CHAPTER 46

TOXIGENIC FUNGI IN THE INDOOR ENVIRONMENT

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46.1 INTRODUCTION

Intense scientific and public scrutiny has focused on hazards associated with exposure to fungi, especially in enclosed environments. Fungi are well known as agents of infection (e.g., histoplasmosis, aspergillosis), allergic disease (e.g., asthma, hypersensitivity pneumonitis), and toxicoses (e.g., tremogenicity, aflatoxicoses, ergotism) (Baxter et al. 1981, Brown et al. 1998, Burge 1985, Dvorackova 1976, Garrett et al. 1998, Juchet et al. 1998, Land et al. 1987, Robertson et al. 1987). Fungi also produce malodorous volatile organic compounds that may cause physical irritation (Pasanen et al. 1998, Walinder et al. 1998). The fungal cell wall is composed primarily of chitin fibrils embedded in a matrix of β-(1 → 3)-D-glucans. Glucan exposure may exacerbate the infectious, allergic, and toxic reactions to fungi (Burge 1989, Flannigan et al. 1991, Rylander et al. 1992). Exposure to mycotoxins in indoor air has become of particular concern because of the potential for both acute and chronic health effects (Flannigan 1987, Hendry and Cole 1993, Jarvis 1990, Miller et al. 1988, Morey 1993, Sorenson 1990, Tobin et al. 1987). The potent health effects elicited in laboratory animals and information from anecdotal case studies have fueled the anxiety. This chapter discusses mycotoxins in general and the fungal species *Stachybotrys chartarum* (syn. *S. atra*) in particular. Although other toxin-pro-

ducing species of fungi can also be found in the indoor environment (Tobin et al. 1987), *S. chartarum* has been the most public, because of the severity of the reported symptoms and the population affected.

46.2 MYCOTOXINS

Throughout history, mycotoxins have played an important role in human and animal health. With its distinctive and grisly symptoms (gangrene, limbs falling off, convulsions, and death), *ergotism*, also known as "Saint Anthony's fire," has been documented as far back as 430 B.C. Ergotism is caused by ingestion of rye products contaminated with a fungus (*Claviceps purpurea*) that produces *ergot alkaloids*, potent mycotoxins affecting the smooth muscles and the central nervous system. However, the pivotal event in the recognition of mycotoxins as a serious cause of animal and human disease occurred in the 1960s with an outbreak of "turkey X disease" in England. The common factor was that affected livestock had been fed Brazilian peanut meal which had been highly contaminated with the fungus *Aspergillus flavus*. Using modern analytic methods, scientists isolated a series of toxins from the meal, which were subsequently named *aflatoxins*. Aflatoxins were shown to be acutely toxic and highly carcinogenic to laboratory animals.

Mycotoxins are secondary metabolites that are derived from a few precursors formed during metabolism (Betina 1989, Jarvis 1989). They have no molecular features in common, and chemical structures range from the simple monilliformin ($C_4H_2O_3$) to complex polypeptides with molecular weights over 2000 daltons (Bu'lock 1980). Mycotoxins are not volatile (Hendry and Cole 1993, Pasanen et al. 1993). It is commonly believed that not all mycotoxins have been identified yet, since more are being discovered as analytic methods are developed.

Mycotoxins are natural products usually produced in response to environmental stimuli only by specific groups of organisms and only during part of their life cycle (Kendrick 1992). The toxins apparently are not necessary for fungal growth, and their exact function has not been clearly established (Bennett and Deutsch 1985, Demain 1989, Lillehoj 1982). They may play a role in regulating competition with other organisms since many mycotoxins inhibit the growth of surrounding microorganisms in culture (Bean and MacFall 1982, Butt and Ghaffar 1972, Janzen 1977, Northolt and Bullerman 1982, Wicklow 1981, Williams et al. 1989).

Particular mycotoxins can be produced by more than one fungal species, or even fungal genus, and conversely, a fungal species typically can produce more than one mycotoxin (Table 46.1). Often, a mycotoxin can elicit more than one type of toxic effect (e.g., antiviral, antibacterial, antifungal, immunosuppressive, carcinogenic, mutagenic, cytotoxic, teratogenic, and neurotoxic), and the degree of toxicity (relative potency) varies greatly.

