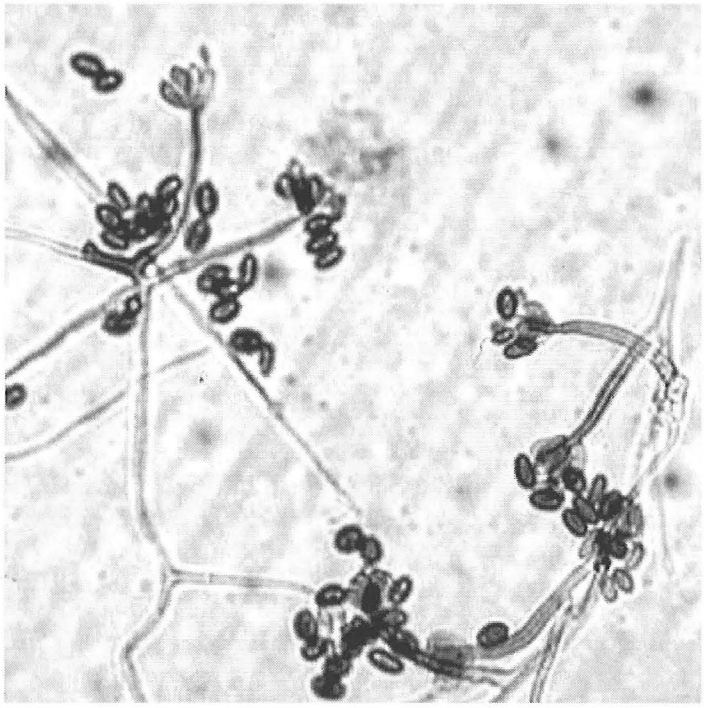
Who's Afraid of *Stachybotrys*?

Clinical Implications of Toxigenic Fungi



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My interests are primarily in Mycology. I am currently studying variables affecting antifungal susceptibility testing and resistance mechanisms in *Candida* bloodstream isolates. I am also interested in phaeohyphomycosis (infections caused by darkly pigmented fungi), as well as the role of melanin in the pathogenesis of fungal infections.

Introduction

There are an estimated 100,000 described species of fungi, which likely represent less than half the total species that exist in nature(1). A small fraction (< 0.1%) routinely cause infection in humans, though several hundred have been reported as agents of disease in the literature. Fungi can be broadly classified as 1) yeasts, which are unicellular and reproduce by budding, 2) filamentous fungi or molds (**Figure 1**), which reproduce by spores and grow in a mycelium with hyphae, or 3) dimorphic fungi, which can exist as either yeasts or molds depending on their environmental conditions. Fungi are characterized by being non-motile, having cell walls, and plasma membranes that contain ergosterol(1). Almost all are aerobes. Despite advances in molecular techniques for identifying most microorganisms, the standard for mold identification remains colony and microscopic examination. Spore characteristics, including size, shape, and type of sporulation are critical for reliable identification to species level(1). This is generally done by experienced reference labs.

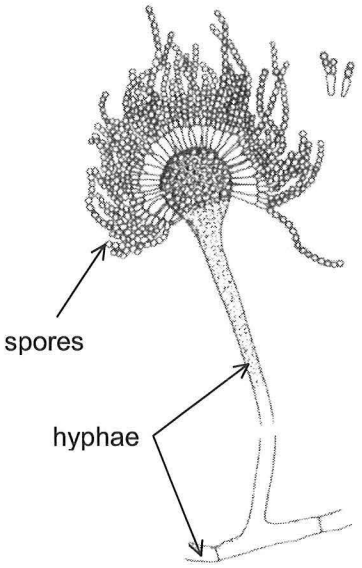


Figure 1. Diagram of *Aspergillus flavus* as seen microscopically. (St. Germain and Summerbell, Identifying Filamentous Fungi,1996)

Abnormal growth of mold has been recognized as unhealthy since ancient times. In the Old Testament, Leviticus, Chapter 14, verse 38, the Lord tells Moses: "If [the priest] finds greenish or reddish streaks of mildew in the walls of the house. . . he shall close up the house seven days, and return the seventh day to look. If the spots have spread in the wall, then the priest shall order the removal of the spotted section of the wall, and the material must be thrown in a defiled place without the city. . . . If the spots appear again, the house is defiled [and] he shall order destruction of the house. . ." Ergotism, the first widespread disease related to mycotoxin exposure, was commonly seen in the Middle Ages and likely much earlier, but not recognized for its fungal origin until the 1800's(2). It was not until 1960 that the formal study of mycotoxins really began with the investigation of the deaths of over a hundred thousand turkey poults in England, eventually linked to aflatoxin in the grains they were being fed(3).

Mycotoxins are secondary metabolites of fungi, usually molds, that can cause injury to animals or humans(4). They are not required for growth of the fungus and are often formed after exponential growth has ceased. Secondary metabolites may be produced by the fungus to reduce competition for growth by other microorganisms. The species that synthesize these compounds are often able to detoxify them in order to protect themselves. Antibiotics such as penicillins, cephalosporins, and griseofulvin are also secondary metabolites of fungi, but are not considered toxins.

Toxigenic fungi have only recently been the subject of detailed study. They have been considered a potential public health concern for several decades, but have not garnered much notice by the lay public. This has changed dramatically in just the past

few years, with fears of 'toxic black mold' gripping news headlines and the public's attention. What really brought the issue into the public spotlight was the report from Cleveland, Ohio of a cluster of infants with pulmonary hemorrhage and their association with toxigenic molds, specifically *Stachybotrys*(5). Since then 'toxic mold' has often been front page news, with claims of health problems and building closures blamed on mold. Couples have burned their houses to the ground as the only way to get rid of mold growth, and one couple in California has recently built a house entirely of stainless steel coated with silver impregnated ceramic material in order to prevent mold growth, at a cost of $3 million(6). In addition, public buildings are regularly closed due to indoor mold growth, and the occupants are only allowed in after remediation (cleaning and/or removal of affected areas) is complete. Apartments have also been evacuated, the residents being told to leave all their possessions to be 'decontaminated'. Insurance companies have raised premiums and refused to cover mold related claims, fearing massive losses due to the perceived widespread problem. Companies have sprung up promising to rapidly diagnose whether there is mold in the home or office, particularly the dreaded *Stachybotrys*, and to decontaminate the building. A recent report from a school in Tennessee found fungal growth of *Stachybotrys* on ceiling tiles, and recommended professional remediation, commenting that "the association of *Stachybotrys* and Sick Building Syndrome (SBS) has been established clearly"(7). In actuality, relatively little is known regarding the effects to humans from exposure to toxigenic molds and their mycotoxins, especially from airborne exposure. The studies often cited are not conclusive and frequently suffer from methodologic problems. The objective of this review is to critically examine the literature on toxigenic fungi and mycotoxins with respect to their health effects in humans.

The Fungi

There are hundreds of fungi that are capable of producing toxins(4). Relatively few produce significant mycotoxins that are known to affect humans. **Table 1** is a partial list of toxigenic fungal genera (pl. of genus), though not all species within a genus will produce toxins. *Cladosporium* is probably the most common fungi found in the

Table 1. Toxigenic fungal genera

Acremonium	*Dichotomomyces*	*Myrothecium*	*Rosellinia*
Alternaria	*Diplodia*	*Microdochium*	*Sclerotinia*
Aspergillus	*Drechslera*	*Monographella*	*Spacelia*
Bipolaris	*Epichloe*	*Nigrosabulum*	*Stachybotrys*
Botryodiplodia	*Epicoccum*	*Nigrospora*	*Talaromyces*
Byssochlamys	*Fusarium*	*Paecilomyces*	*Thielavia*
Ceratocystis	*Gibberella*	*Penicillium*	*Trichoderma*
Chaetomium	*Gliocladium*	*Periconia*	*Trichothecium*
Claviceps	*Gloeotinia*	*Phoma*	*Verticillium*
Colletotrichum	*Khuskia*	*Phomopsis*	*Verticimonosporium*
Curvularia	*Metarhizium*	*Pithomyces*	*Zygosporium*

(from Encyclopedia of Food Mycotoxins, Weidenborner, 2001)

environment worldwide. Colonies are brown to black in color, which is common for many environmental fungi(8). *Penicillium (P.)* and *Aspergillus (A.)* species are also widespread, and certain species are toxigenic. Colonies range from green to gray-green for *Penicillium* and from green to brown/black for *Aspergillus*(8). *Fusarium* is a common mold that contaminates many crops and can produce a variety of mycotoxins. *Stachybotrys (S.)* is a greenish-black mold which is generally found on non-living organic material, especially cellulose in high humidity(9). Although considered by many to be the prototypical 'black mold', other, more common molds are also black in color. There are several species of *Stachybotrys*, but only *S. chartarum* frequently produces mycotoxins(10). Not all strains of *Stachybotrys* produce mycotoxins. Other synonyms for *S. chartarum* are *S. alternans* and *S. atra*. *Stachybotrys* is rarely found in outdoor air, and its spores are not easily airborne. It is associated indoors with areas of water damage, though almost always in much lower levels than *Aspergillus* or *Penicillium*(9). In nature, it has been cultured from hay, straw, soil, and animal hair, though it is usually not the predominant fungus(9).

Mycotoxins and mycotoxicoses

There are estimated to be over 400 mycotoxins, less than 20 of which are considered acutely toxic to mammals (LD_{50} <100 mg/kg)(11). The majority of these can generally be thought of in groups based on their chemical structure: 1) ergot, 2) aflatoxins, 3) trichothecenes, 4) fumonisins, and 5) ochratoxins. Many other mycotoxins have been isolated, though little is known of their effects in humans, and they will not be discussed here. A variety of sources are available that discuss mycotoxins in detail(3,4,12-18). Clinical syndromes relating to mycotoxin exposure have almost exclusively been due to acute or chronic ingestion of preformed toxins in food, usually nuts and grains. Each food source is associated with a limited number of fungi that cause its spoilage(19). The toxins produced are often stable, and may persist through standard food processing(19). Several factors can affect mycotoxin production, including the particular fungal species, mycoviruses, mechanical damage, geographic area, temperature and humidity(2). One of the most important factors in the production of mycotoxins is the water activity of the growth substrate. Water activity (a_w) is a measure of available water for microbial growth, i.e. not bound to food or substrate. In practice it is measured as the equilibrium relative humidity(20). Examples in foods are listed in **Table 2**. Mycotoxins are generally produced in environments with high water activity, usually with an a_w of > 0.85.

Three main genera of fungi are responsible for the vast majority of mycotoxin production in association with food: *Fusarium, Aspergillus,* and *Penicillium*. *Fusarium* is a destructive plant pathogen and infests crops before and during the harvest, while *Aspergillus* and *Penicillium* frequently contaminate crops during drying and storage(18). It is important to remember that the presence of toxigenic fungi does not necessarily mean presence of toxin and absence of toxigenic fungi does not mean absence of toxins, as they can persist long after the fungi have become non-viable(18). Contamination of food with mycotoxins is widespread throughout the world and has seasonal variation(21). Commercial food companies monitor levels of various

mycotoxins in foodstuffs prior to processing, though worldwide standards are not available for most mycotoxins, each country having its own acceptable levels(19,22).

Table 2. Water activity of common foods
(not associated with toxigenic fungi)

Food product	Water activity (a_w)
Fresh meat and fish	0.99
Bread	0.95
Aged cheddar	0.85
Jams and jellies	0.80
Dried fruit	0.60
Biscuits	0.30
Instant coffee	0.20

(Food Science Australia, www.dfst.csiro.au/water_fs.htm)

Mycotoxin exposure outside of food (i.e. airborne exposure) is an evolving area of study, though it has received much attention recently. *Stachybotrys* is the fungus most commonly studied, though other fungi also have potential for airborne exposure. It is important to realize that mycotoxins are generally not volatile compounds, so exposure is likely due to the presence of the toxins in the inhaled spores (23).

Vaccines against mycotoxins have been studied in animals. Anti-idiotype antibodies directed against the binding region of anti-toxin antibodies have been shown to be protective in a murine model, though studies have been limited(24).

Ergot

Ergot poisoning or ergotism has been recognized as a syndrome for over a millennium, though it's relation to a fungus, *Claviceps purpura*, was not discovered until the mid 1800's(2). It was the first mycotoxicosis known to cause epidemics of disease, eventually associated with moldy rye grain. *Claviceps purpura* invades the rye plant, replacing the seeds with a dense fungal mass called a sclerotium, where ergot is concentrated(2). Ergotism was relatively common in Europe from the 9th to the 14th centuries.

Two clinical forms were distinguishable: gangrenous (most common) and convulsive. The gangrenous form was characterized by an initial prickly sensation in the distal extremities, followed by vasoconstriction and hemorrhagic bullae, and finally dry gangrene(2). Gangrenous ergotism was known as 'St. Anthony's Fire' in the Middle Ages, as the afflicted would travel to the shrine of St. Anthony, where many would supposedly be cured(25). As it turns out, the region of the shrine was free of ergot contamination of rye, so it is likely that many did improve from the lack of ergot in their diet. The convulsive form of ergotism was characterized by vertigo, headaches, tinnitus, seizures, gastrointestinal upset and hallucinations(2). It is postulated that this form may have been an impetus for the Salem witchcraft trials in the 16th-19th centuries(25). Interestingly, lysergic acid diethylamide (LSD), the potent hallucinogen, is a simple derivative of ergot alkaloids(2).

There are several ergot derivatives, some of which have had clinical uses. They can be agonists and/or antagonists for norepinephrine, dopamine and serotonin receptors, leading to their varied effects, though severe vasospasm of distal arteries is the most important clinically(2). As a result of variable metabolism, individual effects of

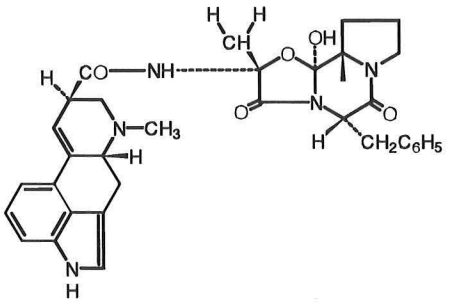


Figure 2. Ergotamine

ingestion can differ widely from individual to individual. Ergotamine (**Figure 2**) and its derivatives are still used for vascular headache, uterine atonia, and migraine(2). Ergotism from contaminated rye has been rare in modern times, due to improved storage and handling of rye grain. The last known outbreak occurred in 1977 in Ethiopia, affecting 140 people(26).

Aflatoxins

Aflatoxins are produced primarily by *A. flavus* and *A. parasiticus*, though not all isolates of *A. flavus* produce aflatoxins(13,27). Aflatoxin B_1 (AFB_1) is the most common and potent compound. It was discovered as a cause of disease during the investigation of the deaths of over 100,000 turkey poults in England in 1960(3). Aflatoxins are produced at high water activity (a_w 0.98-0.99) and production stops at a_w <0.85(18). *A. flavus* grows best at 32-33°C, but can grow from 10-42°C(18). Production of toxin requires higher water activity than growth alone, which is true of most toxigenic fungi(13).

Aflatoxins are a common contaminant of peanuts and grains worldwide, though corn is most commonly affected in the United States(2,28). They may also be in some forms of heroin(26). Aflatoxin B_1 is the only mycotoxin regulated by the Food and Drug Administration. Although it cannot be totally eliminated from foods, <20 parts per billion (ppb) is considered acceptable for food products(2,16). The levels of AFB_1 in food can be reduced by a variety of methods, including treatment with ammonia (which can alter its taste), removing obviously moldy nuts, separating moldy grains in a sodium chloride solution (reduces levels by 75-85%), and dehusking and polishing rice (reduces levels by 70-90%)(16,27,29). Inorganic feed additives can also help decrease absorption by livestock(16).

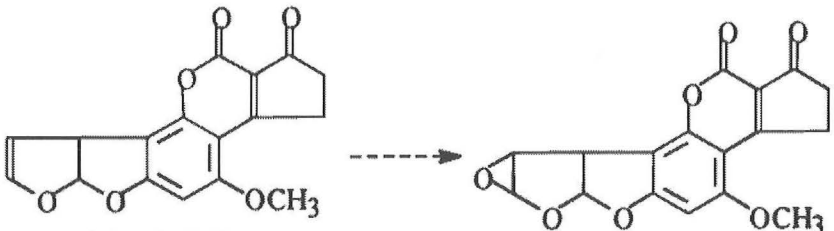
Symptoms of acute ingestion are vomiting, abdominal pain, fulminant hepatic failure, and death from as little as 10-20 mg total AFB_1(25). Human consumption varies from 0-30,000 ng/kg/day, usually 10-200 ng/kg/day(2). In one study in Kenya, maize contaminated with 3.2 and 12 parts per million (ppm) aflatoxin was associated with hepatic failure while unaffected homes had levels < 0.5 ppm(2). In another outbreak in India in 1975, almost 400 people were affected by corn contaminated with aflatoxin(30). The mean daily amount ingested was estimated to be 2-6 mg. The corn was thought to have become infested after unseasonable rains followed a prolonged drought. One

hundred six people died, mostly of acute liver failure over a period of 2-3 weeks. After the harvested corn was completely consumed, no further cases were seen. It was felt that insects may have been in part responsible for transferring the fungi to the corn plants. This can be a particular problem in India, when there is often high temperature, humidity and poor storage conditions(30). In the U.S., warm, humid areas of southeastern states are more commonly involved with aflatoxin contamination(28).

Aflatoxin B_1 is the only mycotoxin that has been strongly linked to the development of hepatocellular carcinoma (HCCA) in humans resulting from chronic ingestion(25,26). It is considered the most potent natural carcinogen known to man(18). In the Qidong district of China, for example, HCCA is the leading cause of cancer deaths, and AFB_1 exposure is widespread(25). In combination with hepatitis B, which is also prevalent in China, exposure to aflatoxin greatly increases the risk of developing HCCA(25).The carcinogenic potential of AFB_1 may be more related to chronic rather than acute exposure. In a well documented case, a woman ingested 5.5 mg of AFB_1 over 2 days, then 35 mg over 2 weeks in a suicide attempt(3). She developed a rash, nausea and headaches, but completely recovered and was well at follow-up 14 years later.

The mechanism of carcinogenesis for aflatoxins has been well studied. AFB_1 must be converted to an epoxide form by cytochrome P-450 dependent enzymes (CYP), which can also catalyze its hydroxylation and demethylation to less toxic forms (**Figure 3**)(31,32). It is activated to the 8,9 epoxide form primarily by CYP 3A4, though CYP 1A2 has higher affinity(32). Variability in which pathway predominates in any given individual is likely the result of genetic differences(32). The epoxide of AFB_1 is highly

Figure 3. Activation of aflatoxin B_1



reactive with DNA, RNA, and protein(31). Its binding to DNA is thought to result in a purine to pyrimidine conversion, leading to point mutations(31). By comparison, benzene binds to DNA with a binding index of 1.7 versus 10,400 for AFB_1(33). In areas of high aflatoxin exposure, HCCA associated with a specific mutation (AGG→AGT at codon 249) in the p53 tumor suppressor gene has been observed, which is not seen in areas with low levels of aflatoxin(18). There is also evidence for immunosuppression related to aflatoxin ingestion(34). This may be related to protein synthesis inhibition, perhaps a by-product of interaction with DNA and/or RNA. In animal models, cell mediated immune responses appear to be more sensitive than humoral responses to low levels of aflatoxins(34).

Conjugation to glutathione by glutathione S-transferase (GST) is an important pathway for detoxification of aflatoxin(31). Oltipraz, an experimental anti-angiogenesis and anti-schistosomal drug, is being studied to decrease the risk of HCCA, as it decreases the conversion of AFB_1 to its active form(25,31). Oltipraz enhances GST activity, and has been shown to reduce the toxicity of AFB_1 in rats(31).

Trichothecenes

Trichothecenes (TCTs) are a group of several dozen sesquiterpene compounds produced by a variety of fungi (**Figure 4**). They are named from the fungus *Trichothecium roseum*, from which the prototypical compound was isolated in 1948(2). *Fusarium* is the most important worldwide fungus that produces TCTs, but other fungi also produce these toxins, including *Stachybotrys* and *Trichoderma*(2,25). *Fusarium* has a gene responsible for detoxifying TCTs as well(27). *Fusarium* toxins are generally produced at lower temperatures, up to 25°C(35).

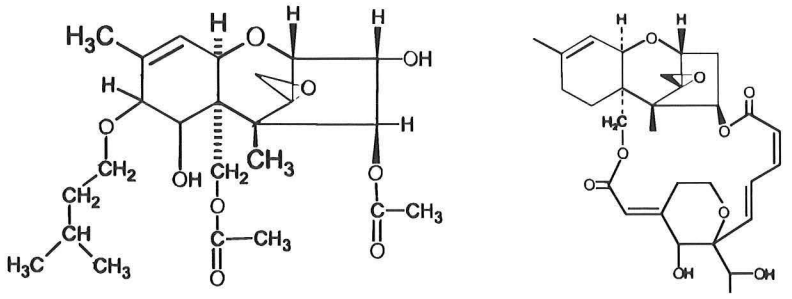


Figure 4. Trichothecene mycotoxins from *Fusarium* (T-2 toxin) and *Stachybotrys* (satratoxin)

Trichothecenes are potent inhibitors of protein synthesis(2). In contrast to aflatoxins, trichothecenes are directly toxic due to the presence of a reactive epoxide group in their structure(32). Their primary site of activity appears to be the 80S ribosomal subunit, though they have been reported to have activity during the initiation, elongation, and termination phases of protein synthesis(2). In murine and human cell lines, TCTs show cytotoxicity that may also be mediated by apoptosis(36). The activation of MAP kinases is thought to be involved(36). They are not associated with carcinogenesis(2).

In animal models, ingestion is associated with gastrointestinal bleeding, anemia, and granulocytopenia(18). Necrosis of the spleen, lymph nodes, thymus has been observed, as well as decreased antibody synthesis(34). They are toxic to rodents with an LD_{50} of 1-5 mg/kg, newborn rodents being more susceptible with an LD_{50} of 0.15-0.20 mg/kg(12). In Hungary, hundreds of sheep became ill and many died after eating straw contaminated with *Stachybotrys*(37). Common symptoms were weakness, bloody nasal discharge, and diarrhea. Satratoxins were isolated from the straw and confirmed by HPLC, though these were not quantitated. Another syndrome associated with TCTs, stachybotryotoxicosis, was described by Soviet scientists in the 1940s(25). Horses would get the syndrome after consuming wheat contaminated with *Stachybotrys*(15). Experimentally, the animals would develop stomatitis with necrotic ulcers, followed weeks later by severe leukopenia, coagulopathy, hypoglycemia, and bleeding from the gastrointestinal tract, lung, and brain with necrosis(15). An atypical form was also observed, with areflexia, hyperesthesia, visual loss, irritability, polydipsia, and no hematological abnormalities(15).

One of the first clinical syndromes in humans linked to ingestion of TCTs was alimentary toxic aleukia (ATA), reported in Eastern Siberia in 1913(2,15,25). It was characterized by development of necrotic ulcers on the oropharynx and gastrointestinal

tract with hemorrhage from the nose, mouth, gastrointestinal tract and kidneys, as well as agranulocytosis(15). There was an association with eating wheat and corn contaminated with *Fusarium*(15). The grains would lie under snow all winter and become contaminated by fungi, with toxins produced during the spring thaw. Symptoms were seen after eating an average of 2 kg of contaminated grain within 2-3 weeks, death seen with >6 kg in 6-8 weeks(15). Those less affected ate other foods as part of their diet and/or thoroughly washed grains prior to consumption.

In agricultural products, the major trichothecenes are T-2 toxin and nivalenol(2). In the U.S., contaminated grains are more commonly found in the northern states as well as Canada, though these are more of a concern to livestock(28). Deoxynivalenol, also known as vomitotoxin, has been associated with outbreaks in China from 1961-1985 of a vomiting illness and in India in 1987 associated with eating contaminated wheat. In the Indian outbreak, thousands were affected(25). Bhat et al reported on an outbreak of mycotoxicosis where 150 families were studied, 39 of whom had one member with a gastrointestinal illness, usually characterized by abdominal pain and bloating within one hour of eating a meal(35). Some individuals had sore throat, diarrhea, vomiting, and a rash. Only persons eating wheat were affected, and the outbreak ended when the harvested wheat had been entirely consumed. Samples taken demonstrated *Fusarium* in all wheat samples, and TCTs were found in half the samples, an average of 0.03-8 µg/gm wheat. However, blood samples were not done. Following the out break it was theorized that a crop of wheat was damaged by rains prior to harvesting, and this crop was mixed with previous good wheat.

Trichothecenes have also been considered as possible threats from terrorism(38). T-2 toxin has an estimated LD_{50} of 1-2 mg/kg, which is relatively less potent than other biological toxins, though toxicity would likely increase with inhalation(38). Since it is not a volatile compound, its delivery would be difficult. Cutaneous exposure can lead to a necrotic rash which may persist for weeks. The toxin is relatively stable, though it may be denatured by heating to 260ºC for 30 minutes, or exposure to 3-5% bleach(38).

Fumonisins

Fumonisins are a recently discovered group of compounds produced primarily by two *Fusarium* species, *F. moniliforme* and *F. proliferatum*(25,39). There are several types of fumonisins, with fumonisin B_1 being the most common(39). These are frequent contaminants of grains, but by far the most common source is corn, worldwide(14,27,39). They were discovered in 1988, following an investigation into an outbreak of equine leukoencephalomalacia in South Africa(25,40). They are also thought to cause porcine pulmonary edema syndrome(18,40). They are structurally related to sphingolipids, and are felt to act by interfering with sphingolipid metabolism and cellular folate uptake (**Figure 5**)(25,39). Sphingolipids are an important intracellular messenger, and can inhibit protein kinase C as well as being an important component of cell membranes(39). Fumonisins inhibit sphingosin-N-acetyl transferase, which alters production of certain sphingolipids, some of which are considered toxic(27). In cultured kidney cells, fumonisin B_1 causes apoptosis at 50 µM, due to a calmodulin dependent pathway (41). Fumonisins are metabolically stable and are excreted unchanged(39).

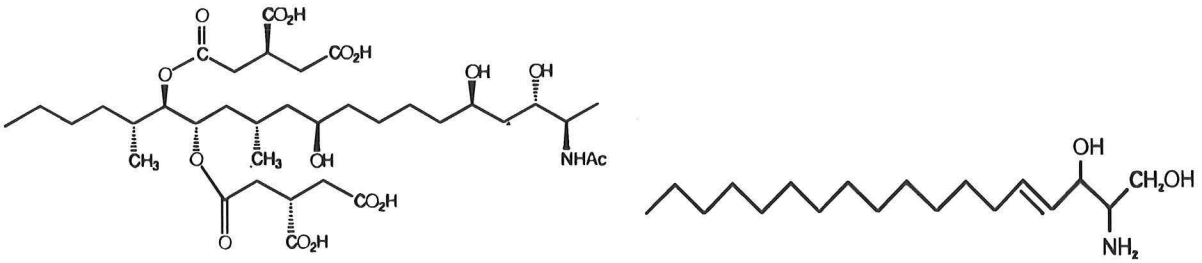


Figure 5. Fumonisin B_1 and sphingosine

Fumonisins are listed as Group 2B carcinogens for possible carcinogenic effects(18). In the Ames test using *Salmonella*, they are non-mutagenic(42). However, they do induce gamma glutamyltranspeptidase and placental glutathione-S-transferase, which may be markers of preneoplastic lesions(42). Short term exposure (<7 days) does not seem to initiate carcinogenesis(42). In rats, chronic exposure does lead to cancers in some studies, but not others(42). Carcinogenesis may be related to cytotoxic or proliferative responses(42).

In animals, specific syndromes have been observed from fumonisin exposure. In horses, a disorder called equine leukoencephalomalacia (ELEM) is characterized by facial paralysis, nervousness, ataxia, and an inability to eat or drink(16,40). In one outbreak, 14/18 horses died after eating 37-122 ppm fumonisin B_1 in their feed(16). Interestingly, only equine species seem to be affected by this syndrome. In one study, levels of fumonisins varied from 0.055 to 5 µg/gm corn, though most samples were negative(40). An atypical form of ELEM is also seen with predominant hepatotoxicity with necrosis and fibrosis(40). These symptoms are seen when the animals are experimentally fed fumonisin B_1 at a dose of 1-4 mg/kg/day. Porcine pulmonary edema syndrome is characterized by dyspnea, weakness, cyanosis and death at levels of 400 µg/kg/day for 5 days or longer(40). In their diet, 175 µg fumonisin/gm feed leads to pulmonary edema, while it is hepatotoxic at 23 µg/gm feed(40). In cattle, few effects are seen(40). Clearly, species specific clinical manifestations are observed with these mycotoxins.

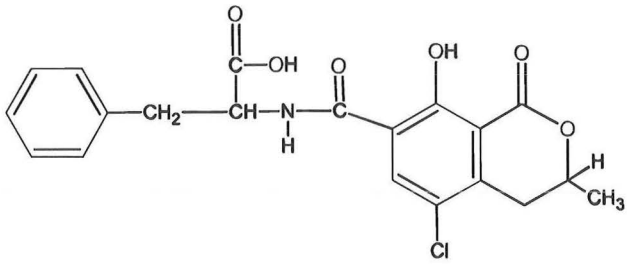
Health effects from ingestion of fumonisins in humans have not been established, but are postulated. In China and Italy, they have been associated with increased rates of esophageal cancer, though this has not been definitively established(18,26,42,43). In addition, no experimental basis has been put forth for this association.

In 1989-91, a cluster of 32 newborns with neural tube defects was seen in South Texas along the border with Mexico(44). This was associated with the heavy consumption of maize contaminated with fumonisins. Women in this area ate much higher amounts of corn based food than elsewhere in the country, which may have contributed to the clinical toxicity. Levels of fumonisins in the local corn in 1989 and 1990 were much higher than normal, and the increased incidence of neural tube defects resolved after two years, coinciding with reduced levels of fumonisins in maize. The causation remains unproven, however, and further studies are needed.

Ochratoxins

A. ochraceous and *P. verrucosum* are the principal fungi responsible for producing ochratoxins, though *A. carbonarius* in the tropics can also produce them (2). *A. ochraceous* grows slowly at 8-37°C and a_w 0.77, but toxin production doesn't begin until a_w 0.80(13). *P. verrucosum* can grow within the range of 0-31°C, but best at 20°C and a_w 0.80, but toxin production doesn't begin until a_w 0.86(13). Ochratoxins are

Figure 6. ochratoxin A



derivatives of isocoumarin linked to phenylalanine, ochratoxin A being the most potent (**Figure 6**)(12). They were discovered in 1965 and are nephrotoxic in all animal species studied(45). Ochratoxin A is thought to inhibit protein synthesis at the level of translation(45). It competitively inhibits phenylalanine-tRNA synthetase as it contains phenylalanine in its structure(45). In addition, ochratoxins may be teratogenic and carcinogenic(45).

The primary target organ is the kidney, where it is concentrated 200 fold intracellularly, particularly in the proximal tubule(12,18). Ochratoxin A produces a nephropathy in pigs that is characterized by polyuria, glucosuria, and proteinuria(45). It is not mutagenic by the Ames test, but can cause kidney and liver tumors in mice(45). In rats it is associated with renal carcinoma in doses of >70 µg/kg/day, with an LD_{50} of 22 mg/kg (45). In animals studies, lethal toxicity is reduced with administration of phenylalanine(12,45). It may have some immunosuppressive effects, but these have generally been observed at high doses in animal studies, with questionable clinical relevance(34).

Ochratoxins are widespread in foodstuffs, such as meats, cereals, and milk. They are not a common contaminant of food in the U.S(28). The World Health Organization has proposed a limit of 5 µg/kg in cereals(45). The estimated daily tolerated intake is ~5-16 ng/kg(27).

Balkan Endemic Nephropathy (BEN) is a chronic tubulointerstitial nephropathy seen primarily in Bulgaria, Romania, Serbia, Croatia, and Bosnia and is suspected to be caused by ochratoxin exposure(46). This is because the syndrome is similar to porcine nephropathy, which is known to be caused by ochratoxins. BEN is seen sporadically with clusters of cases in villages, with neighboring villages unaffected. There is familial aggregation without Mendelian inheritance patterns. Other features include: only adults (age 30-50) affected, with almost no children or individuals over 70; long incubation period, with newcomers to region not affected until 10-15 years of residence; primarily rural farming regions with no ethnic or religious differences; and a slight female to male predominance of 1.5:1(46). In affected patients, there is a much higher incidence of upper urinary tract tumors(46). Anemia is common, but other symptoms are non-specific. However, the nephropathy is not seen in other areas where ochratoxin is also

present(46). The role of ochratoxins in this disease has not been proven. Other environmental factors are being studied as well, but none have proven conclusive as the etiology. This remains a medical mystery.

Fungi in Indoor Air

Fungi are ubiquitous in the indoor and outdoor environments. Mold spores are the only allergens present year round in outdoor air. Most spores are easily airborne and small enough (<5 μm) to reach the lower airways and alveoli with the result that everyone is constantly exposed to them. Generally, the same fungi prevalent in outdoor air are present in indoor air. Some individuals can develop bothersome allergic symptoms and even hypersensitivity pneumonitis depending on the exposure level and individual predisposition. Recently, much attention has been given to the concept that abnormal fungal growth in indoor environments may cause health effects in building occupants, including pulmonary hemorrhage in infants, which will be discussed later. Homes with water damage and mold growth have been associated with cough and wheezing symptoms, but other symptoms such as fever, headache, fatigue, cognitive defects, and gastrointestinal symptoms have been less well established as being caused by mold(49).

Sick building syndrome is a widely described but poorly understood constellation of symptoms which has been observed in certain individuals in 'sick' buildings(47). Symptoms include headaches, poor concentration, fatigue, memory loss, dry skin, and itchy eyes(47,48). A variety of causes have been attributed to this syndrome, including poor ventilation, environmental factors, volatile organic compounds (VOCs), abnormal fungal growth, toxic compounds, and mass hysteria(48). No one factor has been clearly responsible.

Before approaching the issue of health effects from indoor fungi, one should consider what are 'normal' levels of fungi in indoor air. Standards for assessing which fungal species and how much should be present in indoor air are not available, mainly because reliable data have been lacking. What is considered 'normal' in indoor air varies from <100 colony forming units (CFU)/cubic meter (m^3) to <10,000 CFU/ m^3, depending on the study(50). These levels are not based on known health effects. In addition, personal exposure to molds is difficult to measure(50). However, a generally accepted principle is that indoor airborne fungal concentrations should be less than outdoor concentrations, with similar fungal species present(50).

Baseline data

Recently, a nationwide survey was performed to address this issue. Shelton et al analyzed data from over 12,000 fungal air samples collected from the entire United States for a variety of indications(51). Eighty percent were indoor samples, while 20% were outdoor samples. The median concentration of airborne fungi was 540 CFU/m^3 for all outdoor samples and 82 CFU/m^3 for all indoor samples(51). The most common genera were *Cladosporium, Penicillium*, and *Aspergillus*, which were seen in all regions and all seasons. *Stachybotrys* was detected in 6% of all indoor samples and 1% of all

outdoor samples. **Table 3** lists fungi by frequency of isolation for all buildings, and a subset with specific reasons for air sampling. Concentrations of fungi in indoor and outdoor air were highest in fall and summer, lowest in spring and winter, and varied by geographic region. Despite variation in absolute levels depending on season and region, the ratio of indoor fungal concentration to outdoor fungal concentration was

Table 3. Prevalence of fungi in indoor air

Fungi	% Buildings(n=1717) (median CFU)	% Buildings (by reason for air sampling)			
		Health complaints (n=45)	Visible mold (n=8)	Water damage (n=8)	'Proactive' (n=16)
Cladosporium	86(40)	87	100	63	94
Penicillium	80(30)	82	100	63	88
Aspergillus	62(20)	71	83	75	69
Stachybotrys	6(12)	4	38	25	0
Curvularia	4(16)	22	13	0	6

relatively constant. This suggests that both measurements should be taken when conducting air quality investigations. There was no significant association between fungi and reported health complaints, though the study was not designed to evaluate this issue(51).

In a study from Denmark, 44 homes were sent agar plates every month to expose to indoor air and examined for fungal growth(52). *Cladosporium* and *Penicillium* were present in all homes, *Aspergillus* and *Alternaria* in >50%, and *Stachybotrys* in <10%. Highest colony counts occurred during the summer. Several factors appear to impact on levels of fungi in indoor air and should be considered when analyzing such data.

Indoor fungi and health complaints

Many studies have examined the link between the presence of fungi indoors and health complaints. In a study from the Czech Republic, 48 investigations were conducted for health complaints or for concerns over visible mold growth; an additional 20 investigations were carried out in control rooms(53). They found that there was no relation to health complaints whether mold was present or not. The presence of visible mold did correlate with significantly higher airborne fungal counts and higher relative humidity. In a study from California, 68 homes were surveyed for airborne mold, with a range of 36 – 5984 CFU/m^3(54). The mean counts of *Cladosporium* and *Penicillium* were ~600 CFU/m^3. Five homes had mold and water damage, but no symptoms were noted in the occupants, and no difference seen in spore counts vs. homes without water damage(54). In a study from Denmark, 29 offices and 21 schools were examined for fungi by surface and air sampling(55). Areas with carpet had higher surface fungal counts, but similar airborne fungi to bare floor areas. *Cladosporium, Penicillium*, and *Aspergillus* were the most common fungi, and health complaints always came from carpeted areas(55). Harrison et al studied 15 buildings, 11 with closed ventilation and 4 with 'natural' ventilation systems(56). Airborne fungal levels were 10x higher in naturally

ventilated buildings than closed buildings, though none had complaints related to Sick Building Syndrome (SBS). There was a correlation between symptoms and closed ventilation systems. The conclusions were that airborne fungi or bacteria were not likely to be a cause of SBS symptoms. In another study by Dales et al, 400 families were studied who had children in elementary school(57). Air samples were taken from the homes, but not for mold. Mold exposure was assessed instead by a positive answer to the question "Has there been mold on any surface within the past 12 months?" Symptoms of respiratory complaints were higher in homes with reported mold, and the authors concluded that mold was likely responsible, despite no direct evidence. However, there are a number of cases of asthma in children that improve with remediation of mold growth(58).

Health effects from Stachybotrys *and mycotoxins in the indoor environment*

Several articles suggest the involvement of airborne *Stachybotrys* and/or mycotoxins in the adverse health of building occupants. The cases are summarized in **Table 4**. These studies in general suffer from a lack of direct evidence of mycotoxin exposure. None measure the presence of specific mycotoxins in either blood, tissue, or air samples, which would be necessary to demonstrate a causal link between health effects and toxin exposure. Even so, the symptoms described are often vague, without a specific pathologic diagnosis or pathophysiologic explanation. Nevertheless, some authors conclude that mycotoxin exposure is the most likely etiology for the patients' clinical syndrome. In addition, several studies did not perform air samples for fungi in control buildings. Surface sampling alone may be misleading. In one study, moldy building materials were found to contain mycotoxins that did not correlate with the fungi found on the material(59). Analyses for both fungi and toxins should be performed when assessing for mycotoxin exposure.

There have also been rare reports of clusters of leukemia in unrelated individuals from the same house where toxigenic fungi were present(60,61). One house associated with a report was positive for *A. parasiticus* and *Trichoderma* which both produced mycotoxins(60). However, there was no mention of water damage, visible mold growth or how the occupants may have been exposed.

Recommendations for future studies

As can be seen from the studies presented above, many problems exist with regard to determining the health effects of exposure to indoor fungal growth. Simple visual inspection to look for water damage and mold growth is critical. Sampling of air, surfaces and bulk materials can be performed, but 'aggressive sampling', where bioaerosols are artificially generated, is not recommended. Other potential sources of indoor air pollution should also be measured, including allergens, carbon monoxide, nitric oxide, tobacco smoke, endotoxin, etc(62). Reliable and quantifiable methods to assess fungal exposure in humans are lacking. Suggested criteria to support causation of health symptoms and mold and/or mycotoxin exposure are(62) : 1) similar results with multiple cohort studies, 2) high odds ratio, 3) cause clearly precedes the effect,

Table 4. Exposure to *Stachybotrys* and/or mycotoxins and health effects

Reference	Health complaints	*Stachybotrys*		Mycotoxins		Comments
		Air	Surface	Air	Surface	
Croft(63)	Upper respiratory infections, diarrhea, headache, rash, fatigue	+ (micro-scopy)	+ (micro-scopy)	+ ('crude test')	+	No mention of other fungi; no cultures taken; no quantitation of fungi or toxins; no correlation of symptoms with known effects of specific toxins
Hodgson(64)	Fevers, myalgias, 'chest symptoms', interstitial lung disease	+	+	not done	+	Cases selected by insurance carrier; smoking assoc. with abnormal PFTs; *Stachybotrys* in air after building vacated using 'aggressive' sampling
Andersson(65)	Nasal and eye irritation	not done	+	not done	+	No air samples taken; high bacterial counts also seen; symptoms consistent with environmental allergies alone
Sudakin(66)	Upper respiratory infections, fatigue, headache, depression, poor concentration	-	+ (1/19 samples)	not done	not done	High levels of bacteria also present; neurobehavioral symptoms difficult to relate to specific exposure
Cooley(67)	Nasal and eye irritation	-	+	not done	not done	Bacteria not cultured; *Penicillium* significantly assoc. with symptoms, which were consistent with environmental allergies alone
Smora-giewicz(68)	Fatigue, headache, upper respiratory infections	not done	not done	not done	+	No cultures taken; neurobehavioral symptoms difficult to relate to specific exposure; techniques could have been useful in air samples
Trout(69)	Fever, dyspnea, cough	+	+	not done	+	*Penicillium* and *Aspergillus* present with higher frequency, though airborne levels not given; syndrome consistent with hypersensitivity pneumonitis, not related to mycotoxins

4) correlation of data from different studies, 5) no or few other obvious causes, 6) dose-response effect, and 7) consistent with known mechanisms of disease.

Several methods are available to assess for the presence of fungi in the environment. Culturing is very sensitive (<10 CFU/m^3 (air), 0.05 CFU/cm^2 (surface), 5 CFU/gm (bulk)), but species level identification should be performed as well. Measuring outdoor air levels of fungi is important, as these vary by region and season, and would be expected to affect indoor levels as well. Fungal genera often found on indoor contaminated building materials include *Stachybotrys*, *Chaetomium, Memnionella, Aspergillus,* and *Penicillium*(62). *Stachybotrys* may not grow well on some culture media, so levels may be underestimated if cellulose-based media is not used(62). Direct fungal spore counts may be useful, but labor intensive, and definitive identification is usually not possible(62). Surrogate markers, such as measurement of ergosterol or other cell wall constituents or polymerase chain reaction (PCR) are in development. Mycotoxin quantitation by high pressure liquid chromatography (HPLC) or mass spectroscopy is time consuming, but can be done.

Pulmonary hemorrhage in infants and *Stachybotrys*

A cluster of idiopathic pulmonary hemorrhage/hemosiderosis (IPH) cases in infants was seen in a limited geographic area of Cleveland, Ohio from 1993-1994 and was reported in the Morbidity and Mortality Weekly Report (MMWR) in 1994(70). This led to a case-control study of the environments of the infants' homes, from which the authors concluded that mycotoxins from *Stachybotrys* were the most likely cause of the pulmonary hemorrhage(5). This has become one of the most influential studies relating to mycotoxins and human health. Public perception and policy have changed dramatically since its publication. Its conclusions were recently challenged by two expert panels convened by the CDC, one internal and one composed of outside experts. Both concluded that there were several methodologic flaws in the study which weakened the authors' conclusions with respect to an association between IPH and exposure to *Stachybotrys*(71).

Idiopathic pulmonary hemosiderosis

Idiopathic pulmonary hemosiderosis is a disease of unknown etiology, first described in 1864 by Virchow(72). It is characterized by recurrent pulmonary hemorrhage, and the presence of hemosiderin-laden macrophages in bronchoalveolar lavage fluid(73). It is a rare diagnosis, with an estimated incidence of 0.24-1.23/million population(72). It is considered to be immune-mediated and has a variable course, despite therapy with steroids or immune modulators(72). Patients are usually < 10 years old at diagnosis, and mean survival is 2.4 years(73). Pulmonary hemorrhage can also be seen in newborn infants as well, where risk factors may include severe hypoxia, prematurity, surfactant therapy, and congenital heart disease(74,75).

The Cleveland Cluster

From January 1993 to December 1994, 10 cases of idiopathic pulmonary hemorrhage were seen in infants in Cleveland(76). In the previous ten years, only three cases had been observed. This clustering of a rare disease prompted an investigation by the Centers for Disease Control (CDC), which led a case-control study focusing on the home environments of the patients(5). The infants were all African-American, and all ≤ 6 months old, with a mean age of 10.2 weeks. All were previously healthy and presented in respiratory distress with bilateral pulmonary infiltrates. Bronchoscopy showed hemorrhage with no source of bleeding. Significant differences between cases and controls are summarized in **Table 5**. The investigators undertook an environmental survey of the homes for fungal growth with the hypothesis that *Stachybotrys* was responsible for the clinical syndrome. Sampling was done after the patient's illness, which in some cases was many months later. Surface and air samples were cultured 'aggressively' for fungi, and airborne spores of *Stachybotrys* were identified under light microscopy after being collected on a filter. Significantly higher counts of total fungi were found in case homes compared to controls (**Table 6**). The odds ratio (OR) for development of pulmonary hemorrhage for an increase of 10 units in CFU of *Stachybotrys* in the air was 9.83 (CI 1.08-3x10^6). The presence of tobacco smoke with *Stachybotrys* resulted in a OR of 21 (CI 1.07-7.5x10^6). However, it was striking that although mean airborne concentrations of all fungi were much higher in the case homes, none achieved an odds ratio comparable to that for *Stachybotrys*. The authors concluded that infants with IPH were more likely to live in homes with *Stachybotrys* in the indoor air, suggesting that mycotoxins from the spores caused basement membrane fragility and capillary leak in the infants' growing lungs(5).

Table 5. Risk factors for pulmonary hemorrhage in infants

Risk factor	Cases (n=10)	Controls (n=30)	Significance
Male sex	9	15	p<.05
Breastfeeding	0	11	OR=.2 (CI 0-1.2)
Tobacco smoke	9	16	OR=7.9 (CI .9-70.6)
Water damage	10	7	OR=16.3 (CI 2.6-∞)

Table 6. Unmatched Analysis of Filter Samples

	Mean CFU/m3	
Organism	Patient Homes (n = 9)	Control Homes (n = 27)
Aspergillus	23111	445
Cladosporium	1434	27
Penicillium	755	122
Stachybotrys	43	4
Other	3880	109
Total viable fungi	29227	707

(Etzel et al, Arch Ped Adolesc Med, 1998)

Some obvious problems with the study

This study has several important limitations. First, the method of identifying *Stachybotrys* based on examination of airborne spores on a filter alone (third column of **Table 7**) is unreliable. Definitive identification of molds requires careful examination of not only spores, but type of sporulation, including hyphae. The concept of assessing surface growth of fungi is reasonable, but the manner in which it was done could lead to skewed results. Small areas of dense fungal growth will have extremely high spore

Table 7. Detection of *Stachybotrys atra* in Patients' Homes Using Several Methods

Patient No.	*S atra* on Filters Mean CFU/m3	*S atra* on Surfaces Mean CFU/g	*S atra* Spores Present
1	153	2.4×10^7	Yes
2	0	0	Yes
3	32	...	Yes
4	87	1.1×10^8	Yes
5	0	...	No
6	0	0	Yes
7	...	...	...
8	4†	92†	Yes
9	144	0	Yes
10	0	2.2×10^3	No

† These values were imputed because they were below the limit of detection.

(Etzel et al, Arch Ped Adolesc Med, 1998)

counts, whereas other areas may not have as much. A more useful measure might have been the total area of exposed wall and or ceiling covered with visible mold growth, with or without surface cultures. Cultures taken from surface samples in the case homes had enormous skew, with one home at 1.1×10^8 CFU/g, and 3 homes with 0 CFU/g (**Table 7**). Culture results from air samples also covered a wide range, with 4/9 case homes having no detectable *Stachybotrys* spores and 2 having >100 CFU/m^3 (**Table 7**). This likely explains the very wide confidence interval, the lower bound of which approaches one. The above data are indirect evidence of exposure, as the presence of mycotoxins was not assessed in either the air samples or patients.

The CDC Review

Despite the authors' suggestion that further work is needed in this area, the CDC commented that "the findings have influenced closure of public buildings, cleanup and remediation, and litigation." This led the CDC to form a internal working group as well as an outside expert panel to independently review the study data and conduct interviews with the relevant investigators(71). Their findings were: 1) cases were distinct from IPH as described in literature, and should be referred to as 'Acute' IPH, 2) additional cases identified in surveillance do not fit with initial case descriptions, and 3) "available

evidence does not substantiate the reported epidemiologic association between water damage/fungi and AIPHI". The last finding was based on the following:

1. There was a limited description of water damage, and no standard definition or protocol for inspection of the homes.
2. Exposure to fungi or mycotoxins is difficult to interpret, with available methods being of questionable clinical relevance.
3. Methods of analysis were flawed:
    a. The odds ratio of 9.8 for a change of 10 CFU of *Stachybotrys* was "statistically unstable". The mean airborne concentrations of *Stachybotrys* were incorrectly calculated, which decreased the OR to 5.5. One house was sampled months after the others and the value was below the limit of detection and imputed to be 4. This should have been recorded as 0, which decreased the OR to 1.9. Matching on age for airborne fungi was unnecessary and unmatching further reduced the OR to 1.5.
    b. A presumably blinded investigator correctly guessed the identity of the case homes and took twice as many environmental samples from them. The use of aggressive sampling in an unblinded manner introduced potentially significant bias into the sampling results.
    c. Among homes labeled as water-damaged in the study, culturable airborne *Stachybotrys* was found in 4/8 cases vs. 3/7 controls (CDC, unpublished data), suggesting that water damage may have been a confounding factor.

In addition, the CDC report mentions that evidence for an association between *Stachybotrys* and AIPH from other sources is limited: It is not consistent with other cases of human illness relating to *Stachybotrys*, clusters of AIPH have not been reported in other flooded areas with water damage, and this association was not seen in a similar outbreak in Chicago in 1992-1994 (CDC, unpublished data). The above criticisms of the initial Cleveland study do not necessarily disprove the authors' hypothesis, but add a significant note of caution to their conclusions, underlining the need for more well-designed studies and surveillance for additional cases. The CDC has published a case definition for acute idiopathic pulmonary hemorrhage/ hemosiderosis in infants (AIPHI) and is continuing to investigate possible reported individual cases and clusters(77). They consider the etiology of AIPHI to be unresolved.

Additional reports of IPH in infants

Other reports of IPH in infants have also been published. In Chicago from April 1992 to November 1994, 7 cases of IPH in infants were seen(78). Six were African-American and 4 were male. All were previously healthy and presented in respiratory distress with bilateral pulmonary infiltrates. Only one was breastfed. No geographic clustering was seen. Bronchoscopy did not show hemosiderin macrophages. As mentioned above, exposure to mold or water damage was not associated with this cluster of cases. Prior to the Cleveland cluster, individual cases of idiopathic pulmonary hemorrhage have been reported, usually in infants <3 months old without known precipitating factors, and these often did not recur(79-81). Subsequent to the Cleveland study, there have been individual reports of infants with IPH associated with mold

growth in their living environments, but not all with *Stachybotrys*(82-85). IPH in infants may in fact have multiple etiologies, and clearly needs further study.

Elidemir et al reported a case of a 7 year old male with chronic cough, fatigue and recurrent pneumonia who was found to have a RLL infiltrate(86). Bronchoscopy showed hemosiderin macrophages and culture of bronchoalveolar lavage (BAL) fluid80 grew *Stachybotrys*. Environmental assessment of his home showed *Stachybotrys* under a sink near his bedroom, though no airborne samples were taken. After remediation, symptoms resolved. This has been cited as a case of IPH, though clinically it is very different from IPH in infants, and is more suggestive of pneumonitis.

Animal studies and Stachybotrys

There are animal studies with *Stachybotrys* that may support its role in causing pulmonary hemorrhage. In a study of mice, 10^6 spores of *Stachybotrys* were given intranasally(87). A highly toxic strain and a minimally toxic strain as determined by toxin production in vitro were used. The mice given saline and the minimally toxic strain were asymptomatic and no pathologic changes were seen, though the group given toxic spores showed severe inflammation and a hemorrhagic exudate, with 2 animals dying within 24 hours. Of note, adjusting for a 70 kg adult, that would be an inhaled dose of $3.5x10^9$ spores, unlikely to be seen clinically. Another similar study gave mice 10^5 and 10^3 *Stachybotrys* spores intranasally 6 times over a period of 3 weeks(88). No clinical symptoms were observed, though the mice given the higher dose had evidence of inflammation and hemorrhage on histopathology. No antibodies were found to *Stachybotrys* in any mice. In another study, *Stachybotrys,* satratoxin (a trichothecene), and *Cladosporium* were given to mice(89). No clinical effects were seen, though abnormal alveolar cells were present after 48 hr in mice given *Stachybotrys* and satratoxin. Interestingly, the same dose of *Stachybotrys* as a previous study (10^6 spores) was not symptomatic in this study, perhaps reflecting differences in strains. Another study with even higher doses showed similar findings(90). Studies of continuous, chronic exposure have not been done to date, and potential species variability in the effects of mycotoxins should also be considered in interpreting the above data.

In vitro studies and Stachybotrys

In a study of the *Stachybotrys* strains isolated from case and control homes from Cleveland, both were found to produce TCTs(91). Hemolysins have also been found to be produce by *Stachybotrys*. In a study by Vesper, it was suggested that case strains from Cleveland produced hemolysins more frequently and consistently than control strains(92). However, this was found at 37ºC, not room temperature, which is more relevant. At room temperature, all strains except one were hemolytic at some point during an 8 week incubation, though not consistently. In a recent study, 50 strains of *Stachybotrys* were examined for toxin production(93). About 2/3 produced a newly discovered class of compounds called atranones, ~1/3 produced TCTs, and <10% produced neither. It is unclear whether atranones are as toxic as TCTs.

Guidelines for future studies of airborne toxigenic fungi

Given the paucity of reliable data on the issue of airborne exposure to toxigenic fungi, Burge suggested several factors to consider in future studies(94). These include: 1) there are no guidelines for the amount of fungal growth that may cause illness in humans, 2) toxin production depends on multiple factors, 3) likelihood of airborne dissemination needs to be assessed (i.e. *Stachybotrys* does not become airborne easily), 4) fungal bioaerosols are variable over time, 5) toxin exposure should be quantified (i.e. amount of toxin/gm spores or 10^6 spores, etc.), 6) time of exposure, 7) amount of toxin reaching various organs, 8) amount of toxin needed for effect, 9) species variability of effects. Additional considerations were that many studies ignore the presence of other fungi, usually present in much higher concentrations than *Stachybotrys*, and that high exposures are probably necessary acutely, which are unusual outside of agricultural settings. Other reviews also suggest caution in interpreting the current data with respect to *Stachybotrys* and human health(9,95,96).

Final recommendations

The American Academy of Pediatrics released guidelines in 1998 on indoor molds and child health(97). They concluded that this was an evolving field, but that until more is known, pediatricians should consider recommending that infants < 1 year old should not be exposed to chronic, moldy and/or water damaged environments. Pediatricians should also ask about mold and water damage exposure for infants found to have pulmonary hemorrhage, and that these infants should not be exposed to tobacco smoke.

The New York City Department of Health "Guidelines on Assessment and Remediation of Fungi in Indoor Environments" is a widely cited publication (nyc.gov/html/doh/html/epi/moldrpt1.html). It suggests that visual inspection is critical when assessing for fungal growth. Surface or air sampling for fungi is not necessary unless evaluating for a specific medical condition, symptoms or diagnosis. Normal or safe levels of indoor fungi are not known. Recommendations for remediation include 1) correct underlying cause of water damage, 2) clean non-porous surfaces with detergent and discard porous materials unless able to reliably clean them, and 3) use appropriate equipment (i.e. respirator, body suit) according to size of area affected. Infants < 12 months old with dyspnea and living in 'moldy' homes should be considered to be evaluated for idiopathic pulmonary hemorrhage.

The CDC has guidelines (www.cdc.gov/nceh/airpollution/mold/stachy.htm) on *Stachybotrys* and other molds that suggest toxigenic molds be considered the same as other molds, in that all should be removed from the environment. No special precautions are recommended for *Stachybotrys*. Mold growth should be cleaned with a weak solution of bleach, and water damage promptly remediated to prevent mold growth. Other recommendations are 1) keep humidity <50%, 2) use an air conditioner during humid months, 3) maintain adequate ventilation indoors, 4) clean bathroom with mold killing products, 5) do not carpet bathrooms, and 6) remove and replace flooded carpets.

The concern over toxigenic molds has grown dramatically in recent years, prompted by reports linking infant pulmonary hemorrhage to *Stachybotrys* exposure and fueled by extensive media coverage. While many case studies are suggestive of an association between airborne exposure to toxigenic molds and disease, conclusive evidence is lacking, due in part to the inherent difficulties in conducting such studies in a rigorous manner. Further developments in measuring and assessing exposure to airborne fungi and mycotoxins will be required to conduct such studies appropriately. What has not been given as much attention is the effect of mycotoxins on human health through contamination of a variety of staple foods. The known and potential health effects from foodborne exposure to mycotoxins appear to be much more widespread and could affect most human populations, compared with airborne exposure. This is an area that deserves attention as well as sustained research efforts. Well designed and rigorously controlled studies are needed to further clarify these complex issues.

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