Mycotoxins and Indoor Molds

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INTRODUCTION

With a growing awareness of the potential hazards of chemical and biological agents in our homes, schools and workplaces, a greater emphasis has been placed on evaluating the role of mycotoxins and mycotoxin-producing fungi in our indoor environments. Now, more then ever, the potential health effects of airborne molds and other biological contaminants are being given serious consideration in IAQ investigations.

What are mycotoxins?

Simply, a mycotoxin is any toxic fungal metabolite. Fungi produce a variety of secondary metabolites as a byproduct of their metabolism. Those capable of eliciting deleterious effects on other organisms are classified as mycotoxins. Mycotoxicosis can be defined as illness resulting from ingestion, inhalation, or other involvement with mycotoxins. Mold mycotoxins of primary concern here elicit a harmful effect on humans. Other substances such as those produced by poisonous mushrooms are also mycotoxins, and mycetismus is the term applied to mushroom poisoning as a distinct category of mycotoxicosis. The discovery of antibiotics produced by fungi revolutionized the treatment of disease because the compounds are sufficiently non-toxic to the host. In nature, mycotoxins are a chemical defense for fungi and have evolved as mechanisms for antiherbivory and to provide a competitive advantage when colonizing new substrata.

Historical Mycotoxicoses

The study of mycotoxins originally centered on the health effects of ingestion of toxic fungal byproducts from growth of fungi on food and livestock feed. A review of the historical significance of mycotoxins sets the stage for more recent work with inhalation myctoxicosis studies.

Ergotism or St. Anthony's Fire, is one of the oldest known mycotoxins. Early records attributable to the disease inlcude the Spartan war with Athens in 430 BC. In 1093 the Order of St. Anthony was established for victims of St. Anthony's Fire, a crippling disease of unkown origin. In 1673 in France, the disease was linked to consumption of grain infected with ergot (sclerotia of *Claviceps purpurea*), and in 1770 an epidemic resulted in the first ergotism control measures. Today, the FDA has set limits on ergotalkaloid levels, and food grains are strictly monitored. Two forms of the disesase, gangrenous and convulsive ergotism, are associated with prevalence of different ergotalkaloids.

Stachybotryotoxicosis was one of the first mold mycotoxicoses to draw scientific study and paved the way for a broader understanding of the hazards posed by mycotoxins. It was first recorded in the Ukraine in early 1930's, primarily affecting horses fed hay infected with *Stachybotrys chartarum* containing trichothecene mycotoxins. Symptoms included irritation of oral/ nasal passages and necrotic lesions of respiratory and digestive tracts, often proving fatal within 24 hr.

Alimentary Toxic Aleukia (ATA) caused by T-2 toxin, another potent trichothecene, reached epidemic proportions during World War II in Russia. In some districts 10% of the population and livestock contracted the often fatal illness. ATA was caused by ingestion of grain infected with *Fusarium poae* and *F. sporotrichioides* that had overwintered in the field. Symptoms include subcutaneous haemorrhages, leukopenia, lymphocytosis, and acute degeneration of internal organs.

Aflatoxin, perhaps the most famous of all mycotoxins, remains one of the most potent carcinogens of natural origin known to man. In 1952, an outbreak of 'moldy corn toxicosis' was

caused by the consumption of mouldy corn by swine in southern USA. Another outbreak in 1960, Turkey 'X' disease, caused the death of 100,000 poults in England. Aflatoxins are potent hepatocarcinogens produced by *Aspergillus flavus, A. parasiticus*, and *A. nomius*. Symptoms include anorexia, lethargy, muscle weakness, liver haemorrhages and necrosis, engorged kidneys and liver cancer. There is at least one human case of acute aflatoxicosis (severe hepatitis) in India. Long term effects of diets containing aflatoxin are correlated with high incidence of liver disease in certain regions. While acceptable levels of aflatoxin in food are about 15 ppm, samples of contaminated food in Nigeria measured 100 ppm, maize in India 15,000 ppm, and corn in USA reached 320,000 ppm.

Yellow Rice Disease, or Shoshin-kakke, was prevalent in Japan during Sino-Japanese war and after World War II. This potentially fatal, agonizing disease resulted from consumption of rice infected with *Penicillium citreoviride*. The toxin, citreoviridin, causes paralysis, cardiovascular damage, and respiratory failure.

Ochratoxin mycotoxicoses is linked to endemic Balkan nephropathy, a fatal kidney disease of young people living near the Danube River. Ochratoxin, a powerful nephrotoxin and hepatotoxin, is also implicated in an outbreak of procine nephropathy (swine are extremely sensitive) in Denmark in the 1920's. *Aspergillus ochraceus*, *Penicillium verrucosum* and related species commonly produce ochratoxin in grain, dried beans, peanuts, green coffee beans, and hay. Estimates suggest that most European pork has trace amounts of ochratoxin, primarily from *Penicillium verrucosum* and *P. nordicum,* species particularly prevalent in northern climates.

Case Study: Rubratoxicosis

First recognized in the 1950's as 'Hemorrhagic Syndrome in Poultry', the mycotoxicoses caused by rubratoxins after consumption of moldy feed resulted in 40% mortality. Other agricultural syndromes include "Hepatitis X" in dogs and swine. All are characterized by congestion and hemorrhage in various organs and histological lesions in liver and kidneys.

Recently, together with medical mycologist Lynne Sigler (Univ. Alberta Microfungus Collection, Edmonton, Canada) and mycotoxicologist Jens Frisvad (Technical Univ. Denmark, Lyngby), I was involved with the first confirmed report of human rubratoxicosis (Sigler et al. 1996). Three teens drinking toxic moldy homemade rhubarb wine became critically ill with rapid onset of fever, chills and severe vomiting and were diagnosed with acute liver failure. One received an immediate liver transplant. Mycotoxins in the wine were suspected since mold had been noted during the wine making process.

Initial inspection of the wine and containers showed no visible mold growth, but the wine was yellowish, unlike the typical rosé color of rhubarb wine. Microbiological analysis included transferring portions of the wine to agar media. Colonies of a blue-green mold were isolated and identified by toxin profile and by macroscopic and microscopic features as *Penicillium crateriforme*.

Animal studies at the Univ. Alberta Hospitals provided evidence that a mycotoxin was responsible. Two adult mice were inoculated with 0.5 and 0.2 ml of filter sterilized wine. The mouse that received the higher dose died within 36 hours.

Wine samples and a mold isolate were analyzed for mycotoxins. The main mycotoxin in the wine was rubratoxin B. Analysis of cultures showed the presence of additional toxins, including rugulovasine A and B, and luteoskyrin, another known hepatotoxin, but these were not present in the wine. *P. crateriforme* is capable of breaking down colored matter from the rhubarb accounting for the wine discoloration . The high level of rubratoxin in the wine may be due in part to the acidic environment and solubility in alcohol.

Although ingestion of mold contaminated food rarely has consequences as serious as the liver failure reported here, this case demonstrates potential risks associated with common indoor molds.

HEALTH EFFECTS OF AIRBORNE TOXIGENIC FUNGI

Organic Dust Toxic Syndrome (ODTS)

Inhalation exposure to mycotoxins was first recognized in agricultural workers exposed to extremely high levels of airborne molds. A variety of fungal and bacterial toxins were potentially involved in the acute toxicological disease termed ODTS.

'NIFIES' and 'Sick Building Syndrome' (SBS)

The health effects of mold exposure have been well described but the specific mechanism of disease remains incompletely understood. Extensive attention has been given to airborne molds as potential allergens or infectious agents. However, the few published studies of occupants of mold-contaminated buildings have not convincingly demonstrated the clinical syndrome is consistent with an immediate (e.g., allergic rhinitis, asthma) or delayed (e.g., hypersensitivity pneumonitis) hypersensitivity reaction, with no immunological markers correlating with clinical illness. Indeed, the consistent finding that most occupants of mold-contaminated buildings experience rapid onset of symptoms upon entering the building and rapid resolution each time they leave strongly suggests a toxicological, rather than allergic mechanism. SBS was applied to unexplained symptoms of occupants primarily in office buildings, and the disorder has been described as a "building-related illness arising from microbial contamination of building materials caused by condensation and leaks" (AIHA 1996) and more vaguely as "building-related symptoms" (ACGIH 1999). A more specific term, Non-Infectious Fungal Indoor Exposure Syndrome (NIFIES) has been proposed to describe the illness typically first called SBS (Craner 1999). Symptoms include eye, nose and throat irritiation/ inflamation, respiratory symptoms such as cough and chest tightness, fatigue, papular rash, and neurocognitive symptoms such as short-term memory loss and concentration problems. Medications such as antibiotics, antihistamines, and asthma drugs are ineffective because mycotoxicoses are non-infectious, non-communicable, and do not elicit measurable immune response. Generally, individuals have complete clinical improvement shortly after removal of the mycotoxin source, either through relocation or remediation of the mold contaminated environment.

Route of exposure

Mycotoxins affect occupants in buildings primarily through inhalation. Many small mold spores (2-10 microns) are respirable into the alveoli, the terminal portion of the lungs where oxygen exchange between the lungs and blood occurs, and in which soluble toxins contained in the spores enter the blood stream. However, the toxicology and mechansim by which mycotoxins enter and distribute throughout the body and selectively produce symptoms remains poorly understood. Currently, no reliable biological marker (e.g., blood test) has been developed to demonstrate the presence of such agents in the body.

Epidemiology

The validation and circumscription of widespread health effects in cases of SBS has been hampered by inadequate occupant questionnaires, incomplete environmental investigations and small sample sizes. New software, EpIAQ™ (Epidemiological Indoor Air Quality) developed by at Verdi Technology Associates (Verdi, NV) allows investigators to quickly and objectively identify the nature, extent, and distribution of building-related health complaints. Investigations (publications pending) utilizing the EpIAQ™ software have objectively demonstrated the direct association between indoor toxigenic fungal exposure and SBS/NIFIES illness.

MYCOTOXINS IN INDOOR ENVIRONMENTS

Referred to simply as 'stachy' in the trade, *Stachybotrys chartarum* has served as the flagship for mycotoxin-producing molds in indoor environments. The sinister reputation as the 'toxic black mold' may be well founded in the potency of the suite of trichothecene toxins produced. Trichothecenes, including the potent satratoxin, are cytotoxic compounds, capable of killing cells. Carcinogenic effects are occasionally reported, but there is no evidence of carcinogenicity of these toxins. Other immune suppressive compounds have also been isolated from *Stachybotrys*. Although *S. chartarum* is by far the most frequently encountered species, others such as *S. cylindrospora* and *S. echinata* are also found occasionally indoors and are both known to produce similar toxins. The first cases of human stachybotrytoxicoses were a result of inhalation exposure of the spores by handlers of contaminated hay and straw. Without adequate PPE, remediators of mold-contaminated buildings are at similar risk of high mycotoxin doses. (See the article devoted to *Stachybotrys chartarum* in the Feb 2001 issue of Indoor Environment CONNECTIONS for more information)

Other trichothecene producing molds can also be found colonizing wet, cellulosic substrata in indoor environments. These include *Fusarium, Myrothecium, Trichothecium* and *Cylindrocarpon.* Additionally, *Trichoderma* species are known to produce sesquiterpenes, toxins very similar to trichothenes.

Penicillium and *Aspergillus* species are common and important molds in the human environment and are among the main agents of spoilage in human and animal food. Many species are known to produce a diversity of toxins (Table 1), making them among the most important indoor molds to recover and identify. Carcinogenic effects of inhaling *Aspergillus flavus* spores have been confirmed by animal studies.

A number of common molds on decaying leaves (phylloplane fungi) are able to colonize cellulosic building materials. Some species of *Alternaria, Cladosporium, Bipolaris* and *Arthrinium* are known to produce toxins. Moderate levels of these fungi are common as a result of outdoor air exchange with the interior of the building. Several species are also well known allergens and active indoor growth is potentially problematic. Other cellulose degrading species such as *Chaetomium* and *Phoma* may produce toxins. *Chaetomium* is an ascomycete that is especially common on water damaged paper and wood products.

Sampling for fungi

Currently, the method of choice for assessing potential exposures to mycotoxins in indoor environments involves the detection of mycotoxin-producing fungi through collection and identification of fungal propagules. Determining types and prevalence of various species of fungi present on surfaces and in air allows for assessment of active growth within buildings and the potential for mycotoxin production based on the species identified can be inferred. In many cases, the underlying question is simply "Is active mold growth occurring indoors"? A genus level identification is sufficient in most cases, but for particular cases where correlations are being made to health effects it may be necessary to have all fungi identified to species. This can be especially important in groups such as *Penicillium* or *Aspergillus* where different species produce vastly differing types and quantities of toxins.

Indoor sampling protocols should involve a variety of sample types to get a well-rounded assessment. Areas of visible mold may be sampled directly by sending bulk material or tape-lift samples for identification. Sampling should include air monitoring in selected problem and non-problem areas with an outdoor comparison sample. Culturable and non-culturable methods have differing sensitivities or capabilities. Although either method will usually detect major problems, a combination of the two provides for the most reliable interpretation. The culturable method employs samplers such as the Andersen N6, Biotest RCS, SAS, EM Science MAS 100 and others that draw air across agar media, impacting the fungal spores. Cultures are incubated, allowing identification of the fungi that grow. The main advantage is that precise identifications are possible, crucial for species ID of *Penicillium* and *Aspergillus*, and important for the recovery and recognition of a wide variety of potentially toxigenic molds such as *Paecilomyces, Fusarium, Trichoderma, Phoma, Acremonium,* and *Wallemia*. Non-culturable or 'spore-trap' samplers such as the Zefon Air-O-Cell, Burkard, Allergenco, BioSIS, cyclex-d and others have demonstrated excellent ability to allow sensitive detection of *Stachybotrys* spores present in low levels. They allow for rapid analysis when required and adequately determine the levels and proportions of various spore types determined to genus or broad category. Although they lack some specificity in identification, they recover types of spores that do not grow or compete well in culture. They have the additional advantage of collecting all airborne particles for microscopic observation and non-fungal biological elements such as pollen, insect parts, epithelial cells, fiber glass and carbonaceous debris may be detected to further broaden the scope of the IAQ investigation.

Analysis for mycotoxins

Although sampling for fungi is standard in IAQ studies, direct or indirect analysis for toxins is also possible. Isolates recovered from indoor samples can be assayed for toxin production, but given the variable nature of toxin production based on environmental conditions such as medium, temperature and competition with other microbes, the presence or absence of toxins produced under experimental conditions does not confirm toxin production was occurring in situ. Examination of bulk material for toxins is also possible, but more complicated due to interference of background material and mixed

species assemblages. Perhaps the most limiting factor is the enormous diversity of toxins produced by fungi, including many that remain uncharacterized (Table 1). Toxicological tests utilize a comparison standard, so unknowns must be run for each individually profiled mycotoxin, making analytical costs prohibitive.

Sampling for volatiles, or mVOC's, given off by microbes as a byproduct of their metabolism has also been used to determine fungal growth in buildings. Many of these compounds are alchohols, aldehydes, ketones and other organic chemicals that are not mycotoxins and likely have little health effects on occupants at the levels present. mVOC testing offers an additional parameter in cases where traditional means are inconclusive, but cost and lack of specificity tend to preclude its use in routine investigations.

Summary and Current status in IAQ industry

Despite a growing body of evidence linking mycotoxins produced by indoor molds to adverse effects on human health, a recent AIHCE/AIHA panel concluded that there is "at this time not enough evidence to support an association between mycotoxic fungi and a change in the spectrum of illness, severity of illness or an increase in risk of illness" (Kirkland 2001). Reminiscent of early doubts regarding health effects of other exposure related illnesses such as smoking, we await more detailed scientific studies to corroborate and explain the expanding pool of increasingly convincing observational data that we are confronted with in this field each day.

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Chaetomium globosum

TABLE 1: Selected mycotoxins produced by some common indoor molds and other economically important fungi.

Acremonium	citrinin
Alternaria	altenuene, altenusin, alternariol, altertoxin, tenuazonic acid
Arthrinium	nitropropionic acid
Aspergillus	aflatoxin, austin, citrinin, cytochalasin, fumitoxin, nidulotoxin, ochratoxin, patulin, sterigmatocystin, tremorgenic mycotoxins (fumitremorgen, penitrem, territrem, verruculogen), viomellein, vioxanthin, xanthomegnin
Bipolaris	cytochalasin, sporidesmin, sterigmatocystin
Chaetomium	chaetoglobosin, chetomin, chaetochromin, chaetosin, cochliodinol, sterigmatocystin
Cladosporium	cladosporic acid
Claviceps	ergotalkaloids (egrine, ergometrine, ergonovine, ergotamine, ergotoxine, lysergic acid), secalonic acid
Cylindrocarpon	macrocyclic trichothecenes
Diplodia	diplodiatoxin
Fusarium	fumonisin, fusaric acid, fusarin, fusarochromanone, moniliformin, trichothecenes (deoxynivalinol, T2 toxin), zearlenol, zearalenone
Gliocladium	gliotoxin
Myrothecium	trichothecenes (roridin, verrucarin)
Paecilomyces	patulin, viriditoxin
Penicillium	citrinin, citreoviridin, citromycetin, erythroskyrin, ochratoxin, griseofulvin, luteoskyrin, oxaline, patulin, penicillic acid, roquefortine, rubratoxin, rugulosin, rugulovasine, tremorgenic mycotoxins (penitrem, territrem, verruculogen), verrucosidin, viomellein, viridicatin, xanthomegnin
Phoma	brefeldin, cytochalasin, secalonic acid, tenuazonic acid
Phomopsis	macrocyclic trichothecenes
Pithomyces	sporidesmin
Rhizoctonia	slaframine
Rhizopus	rhizonin
Sclerotinia	furanocoumarins
Stachybotrys	griseofulvin, trichothecenes (isosatratoxin, roridin, satratoxin, trichodermol, trichoverrol)
Torula	cytotoxins
Trichoderma	gliotoxin, koninginin, trichodermin
Trichothecium	roseotoxin, trichothecenes (trichothecin)
Wallemia	walleminol
Zygosporium	cytochalasin