46.3 EXPOSURE ASSESSMENT

Production of Mycotoxins in the Indoor Environment

Some species of fungi can produce mycotoxins on various building substrates (Larsen and Frisvad 1994, Nielsen et al. 1998, Nikulin et al. 1994). Although the majority of individual strains of toxigenic fungal species are genetically capable of toxin production, factors controlling the expression of this potential are not well understood (Lillehoj 1982, Tantaoui-Elaraki

TABLE 46.1 Some Mycotoxins and Associated Effects, Fungal Species, and Occurrences

Mycotoxin	Health effects*	Some toxin-producing fungal species	Known occurrences
Aflatoxins	Carcinogenic, mutagenic, acute toxicity	*Aspergillus flavus, A. parasiticus*	Peanuts, beans, milk, grains
Citrinin	Nephrotoxicity	*Penicillium fellutanum, P. viridicatum, P. citrinum*	Rice
Ergot alkaloids	Gangrenous, convulsive, hallucinogenic	*Claviceps purpurea, C. paspali, C. fusiformis*	Rye
Fumonisin	Carcinogenic	*Fusarium moniliforme*	Corn
Ochratoxins	Nephrotoxicity, carcinogen	*Aspergillus ochraceus, Penicillium viridicatum*	Barley, oats, rice, coffee
Patulin	Antibacterial, carcinogenic, mutagenic, neurotoxicity	*Penicillium expansum, P. urticae, P. patulum, P. roquefortii; Aspergillus clavatus*	Apples, pears
Penitrem A	Tremorgenic	*Penicillium cyclopium*	Grains, grasses
Sporidesmin	Hepatotoxicity	*Pithomyces chartarum*	Forage grasses
Sterigmatocystin	Carcinogenic	*Aspergillus versicolor, A. flavus, A. amstelodami, A. nidulans; Chaetomium theilavioideum*	Rice, corn
Tenuazonic acid	Nephrotoxicity, hepatotoxicity	*Alternaria alternata*	Apples, tomatoes
Trichothecenes: T-2 Toxin, Roridins, Satratoxins, Verrucarins, Verrucarrols, Trichoverrols, Deoxynivalenol	Emetic, hemorrhagic, acute toxicity, alimentary toxic, aleukia, skin necrotization, neurotoxicity	*Fusarium poae, F. sporotrichioides; Stachybotrys chartarum; Myrothecium roridum, M. verrucaria; Trichoderma viride; Dendrodochium toxicum; Cephalosporium crotocinigenum; Cylindrocarpon* sp.; *Memnoniella echinata*	Corn, hay, rice, wheat, wallpaper
Zearalenone	Estrogenic	*Fusarium graminearum, F. oxysporum, F. solani*	Corn

*Documented in animals and/or humans via ingestion, inhalation or dermal contact.

Sources: Busby and Wogan (1970), Hawksworth et al. (1995), Kendrick (1992), Smith et al. (1995), Udagawa et al. (1979), Ueno (1983), Vesonder and Horn (1985).

1992). Mycotoxin production is highly dependent on fungal species and strain, environmental conditions (e.g., temperature cycling, water activity, light, presence of other microorganisms), and growth substrate (Bennett and Deutsch 1985, Bennett et al. 1981, Buckle and Sanders 1990, Butt and Ghaffar 1972, El-Kady and Moubasher 1982, Faraj et al. 1991, Joffee and Lisker 1969, Northolt and Bullerman 1982). Thus, growth of toxigenic fungal species in an indoor environment will not necessarily indicate whether mycotoxins are present. An isolate may produce high levels of toxin in its natural state but yield only low levels in the laboratory. Conversely, an isolate may produce high levels in the laboratory, but not in the field. In addition, some toxigenic isolates can lose (or gain) the ability of mycotoxin production over time under artificial culture conditions (Mayne et al. 1971, Tantaoui-Elaraki 1992). Therefore, the identification of a fungus as a "toxigenic species" is a guide to the type of mycotoxins that might be produced, but does not establish the presence of those toxins. Verification of toxins remains a matter for analytic chemistry (Bu'lock 1980).

Exposure to Mycotoxins

Toxigenic fungi can accumulate mycotoxins in the fungal spores and the fungal filamentous structure (mycelia) and can excrete mycotoxins into the growth substrate. Mycotoxins have not been shown to be volatile (Pasanen et al. 1993). Toxin partitioning between the spores, mycelia and growth substrate is highly dependent on fungal species and strain and environmental conditions (Palmgren and Lee 1986, Sorenson et al. 1987, Wicklow and Shotwell 1982). The primary routes of indoor environmental exposure for mycotoxins are most likely dermal and inhalational. Significant exposures probably occur when fungal spores, fungal mycelia, and contaminated growth substrate are aerosolized, especially as a result of handling of moldy material.

Analytic Methods for Detecting Mycotoxins

The currently available analytic methods were designed for agricultural products, and the crossover to uses in the indoor environment may be problematic in some instances (e.g., air samples with low concentration and low bulk). Analytic methods include analytical chemistry techniques, immunoassays and bioassays (e.g., tissue culture, cell culture, lethality testing in animals) (Table 46.2) (Babich and Gorenfreund 1991, Chu et al. 1984, Gilbert 1993, Krishnamurthy et al. 1989, Panigrahi 1993, Visconti et al. 1991). Extraction using organic solvent and mechanical disruption of samples, sample purification to prevent interference from other compounds, detection and determination, and chemical confirmation are the routine in mycotoxin analyses (Scott 1995).

Analytic Chemistry Methods. The necessary reference standards, equipment (which can be very costly), and technical expertise required for analytic chemistry analyses may not be available to most bioaerosol laboratories, which somewhat limits the use of these techniques. Also, the results from these methods seldom give a complete description of the toxic capabilities of the fungus. Many mycotoxins are still unidentified (therefore no reference standard is available), and even less is known about the effects of mycotoxin mixtures (fungi often produce more than one mycotoxin at a time).

Immunologic Methods. Immunoaffinity chromatography is a relatively new technology that uses highly specific antibodies to separate mycotoxins from a sample (as opposed to organic solvent fractionation and cleanup). The commercially available assay kits are sensitive, rapid, and easy to use. However, commercial assays exist for only a few agriculturally

TABLE 46.2 Methods to Detect, Identify, and/or Quantify Mycotoxins

Method	Assay	Advantages	Disadvantages
Analytic chemistry techniques	Thin-layer chromatography (TLC)	Relatively simple	Insensitive Susceptible to interference compounds Qualitative
	High-performance liquid chromatography (HPLC) Tandem mass spectrometry (MS/MS) Gas chromatography, liquid chromatography or supercritical fluid chromatography in combination with mass spectrometry (GC/MS, LC/MS, and SFC/MS)	Quantitative Identification	Specialized equipment and expertise Reference standards
Immunoassays	Enzyme-linked immunosorbent assay (ELISA) Radioimmunoassay (RIA)	Quantitative Specific Sensitive Realtively simple	Commercial kits available only for some mycotoxins
Bioassay	Toxicity testing Lethality Functional changes Morphological changes Mutation	Simple Rapid	Nonspecific Qualitative

significant mycotoxins (i.e., aflatoxin, ochratoxin, zearalone, T-2, deoxynivalenol, and fumonisins). The accuracy of some of the kits are comparable to HPLC analysis (Dorner and Cole 1989).

Biological Assays. As the detection of many mycotoxins by analytic chemistry methods is not adequate at present and the feasibility of testing for all known mycotoxins chemically is not practical, biological assays have been employed as a detection (qualitative) method (Buckle and Sanders 1990, Robb et al. 1990). Biological assays have been developed for detection of mycotoxins in food and feed using terrestrial animals (e.g., chick embryo, rabbit skin, insects), aquatic animals (e.g., brine shrimp, trout), organ and tissue culture (e.g., fibroblasts, liver cells, lung cells, fetal cells, tracheal explants), microorganisms (e.g., yeasts, bacteria, other fungi), or plants (e.g., seeds) (Abbas and Shier 1984, Babich and Gorenfreund 1991, Madhyastha et al. 1994, Panigrahi 1993). Although there have been attempts to increase the quantitative capability of bioassays (by different cleanup methods), the most feasible purpose is primarily as a qualitative screening method.

Indoor Concentrations and Normal Ranges

Fungi are a common contaminant in the indoor environment. It is the amplification of fungi and dissemination of fungal spores in high concentrations that are unusual. Most fungal species found in the indoor environment have no intrinsic ejection mechanism for spore dispersal, and aerosolization of spores from a surface is determined mostly by physical, not

biological, constraints. Aerosolization is highly dependent on water content of the substrate and physical disturbance (Ward et al. 1995, Wilkins et al. 1998). Thus, aerosolization usually occurs only when surfaces or reservoirs supporting growth are mechanically disturbed (e.g., remediation work).

Virtually all reported data on fungal concentrations in air are based on culture of air samples. Therefore, recovery is dependent on the culturability of the spores, which is probably independent of toxin content of the spores. Neither fungal growth on a surface nor the presence of airborne culturable spores is a reliable indicator of toxin presence.

Given these limitations, there are several published guidelines intended to limit exposures to toxigenic fungi in the indoor environment (Rao et al. 1996). Acceptable levels of fungi range from "presence not acceptable" to less than 1000 colony forming units (CFU/m^3 (Eastern New York Occupational Health Program 1995, Nathanson 1993, World Health Organization 1988). In addition, environmental controls have also been recommended in order to decrease the probability of fungal amplification (e.g., moisture control) (American Society of Heating, Refrigerating, and Air-Conditioning Engineers 1999, Maroni et al. 1995, Standards Australia 1989, U.S. Environmental Protection Agency 1993).

46.4 HEALTH EFFECTS FROM MYCOTOXIN EXPOSURES

Route of Exposure: Inhalational

The most commonly encountered mycotoxins have health effects that include carcinogenicity, induction of tremors, or damage to the immune system or major organs (Table 46.1) (Corrier 1991, Hendry and Cole 1993). Most documented cases of human mycotoxicosis have appeared in rural or agricultural settings as a result of ingestion of contaminated food and/or from skin contact (Akkmeteli 1977). In vitro testing of human skin indicates slow absorption that would not be likely to lead to systemic effects. However, risk of systemic toxicity increases in the presence of high toxin concentration, prolonged dermal exposures, and vehicles that enhance penetration (such as an organic solvent) (Kemppainen et al. 1988/89). Most risk of systemic effects associated with exposure to aerosolized toxin-containing particles is thought to occur through inhalation exposure (Creasia et al. 1990, Jarvis et al. 1989). However, evidence that relates disease to the inhalation of mycotoxins is limited. Some anecdotal human data have indicated that acute, high-level inhalation of mycotoxins can have serious health effects (Dvorackova 1976, Dvorackova and Pichova 1986, Hintikka 1978, Land et al. 1987, Samsonov 1960). Data are limited on chronic, low-level inhalation exposures.

Aflatoxins are produced by *Aspergillus parasiticus* and *A. flavus*. The International Agency for Research on Cancer (IARC) has classified aflatoxins as a group 1 (i.e., sufficient evidence) human carcinogen based primarily on ingestion exposures (International Agency for Research on Cancer 1993). Epidemiologic studies correlate aflatoxin exposures in farming and food processing settings to various forms of cancer (Baxter et al. 1981, Burg et al. 1981, Hayes et al. 1984, Olsen et al. 1988). Anectodal evidence has linked the presence of *Aspergillus flavus* in the environs or the presence of *A. flavus* precipitins in patient serum to neoplastic diseases, interstitial lung diseases, and leukemia (Aleksandrowicz and Smyk 1973, Dobrowolski and Smyk 1993, Lougheed et al. 1995, Wray et al. 1982). However, because of the issues in assessing airborne exposure, a definitive inhalational causal relationship has yet to be established (Akkmeteli 1977, Smith et al. 1995). The development of detection methods for aflatoxin adducts in blood and urine has been useful in characterizing dietary exposures (Scholl et al. 1995, Strickland and Groopman 1995).

Some surveys of "cytotoxic spores" in domestic environments used fungal spores cultured on laboratory media (which probably is not indicative of toxin presence in the natural environment) to characterize exposure. No connection was made to any health effects (Lewis et al. 1994, Smith et al. 1992). In order to correlate specific mycotoxin exposure to indoor air quality problems, identification and evaluation of health effects from exposure to mycotoxins in indoor air are essential.

Experimental Evidence of Mycotoxin-Related Health Effects

Laboratory studies have indicated that inhalation of mycotoxins can elicit adverse health effects (Bunner 1987, Creasia et al. 1987, DiPaolo et al. 1993, Sorenson et al. 1986, Thurman et al. 1988). T-2 toxin was 2 to 20 times more toxic when inhaled than when injected interperitoneally or applied dermally. However, the types of systemic effects were similar regardless of route of exposure (Creasia et al. 1987, 1990). T-2 toxin, patulin, penicillic acid, and aflatoxin can interfere with rat alveolar macrophage function and with normal immune responses in the lung (Gerberick et al. 1984, Jakab et al. 1994, Richards and Thurston 1975, Sorenson et al. 1986, Sorenson and Simpson 1986). This may help explain the opportunistic bacterial infections that are often associated with chronic trichothecene intoxication in livestock (Harrach et al. 1983, Schneider et al. 1979).

It has been hypothesized that mycotoxins associated with spores are likely to be absorbed via the respiratory epithelium and translocated to other sites, possibly producing systemic effects (Flannigan et al. 1991, Tobin et al. 1987). However, most laboratory studies focus on exposures to a single purified mycotoxin dissolved in a solvent, even though these exposures are unlikely to accurately characterize health effects of inhaled dry spores (Nikulin et al. 1997). Synergistic, additive, or antagonistic effects of multiple mycotoxin exposures may occur, depending on the combination of mycotoxins and the concentration ratios (Koshinsky and Khachatourians 1992, Madhyastha et al. 1994, