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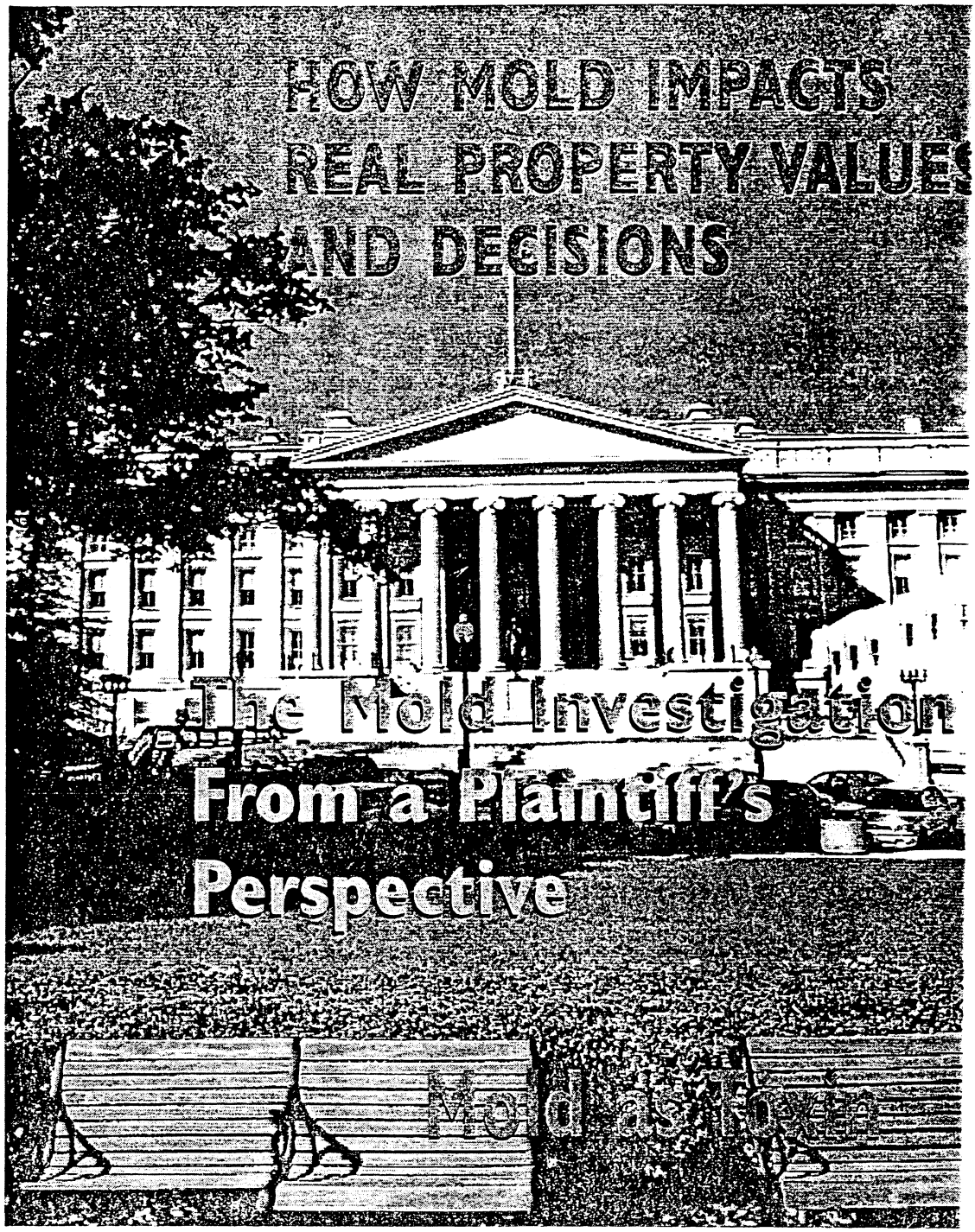

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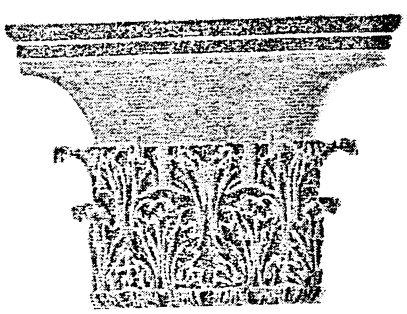

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Health Effects of Mycotoxins in Indoor Air: A Critical Review

Coreen A. Robbins,[1] Lonie J. Swenson,[1] Mark L. Nealley,[2] Ronald E. Gots,[2] and Bruce J. Kelman[1]

[1]GlobalTox, Inc.; [2]International Center for Toxicology and Medicine, Inc.

Industrial hygienists (IHs) are called upon to investigate exposures to mold in indoor environments, both residential and commercial. Because exposure standards for molds or mycotoxins do not exist, it is important for the industrial hygienist to have a broad knowledge of the potential for exposure and health effects associated with mold in the indoor environment.

This review focuses on the toxic effects of molds associated with the production of mycotoxins, and the putative association between health effects due to mycotoxin exposure in the indoor environment. This article contains background information on molds and mycotoxins, and a brief summary and review of animal exposure studies, case reports, and epidemiological studies from the primary literature concerning inhalation of mycotoxins or potentially toxin-producing molds. The relevance of the findings in the reviewed articles to exposures to mold in indoor, non-agricultural environments is discussed.

Although evidence was found of a relationship between high levels of inhalation exposure or direct contact to mycotoxin-containing molds or mycotoxins, and demonstrable effects in animals and health effects in humans, the current literature does not provide compelling evidence that exposure at levels expected in most mold-contaminated indoor environments is likely to result in measurable health effects. Even though there is general agreement that active mold growth in indoor environments is unsanitary and must be corrected, the point at which mold contamination becomes a threat to health is unknown. Research and systematic field investigation are needed to provide an understanding of the health implications of mycotoxin exposures in indoor environments.

Keywords Mold, Indoor Air, Mycotoxins

With increased media attention and public awareness about mold in the indoor environment, both residential and commercial,[1–5] industrial hygienists (IHs) are more frequently called upon to investigate exposures to mold in these situations. The IH is often asked to determine the presence or extent of mold growth and to make recommendations about its remediation. Often when these recommendations are made, there is an implicit assumption that significant health effects may occur if the mold is not removed. Headlines about "toxic molds" have elevated the level of concern and response for certain species of mold which are known to produce mycotoxins. In particular, *Stachybotrys chartarum* (*S. chartarum*) has been the focus of much attention.[6] Because exposure standards for molds or mycotoxins do not exist, it is important for the IH to have a broad knowledge of the potential for exposure and health effects associated with mold in indoor environments.

Large gaps remain in the knowledge base needed to conduct quantitative risk assessments for inhaled mycotoxins.[7] Although a great body of literature exists concerning the ingestion of mycotoxins by animals, there are few such studies of mycotoxin inhalation.[8] In addition, results of these inhalation toxicity studies are conflicting, with some reporting greater potency of mycotoxins via inhalation compared to other exposure routes,[9,10] and with others reporting different results.[11,12] Case reports and studies of agricultural workers indicate that certain health effects occur from inhalation of molds that are due at least in part to mycotoxins;[13–17] however, these exposures are orders of magnitude greater than the typical cases in which mold is found growing in the indoor environment.[17] There are several case reports and epidemiological articles in which toxin-producing molds have been reported to be associated with health effects in indoor environments.[18–21] Although aflatoxin B_1 (AFB_1) is a well-studied mycotoxin, exposures to AFB_1-containing dusts have not been reported in indoor environments, and it is not known whether exposure to AFB_1 poses a health risk in indoor environments.

This review focuses on the toxic effects of molds associated with the production of mycotoxins, and the putative association between health effects due to mycotoxin exposure in the indoor environment. The "indoor environment" here includes the

interiors of offices, commercial buildings, and residences. This article contains background information on molds and mycotoxins, and a brief summary and review of selected animal exposure studies, case reports, and epidemiological articles from the primary literature which concern inhalation of mycotoxins or potentially toxin-producing molds. The relevance of the findings in these articles to exposures to mold in the indoor, non-agricultural environments is discussed.

BACKGROUND

Molds are organisms in the kingdom of fungi (Myceteae).[22] Unlike bacteria and algae, the cells of fungi have a nuclear envelope and thus are eukaryotes. Fungi include molds, rusts, smuts, and mushrooms, and some classifications include the slime molds. Lichens are organisms that consist of a symbiotic combination of a mold and algae.[22]

Fungi have no chlorophyll, and thus they are obligate or facultative saprobes or parasites.[22] They reproduce typically by spores, via sexual or asexual mechanisms. Spores are small, propagating structures that can produce a new individual. Asexual spores are called *conidia*. With the exception of some slime mold life stages, fungi have cell walls, and these almost always contain chitin.

The classification of fungi is based upon phylogeny, reproductive strategies, life cycle, cell structure, and morphology. Their classification is complex, because of the large number and variety of organisms; indeed there is much disagreements about the classification among mycologists.[22] Three major divisions of fungi are:[22]

- Gymnomycota: cellular and slime molds
- Mastigomycota: the "lower fungi" water molds
- Amastigomycota: the "true fungi" yeasts, molds, mildews, cup fungi, rusts, smuts, bracket fungi, puffballs, and mushrooms.

Classes in the division of Amastigomycota are of interest here because all of the common toxin-producing molds are members, and molds from these classes are commonly found in indoor environments. The classes are the Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes. The first three classes are grouped on the basis of different spore-producing structures. The Deuteromycete class is a catch-all group that contains fungi for which the sexual state is unknown, hence the name, "fungi imperfecti." Once the sexual stage is known, the fungi are categorized into one of the first three classes. Thus, a single fungus may be classified as a Deuteromycete and as an Ascomycete. In fact, most Deuteromycete fungi are, or would be, Ascomycete fungi, and include such familiar geni as *Alternaria, Aspergillus, Stachybotrys, Penicillium, Cryptococcus, Histoplasma*, and *Candida*.[22] As a further complication, a single mold species may have more than one name. "Western taxonomists"[23] consider *Stachybotrys atra, S. alternans*, and *S. chartarum* to be the same organism.[24] This grouping will be assumed here, with the three types represented by *S. chartarum* because this name is used in the more recent articles reviewed that include a reference to *Stachybotrys*. For a detailed discussion of the classes of molds in indoor air, see Burge, 1999.[25]

Health Effects of Molds in Indoor Air

Possible health effects associated with fungi generally fall into one of three groups:

1. Allergic: sensitization and immune responses such as allergic rhinitis (hay fever), asthma, or hypersensitivity pneumonitis.[26]
2. Infectious: growth of the fungus in or on the body, as with aspergillosis or histoplasmosis.[27]
3. Toxic: disruption of cellular function and interaction with DNA, as occurs with toxigenic effects, including aflatoxin-induced cancer.[28]

Although the fungi clearly can effect human health in a variety of ways, discussion of the many diseases associated with the fungi is beyond the scope of this review. This article focuses on the toxic effects of molds associated with the production of mycotoxins, and the putative association between health effects due to mycotoxin exposure in the indoor environment. The health effects discussed will be limited to those described in the articles, which report associations between mycotoxin exposure and health effects. The term mycotoxin is very general, and here is defined as a mold-produced secondary metabolite that is injurious to vertebrates upon ingestion, inhalation, or dermal contact.[28–30] Although the toxins from poisonous mushrooms may be included in the general definition of mycotoxins, they will not be considered here.

Mycotoxins

Mycotoxins exert their effect on organisms in many ways, including interference with cellular respiration, interference with carbohydrate and lipid metabolism, and direct binding with DNA and RNA.[31] In general, mycotoxins are large, complex molecules, and thus are not volatile.[32] Mycotoxins have been shown to occur in mycelia, spores, and the matrix in which molds grow.[33,34]

Mycotoxins consist of many diverse compounds produced by a wide variety of molds.[31] A single mold species may produce several different mycotoxins; and conversely, different mold geni may produce the same mycotoxin.[28,31] For example, it is known that several trichothecene mycotoxins are produced by *S. chartarum*, and that the mycotoxin ochratoxin A is produced by both *Aspergillus* and *Penicillium* species.[28] Mycotoxin production for a given species is highly dependent on growth conditions, such as nutrient availability, temperature, and humidity.[29,35,36] There are many molds that produce mycotoxins, and discussion of their health effects on animals and humans are the subject of numerous articles[28,37–42] and texts[31,36,43–46] and a complete treatise or review of these is beyond the scope of this article.

Examples follow of just a few mycotoxins, the molds that produce them, and their role in the history of human disease. An interesting popular press historical account of the impact of the kingdom fungi on human affairs was recently published.[47]

Aspergillus

Aflatoxins are classic mycotoxins produced by *Aspergillus flavus, A. niger*, and *A. parasiticus*.[28] More than 13 aflatoxin compounds have been identified, the most potent of which is AFB_1.[48] Not all strains of *A. flavus* produce aflatoxins, and they are not produced until exponential growth of the colony occurs.[48] *Aspergillus flavus* grows best at 77 to 86°F, and at 80 to 85 percent humidity, but can produce mycotoxins from 54 to 106°F at a relative humidity (RH) of 99 percent.[48]

Foods that can be contaminated include peanuts, pecans, peas, bread, cheese, rice, corn, barley, grain, sorghum, wheat, and cotton seed. Aflatoxins are also found in milk, eggs, and livers of animals that have consumed contaminated feed.[36]

Aflatoxins by the oral route can be teratogenic or carcinogenic in animals, with rats and trout being more sensitive than mice and monkeys.[48] Animal studies indicate that inhaled aflatoxins are immunosuppressive[49] and may also be carcinogenic by the inhalation route in animals.[50,51] The disease outbreak that caused aflatoxin to be identified in the 1960s was "Turkey X disease."[28] Hundreds of thousands of turkeys died after eating aflatoxin-containing peanut meal. Ingestion of aflatoxin has been implicated in human liver cancer, with hepatitis B as a co-risk factor.[52] Aflatoxins have also been indirectly associated with liver cirrhosis and Reye's syndrome.[36] Case reports of inhalation exposure to laboratory workers have associated lung disease and cancer with aflatoxin exposure, and epidemiological studies suggest higher cancer rates in workers involved in peanut processing.[48]

Fusarium, Stachybotrys chartarum, Memnoniella echinata, and others

Trichothecenes have been isolated from cultures of molds including those listed above.[28,53] While these organisms are capable of producing other mycotoxins, the trichothecenes are highly toxic, intensively studied, and include over 100 isolated compounds including: T-2 toxin, diacetoxyscirpenol (DAS), satratoxins (satratoxins G and H have been extracted from straw bedding in animal disease outbreaks[54]), verrucarol, verrucarins, trichoverrins, roridin E, and vomitoxin, to name a few.[31] The main mechanism of action of the trichothecenes appears to be inhibition of protein synthesis.[28] Symptoms of trichothecene poisoning include skin irritation, vomiting, anorexia, diarrhea, hemorrhage, and convulsions, and, in some cases, death results.[28]

Fusarium sporotrichioides is thought to be the mold species responsible for alimentary toxic aleukia (ATA).[36] The probable mycotoxin was T-2 toxin. It is thought that the deaths of hundreds of thousands of people in the U.S.S.R. at the end of WWII were caused by ingestion of grain left under winter snow.[46] People were starving and ate the over-wintered cereal grains. Ten percent of the population was affected; early symptoms of gastroenteritis were followed a few months later by progressive bone marrow damage, leukopenia, pancytopenia, and death due to hemorrhage. The outbreaks were originally thought to be diphtheria or cholera, which have some similar symptoms.[37]

Stachybotrytoxicosis was first reported in 1931 in Eastern Europe and Russia as a fatal hemorrhage disease of horses, with farm workers and those using straw for bedding reporting symptoms as well.[36,55] The moldy straw, which contained *S. chartarum* and other fungi,[56] killed many horses and other farm animals and caused dermatitis, bloody rhinitis, cough, and severe respiratory tract irritation in exposed people.[39] Occupational stachybotrytoxicosis has been reported in farm workers, workers in cottonseed oil plants and grain elevators, and workers at facilities for reprocessing moldy grain, processing malt grain, textile mills using plant fibers, and binder twine factories.[36] Symptoms included chest and upper respiratory symptoms, fever, dermatitis, and leukopenia (in some cases). Recently, associations have been reported for the presence of *Stachybotrys* in indoor environments with pulmonary hemosiderosis and hemorrhage in infants,[20] and with other wide-ranging health effects in adults.[14,21]

Penicillium

Penicillium produces a number of mycotoxins.[33] More than a dozen species of *Penicillium* (and some species of Aspergillus) produce ochratoxin A.[28] It can be teratogenic and has been shown to cause kidney damage in experimental animals.[28] It has been implicated in Balkan nephropathy, a chronic, fatal, kidney disease of humans which occurs in the Balkan valley (Bulgaria, Romania, and Yugoslavia).[28] It is hypothesized that this disease is due to chronic exposures by ingestion of contaminated foods.[28]

Alternaria

Alternaria is a ubiquitous mold.[22] Species of *Alternaria* are known to produce approximately 125 secondary metabolites, one-quarter of which are toxic in animals or cell culture systems.[31] *Alternaria* is an example of how large numbers of mycotoxins may be produced by a single, common mold that is generally considered benign. Although *Alternaria* has a role in allergic disease, whether exposure to its mycotoxins has a significant role in human disease is not known. This also holds for other molds and mycotoxins, because studies of the relationship between inhalation exposure to mycotoxins of different mold species and health effects are few.

Animal Inhalation Studies

Animal studies of inhalation of mycotoxin and mold spores are included in this review because they are used as models of human exposure and response, and they provide a mechanism for comparing the toxicity of different compounds. A great deal of literature exists about mycotoxins in feed and foodstuffs. Because the main route of human and animal exposure

to mycotoxins is by ingestion, the vast majority of animal research on toxicity has been done with ingestion studies.[40] There are few studies that examine the inhalation of mycotoxins or toxin-producing molds.[8,57] and reports from some do not quantify exposure.[58–60] Studies published by Creasia et al. (1987, 1990)[9,10] have been cited in support of the biological plausibility of mycotoxin-related illness from indoor exposure[8,21,61–63] because their results indicate that mycotoxins are more toxic when exposure occurs by inhalation rather than ingestion.

Studies have been conducted on the acute toxicity of T-2 toxin, a potent trichothecene, because of the potential for its use in chemical warfare. In 1986, Marrs reported that for guinea pigs, the T-2 toxin LD_{50} for aerosol exposure (4 mg/kg) was about twice the LD_{50} for subcutaneous exposure (2 mg/kg), but Marrs also reported that effects of acute inhalation and subcutaneous exposure to T2 toxin were quantitatively and qualitatively similar.[11] In contrast, Creasia found later for guinea pigs, that the T-2 toxin LD_{50} for aerosol exposure (0.4 mg/kg) was one-third of the LD_{50} for intraperitoneal injections (LD_{50} = 1.2 mg/kg).[10] These conflicting results may be due to the duration of inhalation exposure for a given total dose. Marrs exposed the guinea pigs for periods spanning 15 to 75 minutes, and noted a decrease in toxicity when dose was delivered over the longer time interval; Creasia exposed the guinea pigs for either 10 or 30 minutes. In the latter study, similarly exposed rats were even more sensitive to inhalation of T-2 toxin, which was 20 times more toxic than by the intraperitoneal route. In another study by Creasia, the sensitivity of mice to inhaled T-2 toxin was similar to rats; and they too were more sensitive to exposures via inhalation (LD_{50} = 0.94 mg/kg) than exposures via the dermal (LD_{50} > 10 mg/kg) or injection (LD_{50} = 4.5 mg/kg) route.[9] In a study of swine, Pang projected that the LD_{50} would be higher by the inhalation route (>8 mg/kg, sub-lethal doses used only) compared to intraveneous exposure (1.2 mg/kg).[12] Pang suggested that this apparent conflict with Creasia's results may be due to differing susceptibilities of the animals, as well as differing time intervals used for the aerosol exposure (pigs were exposed for 45 to 61 minutes).

Aflatoxins have been the subject of extensive research because they are potent liver toxins and are carcinogenic by ingestion exposure. They are found in a wide variety of crops used for human and animal consumption. Studies of acute inhalation exposure (≤120 min) to purified AFB_1 in animals have shown the formation of DNA adducts in the rat liver,[50] and suppression of pulmonary and immune function in rats and mice.[49] Mice exposed chronically (daily for one year) to aerosolized AFB_1 had a 38 percent increase incidence of lymphatic leukemia.[51]

Even though these studies are limited, some general trends are present. The results indicate that rats are more sensitive to inhaled T-2 toxin, followed by mice and then swine. Guinea pigs appear somewhere between the most sensitive (rats) and least sensitive (swine) animals. It also appears that the toxicity resulting from inhalation exposure is dependent on the time interval of exposure for a given total dose, with greater toxicity for shorter exposures. This may be the result of more effective clearance and or metabolism of T-2 toxin by the lung at the lower airborne concentrations associated with longer exposure intervals. Exposures did not result in pulmonary edema or gross histopathological changes in the lung; although the latter effects were seen in other organ systems.[9–12] It has been suggested that toxic effects are not seen in the lung because the toxin or toxin and vehicle is rapidly absorbed by the lung and quickly transported to other organs.[12]

Inhalation studies of mycotoxins in animals were designed to measure acute effects at high exposure levels. These experimental exposures and concomitant effects do not represent exposures to mycotoxins at chronic, low exposure levels from molds in indoor settings. However, the data show decreasing toxicity with longer exposure for a given total dose, indicating that physiological mechanisms can mitigate the effect of exposure at low levels. These results suggest the existence of a threshold for the effects of mycotoxins.

These kinds of studies are useful in determining the range of response for different animals for a particular toxin at different doses and exposure time intervals. However, they are only indirectly useful in examining the issue of exposure to mycotoxins from inhalation of mold spores. Unlike a pure mycotoxin aerosol, a mold spore is a complex assortment of chemicals that may act as synergists or inhibitors in producing toxic effects. In addition, the aerodynamic properties of mold spores (particles) are likely to be different from an artificially generated mycotoxin aerosol. This will most likely cause different deposition patterns in the respiratory tree, which will affect the amount and location of the delivered dose and subsequent toxicity.[64]

In an effort to better model the effect of mycotoxins from inhalation of spores, Nikulin injected mice intranasally with *S. chartarum* spores.[57] Mice were injected once with 10^6 spores in phosphate-buffered saline (PBS) of one of two strains of *S. chartarum*., s. 72, a highly toxic strain, or s. 29, a slightly toxic strain (toxicity determined by cytotoxicity tests). Controls received PBS only. The s. 72 strain contained satratoxins. All mice receiving spores developed lung inflammation; however, there was a significant difference in the inflammation changes between the two strains. The changes in the s. 29-exposed mice were significantly milder than that produced by s. 72, and necrotic changes were seen only in the s. 72-exposed animals.

In another experiment, mice were injected intranasally with 10^3 to 10^6 spores of less toxic (s. 29) and more toxic (s. 72) strains of *S. chartarum*.[65] This treatment was repeated twice weekly for three weeks. A relative increase in pulmonary inflammation (from "−" for no inflammation, to "+++" for extensive inflammation) was reported as the intranasal dose was increased from fewer spores (10^3) of the less-toxic strain, to greater numbers (10^5) of spores of either strain. Severity of inflammatory lung changes was the same in mice instilled with 10^3 spores of s. 72 and 10^5 spores of s. 29. Inflammation was not detected in animals receiving 10^3 of s. 29 spores. Hematological parameters were generally similar between exposed and control animals;

however, unlike the studies with purified mycotoxins, histological changes were seen only in the lungs of exposed animals.

These results highlight the importance of the delivery route and vehicle of mycotoxins in inhalation exposure studies. The significance and applicability of the results to actual inhalation exposures is limited because of the small number of animals used, subjective grading of histological response, nonphysiological exposure technique (injection), and method of spore quantification in the dose. The report further highlights that intranasal inoculation of large numbers of spores is unlikely to model the exposure of humans in even very moldy environments.[57]

CASE REPORTS

Although case reports cannot be used to determine causation for an environmental agent, they can sometimes be used to identify factors that need further investigation. A number of case reports are cited as showing links between exposure to inhalation of mold and mycotoxin-induced illness in humans. Because there are only a few reports of possible mycotoxin exposures in indoor settings, several case reports from agricultural exposures are included in this review. Although the exposure levels were high and occurred in agricultural settings, data from these studies may be relevant to human exposures in indoor environments.

In a review of stachybotrytoxicosis, Forgacs compiled earlier information of human exposure to *Stachybotrys* that had been reported in the Soviet Union during the 1940s.[55] Most of the persons affected had handled contaminated animal feed, but some became ill after using contaminated straw for fuel or mattress stuffing. Some of the scientists investigating animal outbreaks also developed the toxicosis from contact with straw that was naturally or artificially infected with *Stachybotrys alternans*.[55,66] Straw contaminated with other molds did not produce the toxicosis.[66] It was thought that exposure to toxins occurred via inhalation and dermal contact.[55] Symptoms included dermatitis, primarily on the scrotum and in the axillary region, and, less often, on the hands and other body areas. In addition to dermatitis, other symptoms reported were bloody nose, cough, and complaints of throat pain, burning nasal passages, and congestion in the chest. Some patients had elevated body temperature, and some developed leukocytosis followed by leukopenia. Patients recovered rapidly after exposure cessation; however, upon subsequent exposure, the diseased recurred with more serious sequelae.

In the often-cited 1986 report by Croft, a family reported cold and flu symptoms, sore throats, diarrhea, headaches, fatigue, dermatitis, hair loss, and general malaise.[14] The self-reported complaints were thought to be associated with massive growth of mold in the HVAC system and on the ceiling of one room. Although the air sample was not cultured, *S. chartarum* was identified from examination of spores, and trichothecenes were extracted from materials taken from the house. There were no objectively measured disease outcomes, and other possible causes for the reported symptoms were not ruled out. Reported symptoms ceased after the mold was removed. Although the symptoms were attributed to *S. chartarum*, other molds or bioaerosols were not ruled out as possible causes or contributors to symptoms. The presence of other molds is likely because the water infiltration and mold growth had been occurring for four or five years.

In 1987, Brinton reported an isolated epidemic of febrile illness after a college fraternity party.[13] Moldy straw was used on the floor in an enclosed room where the party was held. The airborne dust was so thick that it obscured vision across the room. Fifty-five of 67 partygoers developed fever, chills, cough, shortness of breath, and chest and back pain after attending the party. The risk for illness increased with increasing time spent at the party. Serological tests did not implicate allergic or viral causes. All of the partygoers recovered completely.

DiPaolo (1994) found ochratoxins in *Aspergillus ochraceous*-contaminated wheat that was suspected of causing acute respiratory symptoms and intestinal disturbances in a farmer and his wife who had worked for eight hours sieving the grain.[15] The woman also subsequently suffered acute renal failure possibly due to the inhalation of mycotoxins from contaminated grain dust. The couple recovered completely. The wheat was visibly moldy and covered with dust that was described as acidic and irritating. Several experimental animals subjected to air passing through the contaminated grain died or showed organ damage. The amount of ochratoxin on the grain or in the air flowing through it was not determined and control animals were not used in this experiment.

Emanuel has followed dairy farmers who are thought to have inhaled "massive amount of fungi" from handling moldy hay or corn silage.[16,67] Typical symptoms after exposure include chills, fever, dry cough, and inflamed mucous membranes, with some individuals later developing infiltrates and interstitial disease in the lungs. The authors ruled out allergies as the cause because the farmers did not respond to antigen challenge and were asymptomatic at lower re-exposures. Although five species of mold were cultured from one case lung biopsy specimen, measurements of mycotoxins were not reported. Of the five fungi, one was a *Fusarium* and another was from the genus *Penicillium*. No bacteria were cultured. Lung histology showed a multi-focal acute process in the terminal bronchioles, alveoli, and interstitial cells. Emanuel has suggested that the symptoms and signs were caused by massive exposure to fungi and bacteria, and that mycotoxin, endotoxin, or other constituents were the causal agents.

Measurements of airborne mold were made for a series of cases of farmers with and without symptoms of febrile reactions to inhaled mold dust, referred to as organic dust toxic syndrome (ODTS), and allergic alveolitis (AA).[17] Organic dust toxic syndrome is also referred to as silo unloader's disease and pulmonary mycotoxicosis. It requires intense exposure to airborne dust and results in influenza-like symptoms with leukocytosis and fever but does not require prior sensitization. Antibodies do not develop, and respiratory symptoms may or may

not occur, and there are usually no radiographic changes. Allergic alveolitis, also called hypersensitivity pneumonitis (HP), will not be discussed in detail because it is primarily an allergic response.

Exposure was evaluated within two weeks of medical consultation for ODTS or AA. Samples were collected during normal farming (background) and during handling of materials associated with the reported illness or causing maximal (or worst case) exposure in reference farms. There were 16 cases of ODTS and 17 reference farmers. The average concentration was $1.3 \pm 1.3 \times 10^{10}$ spores/m^3, and $1.2 \pm 2.0 \times 10^8$ spores/m^3, for ODTS cases, and comparison farmers, respectively. The ODTS response was associated with extreme exposure occurring on a single day. There was no correlation of disease with spore type, and the results support the hypothesis that common cell wall components of microorganisms cause the "toxic" symptoms and stimulate immune reactions.

Exposure measurements were unavailable for all but the last[17] of these case reports. For the remaining reports, exposures are presumed to be extremely high, based upon descriptions of the conditions associated with exposure. The measurements from Malmberg's report indicate that exposures associated with acute effects are indeed very high, in the range of 10^{10} spores/m^3.[17] In all the case reports, symptoms were temporally related to exposure, but in most cases the contribution of bacterial endotoxin was not evaluated. Because inhaled bacterial endotoxin has been shown to affect lung function[68] and is also associated with febrile respiratory illness[69] it may have had a role in some of these cases.

As a whole, case reports show that clinically important health effects occur after exposure to conditions associated with high levels of bioaerosols. Historical reports indicate that direct contact with *Stachybotrys*-contaminated straw results in health effects. It is unlikely, though not impossible, that these extreme exposure conditions would occur due to surface mold growth in the indoor working or home environment.[70] Exposure to airborne molds from surface contamination is dependent on the degree of colonization of substrates, how much it is disturbed, and the effectiveness of ventilation,[71] and requires an exposure pathway from the affected area to the occupants.

In two reports,[14,66] the outbreaks are reported to be associated with *Stachybotrys*, but the remaining cases are not reported in association with a particular mold species. The health effects in these case reports may be associated with mycotoxins, endotoxins, other bioaerosol constituents, or a combination of these components, and the contribution of each component to cases of clinical illness remains to be elucidated.[17] Another important common finding among these reports is that symptoms ended upon removal from exposure, and did not re-occur when high-level exposures were avoided.

EPIDEMIOLOGICAL INVESTIGATIONS

There are few epidemiological investigations of inhaled mycotoxins and disease in indoor air settings.[6,18,33,72] Although some purport to show an association between inhaled mycotoxins and health effects,[18,21,63,73] some have been criticized as not having adequate data to support this claim.[6,74] The evidence for health effects in indoor air from mycotoxins associated with *S. chartarum* was recently reviewed and includes the articles summarized here.[6]

Johanning (1996) compared self-reported symptoms and blood and immunology tests among 53 cases from a "problem" building (a building with moisture problems and visible mold growth) and 21 controls from a "non-complaint" building (a building without known problems).[18] This study appears to include results for 43 cases in this building reported previously by Johanning et al., (1993)[73] thus, only the most recent article is discussed here.[18] Cases were further divided into groups depending on whether they worked on the ground floor ($n = 7$), or in the basement ($n = 33$), or sub-basement ($n = 9$) of the building (four subjects without an assigned floor were excluded from sub-group comparisons). The sub-basement had experienced flooding, mold growth, and mold cleanup over several years (unspecified). Tests of the blood, serum chemistry, immunology/antibodies, and lymphocyte enumeration and function were conducted for cases and controls. Results of air samples showed that levels of airborne viable spores were similar on all levels of the building, and these in turn were similar to outdoor concentrations. Elevated spore levels were found in the sub-basement, as compared to the ground level and basement, when aggressive sampling techniques were used. Several mycotoxins, including satratoxin H, were found in bulk building material.

Results of 24 blood and immunological tests are reported for comparisons between cases and controls, and among cases according to building location (ground floor, basement, sub-basement). Results for a second comparison between controls and cases, stratified by building location, are reported for white blood cells (WBC), the proportion of CD3 lymphocytes (CD3%), and natural killer lymphocytes (NK). In this second comparison, cases are stratified differently, as ground floor ($n = 7$), or basement plus sub-basement ($n = 42$) occupants.

In the first comparison, a significant reduction was found in the proportion of CD3 lymphocytes (a measure of the proportion of mature lymphocytes) for cases compared to controls (cases = 73.5%, controls = 75.7%). No differences were found for comparisons by work location. In the second comparison, NK cells were higher for the new basement grouping (basement plus sub-basement, $p = 0.03$); however, it is unclear whether the difference is between cases and controls or between the two case groups (ground floor, new basement). The first comparison of total WBC did not show significant differences. In the second comparison, the new basement group has the highest WBC ($p = 0.024$); but again it is unclear whether the comparison is being made between cases and controls or between the case groups. There was no significant difference between the number of cases and controls with an immune response to *S. chartarum*, *Penicillium*, or *Apergillus* antigens.

The CD3% levels and pokeweed mitogen (PWM) proliferation scores were significantly lower in cases reporting a history of

upper respiratory infections ($p < 0.05$). Significant differences were found for self-reports of lower respiratory, constitutional, eye, and chronic fatigue symptoms. Among the building occupants, a larger proportion of sub-basement occupants reported symptoms. The authors conclude that their results suggested an immune competency dysfunction associated with prolonged and intense toxigenic mold exposure.

The studied disease end points included reported symptoms and reported excess of infections. Clinical evaluation or reviews of records to confirm the patient's perceptions were not reported. In a situation where a perceived hazard exists, the mold in this case, symptoms may be over-reported and must be objectively confirmed before reported symptoms can be considered to reliably represent the incidence of illness or disease. The level of mold exposure was assigned based upon the presence and amount of mold contamination reported. Whether exposure to mycotoxins occurred in the studied population is not resolved, and the authors state that there was no relationship between IgE antibodies to *S. chartarum* and the reported symptoms. This finding does not support an exposure–disease relationship for *S. chartarum* and reported symptoms and infections.

Toxins associated with *S. chartarum* are not known to affect the CD3 population specifically; however, it is known that stress, medications, and infection can all affect various component of the T-cell population.[75] Because the cases reported more infections, this could account for the change in the T-cell population. Thus, whether the change in CD3% was caused by exposure to mycotoxins cannot be determined from these data.

The meaning and importance of differences in the WBC is uncertain. While the WBC was higher for the new basement group (6.29 k/μl), the controls had higher counts (6.06 k/μl) than ground floor cases (5.05 k/μl). More importantly, the reported values all fall within the normal range for adults.[76] This same situation occurs with the NK counts, with the values for new basement group (55.45) >controls (41.83) >ground floor (23.59). This is inconsistent with an exposure–response effect because exposures are expected to increase from controls < ground floor < basement.

The major finding of this study, a significant association between reported symptoms and building occupancy, may be due to recall and reporting bias because conditions in the building had been the subject of investigation and remediation prior to this study. The clinical importance of the statistically different laboratory results is uncertain because the WBC values are within the normal range for adults,[76] and these and the NK results do not follow an exposure-related pattern. The basis for analyzing the laboratory data after changing the case groups (combining the basement and sub-basement cases) is not explained.

The issue of mycotoxins in indoor air received national media attention after the reported association of *S. chartarum* in homes with sick infants.[1] In 1994, eight cases of infant idiopathic pulmonary hemorrhage and hemosiderosis (IPH) were reported in Cleveland, Ohio.[77] Subsequently, the cases were investigated with the assistance of the Centers for Disease Control and Prevention (CDC) and three separate articles were published that examined the occurrence of IPH with environmental factors,[19] and with the presence of mold and *S. chartarum*.[20,63] Risk factors previously implicated in IPH include smoking, pesticide exposure, and familial history of hemoptysis.[78–80]

Testing for the presence of airborne *Stachybotrys* was conducted using a method based upon the method for collecting and analyzing air samples for asbestos.[81] Samples were collected on membrane filters that were mounted on slides and cleared with acetone vapor. Filters were examined with a phase-contrast microscope, and *S. chartarum* was identified by comparing the image on the filter to a photomicrograph of *S. chartarum* conidia. The CAMNEA method[82] was used to estimate viable airborne mold concentration.[63] Samples were collected on Nuclepore filters, diluted and plated on growth media. Cultures were examined and colonies were categorized as either *Aspergillus, Cladosporium, Penicillium, Stachybotrys*, or other.[63] Surface samples were collected from areas of suspected mold growth; the scrapings were diluted and plated. Colonies were counted, and results were expressed as CFU/g.[63]

The first report of the case-control study included the original eight cases, plus two more that appeared later, all compared to 30 controls.[20] Dearborn et al. (1997) reported that significant factors associated with IPH were male gender ($p < 0.05$) and living in a water-damaged home (OR = 16.3, CI = 2.6–infinity).[20] Other factors which were not statistically significant included the presence of smokers in the home (OR = 7.9, CI = 0.9 to 70.6), and *S. chartarum* in the home (OR = 1.6, 1.0 to 30.8).[20] Trichothecenes were later recovered from cultures taken from case and control homes.[53] In the report of environmental variables (Montana et al., 1997), *S. chartarum* is not discussed; however, the authors concluded that environmental risk factors may contribute to pulmonary hemorrhage.[19] In addition to the statistics reported by Dearborn, Montana reported that cases were more likely to have a relative who coughed blood (OR = 33.14, CI = 5.1–infinity), and that cases and controls were significantly different in terms of gender and race ($p < 0.05$).[19] Nine of ten cases were male and all were black, whereas half the controls were male and 83 percent were black. Cases were also less likely to have normal birth weight ($p < 0.05$), and none were breastfed ($p = 0.04$). Additional analyses of the case-control study data were reported by Etzel et al. (1998).[63] The matched odds-ratio for a change of 10 units in the mean concentration of *S. chartarum* was 9.83 (CI = 1.08 to 3×10^6). Higher concentrations of *S. chartarum* on surfaces in case homes (case homes, 20×10^6 CFU/g, control homes, 7.0×10^3 CFU/g) were also reported. Etzel concluded that infants with IPH were more likely to live in homes with toxigenic *S. chartarum* and other fungi in the indoor air.

The odds ratio for *S. chartarum* and IPH is not significant until the matched odds ratio analysis is used.[63] The utility of the matched odds ratio analysis is uncertain, because the comparison is made for units of 10 CFU/m^3. It is difficult to interpret the outcome of a statistical test that is based upon the

comparisons of quantities that differ by less than the reported limit of detection (LOD) of the method (70 CFU/m^3).[81] The interpretation of the study outcome is further complicated because the duration, amount, and source of water damage was not reported, and the extent of mold growth and the putative exposure pathways are not described. Sampling results are not likely to represent exposure conditions because the study was conducted using "aggressive" techniques, and in many cases was conducted months after the IPH was reported. The significance of differences between surface sample concentrations is difficult to interpret because the quantities of mold scraped from an area of suspected mold growth are not likely to represent overall surface contamination in a home. The meaning of these surface sample data in terms of exposure potential is unclear. The CDC recently published a summary of an internal and external review of the investigation of mold exposure and IPH, and in it concluded that the association between IPH and exposure to *S. chartarum* "was not proven."[83] However, the CDC will continue to consider possible associations between IPH and many other possible etiologies, including exposure to molds.

In 1998, Hodgson et al. reported the results of a study of occupants of a courthouse in Florida that had previously had extensive mold growth in its walls.[21] They evaluated questionnaire results, lung function, and blood and immunology test results among occupants of the courthouse and occupants from other non-complaint buildings. Participants in the study included 14 courthouse occupants identified by the workers' compensation carrier for examination; 197 questionnaire respondents from which 30 cases reporting at least two interstitial lung disease (ILD) symptoms were selected for the case-control study; and 47 courthouse volunteers who participated in a clinical screening for building-related disease. The case-control study compared 24 cases selected from the questionnaire study to controls (n = 26) selected from two buildings thought to be free of building-related disease. The case-control subjects underwent full pulmonary function testing and neuropsychological testing.

Air and bulk material sampling was conducted in the complaint building but not in the control buildings. Satratoxins G and H were isolated from ceiling tiles removed from the courthouse. Air samples revealed lower viable spore levels indoors compared to outdoors. *Aspergillus versicolor* and *A. glaucus* were found indoors but not outdoors. Aggressive sampling resulted in indoor air spore concentrations ranging from 10^4 to 10^5 CFU/m^3. *Stachybotrys chartarum* was found in air samples collected while books were being handled, and was also found in bulk samples of water-damaged ceiling tiles.

The 14 subjects identified by the insurance carrier described symptoms consistent with work-related asthma in three cases (decreased FEF_{25-75} in one[a]), interstitial lung disease (ILD) in one, and rhinitis that improved over weekends in six. Of 44 subjects from the clinical screening, 16 reported symptoms consistent with ILD, and had either single-breath carbon monoxide diffusing capacity (DLCO), functional residual capacity (FRC), or total lung capacity (TLC) less than 80 percent of predicted. However, seven of the 16 subjects were smokers, and smoking has been associated with ILD.[21] The questionnaire survey had a 90 percent response rate and was dominated by women (81.8%) in lower-status job categories who were more likely to smoke. Statistically significant increased symptom reporting was found for courthouse occupants for individual symptoms and grouped symptom categories, except for wheezing. Symptoms of ILD were reported more often among case building occupants.

In the case-control study, cases had significantly higher levels of mean reserve volume (RV)/TLC ratios; however, ANOVA indicated that the differences were due to smoking. Immunology panels did not provide useful information. Persons reporting more than two symptoms had significantly higher levels of antibodies to *Alternaria, A. fumigatus*, pigeon serum, pigeon droppings, and bovine serum, but not to the agents identified in the buildings. Results of neuropsychological testing showed no difference in cognitive function; however, subjects with ILD symptoms were more likely to endorse symptomatology reflecting intense moods, anxiety, restlessness, irritability, and other related symptoms.

There were two positive associations reported between courthouse occupancy and outcome variable; (1) in the questionnaire survey, symptoms of ILD were reported more often among case building occupants; and, (2) statistically significant increased symptom reporting was found for courthouse occupants for individual symptoms and grouped symptom categories, except for wheezing. Results from objective measures of health (lung function) or exposure (immune tests) do not appear to support an association between exposure (building occupancy) and health effects.

It is unclear whether the insurance exam participants, questionnaire respondents, clinical screening, and case-control participants represented distinct groups, or if the groups overlapped (except that three of 47 clinical screen subjects were removed because they appeared in other groups). Thus, it is unclear how many occupants participated in the study.

Selection and recall bias were likely to occur in this study because the building had been undergoing mold remediation and sampling prior to the study, and some occupants were actively involved in litigation concerning mold in the building and claimed health effects. In addition, building occupants without symptom complaints would be less likely to participate because subjects were acquired by posting a memorandum describing the purpose of the investigation. Although the authors concluded that a mycotoxin-induced effect was the most likely explanation for increased symptoms in the problem building, support for this conclusion is weak due to the limitations described above.

Epidemiological studies have linked ingestion of aflatoxin-containing foods to liver cancer in humans. However, its exact role in human liver cancer is unclear due to coincidence with hepatitis B virus.[52,84] There is some evidence that the human

[a] FEF_{25-75} = forced expiratory flow of during the middle half of the FVC, the average rate of flow during the middle two quarters of the forced expiratory effort.[94]

lung is also a target tissue for the action of AFB_1,[84,85] but the epidemiological data for AFB_1 as a pulmonary carcinogen is contradictory.[84] Workers exposed to potentially high levels of AFB_1-containing airborne dust from aflatoxin-containing agricultural products have been studied. Two studies of workers at a peanut- and linseed-processing plant (exposed to 0.04 to 2.5 μg AFB_1 over a 45-hour week), showed higher incidences of respiratory tumors compared to an unexposed cohort.[84,86] No excess lung cancer risk was found in a study of proportionate risk of cancer in exposed (170 ng per day) livestock feed processing plant workers; however, the workers had increased risks of biliary and liver cancer.[87] There are also several case reports linking AFB_1 inhalation and human cancer.[88,89]

Although there are currently no published data regarding the exposure to aflatoxin-containing dusts in indoor environments, they are included here because they are potent mycotoxins and have been studied in other environments. It is not known if exposure to aflatoxin-containing dusts in indoor environments poses a health risk to building occupants. Sterigmatocystein, an aflatoxin precursor, has been detected in bulk samples in a mold-contaminated building.[21]

DISCUSSION

Studies of animals exposed to purified mycotoxins demonstrate their potency, but the evidence from these studies that mycotoxins are more toxic via inhalation exposure is equivocal. Studies of intratracheal instillation of mold spores indicate increased inflammatory response in the lung due to the presence of large numbers of toxic mold spores; however the applicability of these results to human exposure in indoor environments is limited due to the lack of comparability of the animal to human physiology, artificial exposure route, high exposure level, and the subjective grading of the response. Case reports indicate that exposures to high levels of airborne molds (e.g. 10^{10} spores/m^3) and/or bioaerosols are associated with acute clinical illness, which is likely due in part to mycotoxins. However, there is no single mold genus that is consistently implicated in these cases and there is evidence that many common molds can produce toxic metabolites.[31] Epidemiological studies which report associations between health effects and exposure to mycotoxins in indoor environments are limited, and generally do not contain strong evidence to support this association. Although there is evidence of a relationship between high levels of inhalation exposure or direct contact to mycotoxin-containing molds or mycotoxins, and demonstrable effects in animals and health effects in humans, the current literature does not provide compelling evidence that exposure at levels expected in most mold-contaminated indoor environments are likely to result in measurable health effects.

More information is needed concerning the health effects of exposure to molds, including putative toxigenic species, in indoor environments. Studies of mold inhalation exposure using animal models (animals having respiratory systems with physiology similar to humans) could provide a better understanding of the transport, fate, and toxic effects associated with inhaling mycotoxin-containing mold particles. Extrapolation of results from animal studies to human inhalation exposure is difficult because of the exposure vehicle, exposure route, and type of animal used. Studies have involved inhalation exposure to purified mycotoxin aerosols, rather than mycotoxin-containing mold particles in different animals (rats, mice, guinea pigs, swine), or have involved intratracheal instillation of mycotoxin-containing mold particles in rodents, rather than inhalation exposure in larger animals. This is limiting because the physiology of the respiratory system of the mouse, rat, and guinea pig are not similar to those of a human, and this affects the dose delivered from a particular airborne exposure level due to differences in terms of particle inhalation, deposition, and retention.[64] Although swine have lung physiology more similar to humans, studies with them have been of inhalation exposure to purified mycotoxins, and this exposure is unlike inhalation exposure to mycotoxins contained in or on a mold particle.

Epidemiological studies of exposure and health effects of potentially highly exposed remediation workers may be useful. This type of investigation is warranted on the basis of the historical record of detecting sentinel health effects in highly exposed populations. The ability of these studies to measure potential health effects of mycotoxins will depend upon the development of methods to quantify exposures to airborne mycotoxins.

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, allergenic, and toxic components (for inhalation and dermal exposures) of molds. Currently, widely available methods allow for quantitative estimates of airborne viable or non-viable mold particles. Viable methods allow for species identification but do not include dead propagules that may continue to have antigenic or toxigenic properties. Non-viable methods give an indication of the total antigenic burden (since they include viable and non-viable propagules), but only allow for putative identification of some mold genera (and a few to species). Neither method is useful for determining the mycotoxin content of the sampled bioaerosol. Only recently has a method been proposed to estimate trichothecene mycotoxins in air samples,[90] and this technique remains to be validated. (Note that this method does not measure trichothecenes directly, but quantifies the level of protein inhibition from material collected on an air sample.) Standard methods for quantitative sampling of surfaces do not yet exist; nor is there a model that would allow the interpretation of surface sampling results to estimate inhalation or dermal exposure. In current practice, standard surface sampling techniques for determining the "cleanliness" of potentially mold-contaminated surfaces and objects are needed.

In addition to developing more standard sampling and analytical techniques, there is a need to develop an empirical database of typical indoor and outdoor molds (amounts and types, airborne and surface) in non-problem buildings in different areas

of the country. Data collected in suspect buildings could initially be compared to these control data to identify similarities and differences that may be helpful in determining if a problem exists.

Health-based exposure standards for molds[91] and mycotoxins do not yet exist. While there is general agreement that active mold growth in indoor environments is unsanitary and must be corrected, the point at which mold contamination becomes a threat to health is unknown. Better information about potential health effects and improved sampling techniques will help in determining *what are* the acceptable levels of exposures to mold in indoor environments. In the future, this will help to determine how clean an environment must be to avoid health effects, and thus, provide guidance on when cleanup is required and "how clean is clean."

Whether molds such as *Stachybotrys* should be treated differently than other molds, when considering cleanup or sampling and exposures issues, is also a controversial subject. Cleanup and repair of mold-contaminated buildings is often conducted using asbestos abatement-like methods and clearance sampling.[92,93] These procedures are often triggered, and sometimes buildings are evacuated, because *Stachybotrys* has been found in the building,[2,3] at times without regard to the extent of contamination or exposure potential. The information about health effects and exposure in the current scientific literature does not warrant the use of more conservative measures for mold remediation based on the presence of a particular species of mold. Instead, the available data indicate that evacuation and immediate cleanup is needed, regardless of the genus or species identified, if contamination in indoor environments results in extremely high airborne mold levels.

With or without mycotoxins, the issue of mold exposure is important from a health standpoint, and can potentially effect anyone in the indoor environment. Research and systematic field investigation are needed to provide an understanding of the health implications of mycotoxin exposures in indoor environments. The appropriateness of the amount of time, energy, and other resources that will be spent in the future on controlling exposures to mold in indoor environments will hinge upon the accurate collection and dissemination of information about the health risks associated with mold and mycotoxin exposures in these environments.

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