children.

OBJECTIVES: To examine the relation between dampness, mold growth, and symptoms of ill health.

METHODS: Homes with at least one family member under the age of 16 were assessed for dampness and mold. The tenants completed a 16-symptom questionnaire. House conditions were divided into categories based on the way homes were rated for dampness (*i.e.*, dry, damp, and moldy). The homes were assigned a score based on a four-point severity scale for dampness and mold (none, mild, moderate, and severe). Air sampling was analyzed using a five-point scale that categorized the spore concentration per m<sup>3</sup> as follows: low =  $\leq 100$  viable spores/ m<sup>3</sup> air; medium = 101-300; high = 301-1000; very high = 1001-5000; extremely high =  $> 5000$ .

RESULTS: A total of 891 respondents completed the questionnaires (73% of eligible questionnaire recipients). About two-thirds ( $n = 597$ ) of those households who had the health interview were also surveyed environmentally. Variables such as smoking and house crowding did not vary statistically among the house dampness groups. There were significant differences among house dampness groups for the following self-reported symptoms: bad nerves, aching joints, nausea and vomiting, backache, blocked nose, fainting spells, constipation, and breathlessness. After adjusting for length of time at address, responder's economic position, cigarette smoking, and household income, the housing dampness condition (*i.e.*, living in moldy households) still remained a statistically significant risk factor for the total number of self-reported health symptoms. The individual symptoms were no longer statistically significant. The concentration of the air spores was positively associated with high blood pressure, bad nerves, backache, palpitations, and breathlessness and negatively associated with headaches. In children, an association was found between house condition (*i.e.*, dry, damp, moldy) and prevalence of symptoms such as wheezing, sore throat, persistent headache, fever and high temperature, persistent cough, and runny nose. After adjusting for the confounding effects of overcrowding, cigarette smoking, and unemployment, the differences remained significant for wheezing, sore throat, persistent headache, fever and high temperature, and runny nose. Cough was not associated with housing conditions. A similar trend toward severity of symptoms, with the presence of mold, was found in children as well.

COMMENTS: This study was based solely on the residents' response to the questionnaire. Most of the statistically significant findings were based on adjustments for confounding variables, which may amplify small differences between groups. The authors did not attempt to find other possible causes for the variety of symptoms described. No physical examination or laboratory tests were performed. They attempted to minimize the respondent bias by having an expert assess the homes. The authors claimed that there was good agreement between the expert and the respondents' reporting of the condition of the house, although the statistical number,  $K = 0.26$  (agreement between evaluators), does not support this statement. There was also a disagreement between the tenants and the experts regarding the dampness and mold state (30.7%). Collectively, these findings suggest that there was substantial respondent bias affecting the outcome of this study. This study found an association among indoor mold, dampness, and the health complaints of the respondents, but a causal relationship was not demonstrated.

## 17 Environmental risk factors associated with pediatric idiopathic pulmonary hemorrhage and hemosiderosis in a Cleveland community

AUTHORS: Montana E, Etzel RA, Allan T, Horgan TE, and Dearborn DG

JOURNAL: *Pediatrics*

CITATION: 1997; 99:1-8

STUDY TYPE: Case-control study.

**OBJECTIVES:** To present a cluster of 10 infants with pulmonary hemorrhage and hemosiderosis identified in Cleveland, Ohio, and evaluate whether indoor mold played a role in their condition.

**METHODS:** The cases consisted of 10 previously healthy infants, who had episodes of acute, diffuse pulmonary hemorrhage of unknown cause (diagnosed either by lung biopsy or bronchoalveolar lavage) during the first 6 months of life. All cases were patients at the same hospital. For each case, three healthy controls (matched for age and geographic area) were selected from the hospital clinic population and from Cleveland birth certificate records. Medical information was retrieved from their medical records. Guardians completed a questionnaire regarding their child's health and home environment (*e.g.*, water damage, plumbing problems, cigarette smoking, exposure to household pesticides, paints, solvents, and gasoline). Visible signs of water damage were recorded during the initial environmental assessment visit. Indoor air and surface samples were taken from the infant's bedroom and from other areas most frequently used by the child. Samples were analyzed for the presence of molds, insecticides, volatile organic compounds, and fluorocarbons-based solvents.

**RESULTS:** The mean infant age was 10.2 weeks (range, 6 weeks to 6 months). There was a statistically significant greater number of males in the cases (90%) as compared to the controls (50%). Other statistically significant differences between the two groups were: the percentages that were African American (cases—100%, controls—83.3%); mean birth weight (cases—5.7 pounds, controls—7.5 pounds); breast-fed (cases—0%, controls 36.6%); electric fan in home (cases—87.5%, controls—45.8%). Smoking in the home did not differ statistically between the two groups, although the percentages were strikingly different (cases—90%, controls—29.1%). The cases had low hemoglobin, hematocrit, and red blood cell (RBC) counts, all factors that are compatible with bleeding. There were, however, also signs of RBC destruction, a finding that is not necessarily characteristic of pulmonary hemosiderosis. The levels of milk protein allergen-specific IgG antibody for the control infants were significantly higher than the values for the cases. Twelve of 15 case and control infants tested positive for cotinine (a metabolite of nicotine) with levels greater than the limit of detection (*i.e.*, they were exposed to nicotine probably from cigarettes). After conducting a logistic regression analysis, three variables remained statistically significant: 1) more cases were male; 2) more cases had a close relative, who also coughed blood while living in the same home; and 3) more cases had lower birth weight. Water damage was more likely to occur in case homes with an odds ratio of 16.25.

**COMMENTS:** This study reported that the environment may have a negative impact on children with certain characteristics that make them more susceptible to developing pulmonary hemorrhage. Smoking was associated with Idiopathic Pulmonary Hemorrhage (IPH), as was breast-feeding. The authors attempted to associate water damage with IPH; however, they did not provide any actual numbers for the houses having water damage in the control and case groups. The fact that these children did not show a laboratory pattern of iron deficiency anemia, as might be expected from chronic bleeding, but a pattern of RBC's destruction raised the likelihood that there were other plausible etiologies to their condition.



## 18 Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi

AUTHORS: Etzel RA, Montana E, Sorenson WG, Kullman G J, Allan TM, Dearborn DG, Olson DR, Jarvis BB, and Miller JD  
JOURNAL: *Archives of Pediatric and Adolescent Medicine*  
CITATION: 1998; 152:757-762  
STUDY TYPE: Case-control study.

OBJECTIVES: To determine whether there was a relationship between indoor molds, especially *Stachybotrys atra*, and Idiopathic Pulmonary Hemorrhage (IPH) in infants.

METHODS: The case and control infants were previously described by Montana and colleagues. The environmental assessment for the current study included sampling conducted by industrial hygienists (IHs), who were unaware of which homes belonged to cases and controls. The IHs specifically looked for *S. atra*. Air and surface samples were taken from the site(s) where the infant spent the most time over a period of 1 to 2 hours from each home. Total spore counts were determined from dust sampling that was taken over 6 to 8 hours from each home. Spores were identified by bright-field microscopy using a reference slide of *S. atra* spores for comparison. No information was provided regarding the person's experience in identifying *S. atra* spores and whether he was blinded to the source of the samples. Samples were also plated on different agar media. Colony Forming Units (CFUs) were averaged across all media and represented the mean of all means and not the total mean. Using a logarithmic model, the authors calculated the matched odds ratios to determine whether exposure to different types of molds was a risk factor for being a patient. Mathematical models of this type adjust the odds ratio by a log unit (*i.e.*, units of 10 CFU/m<sup>3</sup> of *S. atra* spores), resulting in an exponential increase that may be biologically relevant to the comparison of mold spores in the homes of cases as compared to controls.

RESULTS: Nine case homes and 27 control homes were assessed. The average concentration of total molds in the homes of cases (29,227 CFU/m<sup>3</sup>) was three times the amount in control homes (707 CFU/m<sup>3</sup>). No measure of variability (*e.g.*, standard deviation) was provided, however, so it is difficult to compare these values statistically. For the cases, the predominant genus of mold was *Aspergillus*, followed by *Cladosporium*, *Penicillium*, and *Stachybotrys*. A similar pattern was seen in control homes, except there was more *Penicillium* than *Cladosporium*. *Stachybotrys* was less than 1% of the total mold types found in both case and control homes. *S. atra* was detected in 78% of patient and 32% of control homes. The mean concentration of *S. atra* was 10 times higher for the case homes (43 CFU/m<sup>3</sup>) as compared to the control homes (4 CFU/m<sup>3</sup>), although, again, no measure of variability was provided, hindering statistical comparisons between the values. The matched odds ratio was 9.83 for *S. atra* measured in air samples, but only 1.35 for *S. atra* measured from surface samples. The authors explained the model as follows: if there was a 10 CFU/m<sup>3</sup> increase in the concentration of *S. atra* in the air being breathed by an infant, then the infant was 9.83 times more likely to be a patient. The matched odds ratio was 21 when environmental smoking was included as a variable in this statistical model.

COMMENTS: The authors reported an association between IPH and mold, although these results should be interpreted with caution. First, the authors did not provide raw data to calculate statistical differences. Second, the authors assumed a 10 CFU/m<sup>3</sup> exposure to calculate a theoretical odds ratio. Third, the variability in results between the case and control homes could have simply been due to the fact that there was such great variability in the length of sampling time. This purported association has been rejected by the Centers for Disease Control and Prevention.

## 19 Isolation of *Stachybotrys* from the lung of a child with pulmonary hemosiderosis

AUTHORS: Elidemir O, Colasurdo GN, Rossmann SN, and Fan LL

JOURNAL: *Pediatrics*

CITATION: 1999; 104:964-966

STUDY TYPE: Case report of a child with pulmonary hemorrhage where *S. atra* was isolated from the bronchoalveolar lavage (BAL) fluid (*i.e.*, the lungs are washed and the fluids retrieved are checked).

DESCRIPTION: A 7-year-old white male was referred to a pediatric lung clinic for evaluation of chronic cough and fatigue. Other family members did not complain of symptoms. The patient lived in a 25-year-old farmhouse with a central heating system. They moved shortly before the symptoms started. There were no smokers in the house. Chest X-ray and computed tomography showed a finding (consolidate) on the left lower lobe of the lung. Pertinent laboratory findings included anemia without evidence of red blood cells destruction. The BAL fluid showed a moderate number of hemosiderin-laden macrophages and grew *S. atra* in Sabouraud-dextrose agar medium. Multiple surface cultures from the bronchoscopy suite and mycology laboratory were all negative. The house had suffered from flood damage in the past. Beneath the bathroom sink, adjacent to the patient's bedroom, 2m<sup>2</sup> of the wallpaper was covered with black-colored mold. Cultures from this area and other surfaces grew *S. atra*, *Aspergillus*, and *Penicillium*. The patient was relocated and the house remediated. Within 1 month, the patient's condition improved, his cough resolved, and he gained weight. Ten months later, he remained asymptomatic.

COMMENTS: This is the first reported case of culturable *Stachybotrys* being isolated from human body fluids of a patient with pulmonary hemorrhage. Based on this finding, the authors concluded that this might have been the causative agent. This case report demonstrated the growth of *S. atra* from pulmonary fluids. It is possible that this mold exists in non-ill individuals; however, it is necessary to have a control group and sufficient numbers of patients to prove this. The authors did not provide any information as to whether an attempt was made to isolate mycotoxins from the fungi. Information on the patient's environment and parent's activities also was not provided. These factors are relevant, since other cases of human stachybotryotoxicosis have been mainly described in farmers and this patient lived in a farmhouse. It is plausible that *S. atra* was acquired as a result of working with moldy silage or hay and its presence in on the wallpaper was incidental.

## 20 Pulmonary hemorrhage in an infant following two weeks of fungal exposure

AUTHORS: Novotny WE and Dixit A

JOURNAL: *Archives of Pediatric and Adolescent Medicine*

CITATION: 2000; 154:271-275

STUDY TYPE: Case report.

DESCRIPTION: A 40-day-old, previously healthy Hispanic-Filipino boy spent 2 weeks at his grandmother's house in St. Louis and developed a sudden episode of shortness of breath, strenuous breathing, and cough while on a train returning to his home in Florida. The train car was described as being saturated with tobacco smoke. He was brought to the emergency department because of increasing respiratory distress. He received ventilatory support by way of mechanical ventilation in the hospital. Bright blood was repeatedly suctioned from the endotracheal tube (*i.e.*, the tube through which respiratory support is provided to the lungs).

A bronchoscopy (*i.e.*, a medical tool containing a camera for visualizing the insides of the lungs) was performed. On bronchoalveolar lavage, 10% of the macrophages recovered contained hemosiderin (*i.e.*, a red pigment derived from blood). Cultures for bacteria, viruses, fungi (unspecified), and parasites were all negative. Other laboratory results and imaging studies were unremarkable. Treatment with high-dose steroids (a potent anti-inflammatory medication) was started and a repeated bronchoscopy, done 4 days after the initial one, showed a remarkable improvement. The child recovered satisfactorily and was eventually discharged home.

METHODS: The father smoked cigarettes but he stated that he never smoked indoors. The environmental assessment included discovery that there were moisture problems following rain showers in the basement of the house where the child had resided for 2 weeks. A black discoloration, which covered approximately 12 sq. ft. of a basement wall, was identified. The case infant slept in a bedroom where "mold" grew in the paint and in the crevices of the walls. The house was inspected 4 months after the episode of pulmonary hemorrhage; water damage and fungal growth were observed on the upper floor where the infant's bedroom and living room were located. Air and surface samples were taken. Airborne fungal spores were cultured on maltose extract agar. Some of the fungi were further isolated on Sabouraud dextrose agar.

RESULTS: The 40-day-old child apparently suffered from acute idiopathic pulmonary hemorrhage in infants. Surface samples of various molds were isolated, including *Penicillium*, *Trichoderma*, *Cladosporium*, and *Ulocladium*. Air samples yielded *Alternaria*, *Cladosporium*, *Pseudallescheria*, *Epicoccum*, *Cladosporium*, and others.

COMMENTS: Causation was not proved in this single case report. The association of mold with acute idiopathic pulmonary hemorrhage in infants is not supported by the epidemiology literature or the Centers for Disease Control and Prevention. As with the other reported cases of this condition, smoking might trigger this condition.

**21 Experimental lung mycotoxicosis in mice induced by *Stachybotrys atra***

AUTHORS: Nikulin M, Reijula K, Jarvis BB, and Hintikka EL

JOURNAL: *International Journal of Experimental Pathology*

CITATION: 1996; 77:213-218

STUDY TYPE: Animal study.

OBJECTIVES: The goal of this study was to investigate the early response of lung tissue after exposure to inhaled *S. atra* spores in mice.

METHODS: Two strains of *S. atra* were used: slightly toxic (s.29) and highly toxic (s.72). Extracts of both strains were tested for their toxicity on a cat fetus lung cell line. Toxicity was measured as the ID<sub>50</sub>, *i.e.*, the amount of extract necessary to inhibit 50% of the cells from growing. Mycotoxins were isolated using high-performance liquid chromatography. Three groups of four NMRI (National Veterinary and Food Research Institute, Helsinki, Finland) mice, aged 5 weeks (mean weight =  $21.4 \pm 1.4$  g) were used. One group received intranasal infusion of  $10^6$  spores of strain s.29, the second group received the same dose by the same route of strain s.72, and the third group served as a control group, receiving only the spore carrier-phosphate buffered saline. The mice were sacrificed 3 days later and the lungs excised for pathological examination.

RESULTS: Strain s.29 proved to be slightly toxic on cat fetus lung cells (0.8 mg crude extract/ ml caused 50 % inhibition of cell growth), whereas strain s.72 was highly toxic (0.00006 mg crude extract/ml caused 50 % inhibition). The following mycotoxins were isolated from strain s.72: satratoxins G and H, stachybotrylactone and stachybotrylactam. No satratoxins and only minor amounts of stachybotrylactone and stachybotrylactam were detected from strain s.29.

Mice receiving strain s.29 did not show signs of toxicity whereas the group that received strain s.72 became lethargic. Two mice died 10 and 24 hours following spore administration. The mean weight after the 3-day observation period of the s.29, s.72, and control groups was  $23.5 \pm 1.6$  (for the first two groups) and  $17.7 \pm 0.1$  g respectively. These differences were statistically significant. Pathology evaluation revealed that all mice receiving *S. atra* spores developed inflammatory lung lesions with hemorrhagic exudate; however, the lesions were more severe (including necrosis) in the s.72 group. The control group did not develop any lung damage.

COMMENTS: The highly toxic strain of spore insufflation was able to cause lung inflammation. The control group was not appropriately controlled for potential effects of Organic Toxic Dust Syndrome.

## 22 Effects of intranasal exposure to spores of *Stachybotrys atra* in mice

AUTHORS: Nikulin M, Reijula K, Jarvis, BB, Veualainen P, and Hintikka EL

JOURNAL: *Fundamentals of Applied Toxicology*

CITATION: 1997; 35:182-188

STUDY TYPE: Animal study.

OBJECTIVES: To investigate the histological (tissue) changes in lungs, spleen, thymus, and intestines of mice exposed to spores of highly toxic and nontoxic strains of *S. atra*.

METHODS: The spores and toxins were extracted as described in article #21. Fifty NMRI (National Veterinary and Food Research Institute, Helsinki, Finland) mice aged 5 weeks (mean weight  $21.8 \pm 1.7$ g) were divided into 5 groups of 10 mice each. Two groups received intranasal infusions of 50 ml of  $10^5$  and  $10^3$  spores respectively of the s.29 strain (non-toxic strain) whereas the other two groups received the same dose, by the same route, of the s.72 strain (highly toxic strain). The fifth group served as control receiving 50 ml of phosphate buffered saline (the solution in which spores were dissolved). Instillation was given twice a week for 3 weeks for a total of six treatments. Mice were weighed before each application and blood samples were collected to measure antibodies and cell blood counts, including platelet counts, red blood cells, hemoglobin, hematocrit (the red blood cell concentration), eosinophil counts (a type of white blood cell which participates in allergic responses), and lymphocyte counts (a type of white blood cell which participates in the immune system). After the mice were sacrificed, samples from the lung, thymus (the organ responsible for the maturation of T lymphocytes), spleen, and small intestine were taken for pathological examination. Type IgG antibodies against *S. atra* were measured in all mice groups.

RESULTS: Blood platelets were decreased compared to the control group only with the toxic strain on a dose of  $10^3$  spores. No difference was found with the other doses and the other strain. For white blood cells, the total number did not differ among the three groups. Eosinophils increased only with the  $10^5$  concentration of the toxic strain and with both concentrations of the non-toxic strain, suggesting that this effect may not be related to the toxicity of *S. atra*. Lymphocyte counts increased with  $10^3$  non-toxic strain spores. Hematocrit was increased with all four concentrations (both strains) compared to the control group. Red blood cells showed an increase with the toxic strain at a spore concentration of  $10^5$  and with both concentrations of the non-toxic strain. No antibodies to *S. atra* were detected in the serum of the treated mice. No histopathological changes were detected in thymus, spleen, and intestines in any of the groups of mice. All mice receiving  $10^5$  spores of either strain of *S. atra* or  $10^3$  of the toxic strain showed inflammatory changes in the lungs. The mice receiving  $10^5$  toxic spores intranasally developed severe inflammatory changes within both bronchioles and alveoli. Hemorrhage was detected in alveoli. The lungs of the animals receiving  $10^3$  spores of the non-toxic strain remained similar to those of the control group. The inflammatory changes were more severe in the s.72 group.

COMMENTS: Animal study suggesting that large direct insufflation of *S. atra* spores into the lung may result in an inflammatory response. No other organ system effects were noted.

## 23 Study of toxin production by isolates of *Stachybotrys chartarum* and *Memnoniella echiata* isolated during a study of pulmonary hemosiderosis in infants

AUTHORS: Jarvis BB, Sorenson WG, Hintikka EL, Nikulin M, Zhou Y, Jiang J, Wang S, Hinkley S, Etzel RA, and Dearborn D

JOURNAL: *Applied Environmental Biology*

CITATION: 0665; 64:3620-3625

STUDY TYPE: In vitro study (a laboratory study).

OBJECTIVES: Attempt to isolate mycotoxins and to test their toxicity, from samples taken from the cluster of pulmonary hemorrhage cases in Cleveland, Ohio.

METHODS: Cultures of *S. chartarum* and *M. echiata* were isolated from air and surface samples from homes enrolled in the case-control study of pulmonary hemosiderosis in infants as previously described. Isolates from both types were grown on rice. After an incubation time of 2 and 4 weeks, extracts were prepared from the cultures. These were tested for cytotoxicity (cell toxicity) by using an inhibition of cell proliferation assay with a continuous cell line of feline fetus lung cells. Trichothecenes mycotoxins were isolated from these extracts.

RESULTS: In general, the levels of cytotoxicity observed correlated with the relative concentrations of macrocyclic and trichoverroid trichothecenes. Of the case and control home isolates, three belonging to each group were among the most cytotoxic and three belonging to each group were essentially non-toxic. Most of the rice culture extracts of *S. chartarum* contained macrocyclic trichothecenes (satratoxins) and trichoverroid trichothecenes (roridin L-2 and trichoverrol B).

COMMENTS: Isolates taken from both the control and case houses produced mycotoxins when grown in laboratory conditions. Strains isolated from the case studies did not exhibit cytotoxic effects.

## 24 Stachybotryotoxicosis

AUTHOR: Drobotko VG

JOURNAL: *American Review of Soviet Medicine*

CITATION: 1946; 238-242

STUDY TYPE: Narrative description of stachybotryotoxicosis in humans and horses.

DESCRIPTION: Stachybotryotoxicosis is a disease found in horses and humans, which appeared in Ukraine in the early 1940. Stachybotryotoxicosis equi or stachybotrys disease in horses was described as manifesting itself in three clinical stages: 1) mouth, throat, nose, and lips irritation, extensive desquamation (*i.e.*, shedding) of the epithelial cells (*i.e.*, the cells which line the mucous membranes), 2) leukopenia (*i.e.*, decreased white blood cells) appears and gradually worsens, this stage may last days to month, and 3) fever (104 – 105° F) which remains at this level until death. In this stage, foul necrotic ulcers appear on the mucous surface of the mouth and throat. The majority of cases recover after the 1<sup>st</sup> and 2<sup>nd</sup> stage and seldom passes to the 3<sup>rd</sup> stage.

At autopsy, ulcers are found in the mouth mucosa, throat, and nose, tonsils, stomach, intestines, and appendix. Hemorrhages are seen in almost all organs, including subcutaneous tissue, pleura- lung lining membrane, pericardium- the lining membrane which covers the outside of the heart, endocardium (the lining membrane which covers the inside of the heart), gastrointestinal tract, and spleen. The bone marrow appears pale and microscopic examination shows an almost complete absence of formed blood elements. In summary, the disease in horses was characterized by marked leukopenia followed by bleeding, ulcerations, and necrosis of the mucosa. The source of the disease was thought to be mold-contaminated hay. When healthy horses were fed this hay, the symptoms developed within 36 hours. Analysis of the hay revealed *Stachybotrys alternans*. Horses fed with pure mold cultures also became ill. The severity of the disease was directly associated with the dose (30, 20, 10, 5, 3, and 1 petri dishes). A similar disease was described in humans in Siberia and Bashkeria (former USSR). This illness called "septic angina," "agranulocytic angina," or "agranulocytic hemorrhagic aleukosis," was characterized by bleeding and leukopenia. Necrotic ulcers (similar to those described in horses) of the mouth, throat, nose, and stomach mucosa were observed as well. This disease was associated with grains that had been under the snow during the winter and were later used as food. Patients noted that they complained of a burning sensation in the mouth immediately after consuming the food prepared from these grains. The disease in humans manifested itself by the appearance of skin rash especially in areas with high perspiration. The rash eventually became a moist dermatitis (inflammatory rash). Also, pharyngitis (throat inflammation), rhinitis (runny nose) with bloody exudates (excretion), and cough were reported. Patients complained of pain in the throat, burning of the nose, and chest pain or congestion. Rarely did the body temperature rise above normal. A small number of individuals developed leukopenia (as described in horses). The disease occurred among those who worked or had contact with the contaminated hay.

COMMENTS: These were the first description of animal and human diseases from dermal and inhalation exposure to mold from the *Stachybotrys* genus. The horse experiments were convincing because authors re-challenged the animals with contaminated hay or pure mold culture, inducing the disease. However, there was no control for endotoxins or other possible confounders.

## 25 Correlation between the prevalence of certain fungi and sick building syndrome

AUTHORS: Cooley JD, Wong WC, Jumper CA, and Straus DC

JOURNAL: *Occupational and Environmental Medicine*

CITATION: 1998; 55:579-584

STUDY TYPE: Case-control study.

OBJECTIVES: To examine the role of fungi in the production of sick building syndrome (SBS) in 48 schools in the United States.

METHODS: This was a 22-month study of 48 schools throughout the United States. For each building, the following information was collected: building characterization including measurements of temperature and humidity, examination of the heating, ventilation and air conditioning (HVAC) systems, physical examination of the building, determination of particulate and CO<sub>2</sub>, and inspection for dampness and mold growth. Air samples were taken and swab samples collected from areas of visible mold, which included the HVAC systems, wetted areas, standing water, dead air spaces, and areas of dust accumulation. Formaldehyde, nitrogen dioxide, hydrogen sulphide, sulfur dioxide, and carbon monoxide were also measured. Occupants were asked to complete a questionnaire, including information on health complaints and their nature, symptom patterns, and observation about building conditions, which might explain the symptoms found. Their answers were placed into the following categories: 1) type of symptoms, 2) when the symptoms started, 3) when the symptoms disappeared, 4) when the symptoms worsened, 5) pre-existing symptoms (allergies and asthma), 6) discomfort complaints (noise, temperature, odors), and 7) complaint areas. Non-complaint areas were also identified and specified.

RESULTS: Of the 48 schools surveyed, 40 were elementary schools where the questionnaire was distributed to staff only. A total of 28% of the 622 occupant responders reported symptoms. The most common complaints were nasal drainage, itchy or watering eyes, headaches, increased airway infections (like bronchitis, tonsillitis or infection of the tonsils, and pneumonia), allergies and sinus. The average per 100 employees was > 10 symptoms. With the exception of nausea, there were no significant differences between the reported complaints and symptoms at the different evaluated sites. One third of the responders reported that symptoms aggravated with increased humidity. Complaints decreased after the buildings were remediated. No association was found between indoor and outdoor CO<sub>2</sub> concentrations, particulate measurement and complaints. The percent distributions of outdoor molds were as follows: 81.5% *Cladosporium*, 5.2% *Penicillium*, 4.9% *Chrysosporium*, 2.8% *Alternaria*, 1.1% *Aspergillus*, and others in smaller percentages. The indoor counts in general were lower than the outdoor fungi count and the fungal profile was similar to the outdoor profile. The following trends were found with the specific types: *Cladosporium*: outdoor > indoor non-complaint area > indoor complaint area (the difference between outdoor and indoor was statistically significant; *Penicillium*: indoor complaint > outdoor > indoor non-complaint. Very few *Aspergillus* colonies were isolated from air samples (~ 2-4 CFU/m<sup>3</sup> compared to ~ 700 with *Cladosporium* and 40-60 compared to *Penicillium*). In 20 schools, *Penicillium* was the predominant species in indoor complaint areas compared to outdoor and indoor non-complaint areas. At 11 schools, *Stachybotrys atra* was isolated only from swabs of the visible growth taken from under wetted carpets, interior gypsum board walls, and behind vinyl covering on gypsum walls that had been wetted in indoor complaint areas. Air samples did not yield *Stachybotrys*.

COMMENTS: Study limitations include responder bias and the existence of confounders, such as smoking.



# 4

## References by Topic

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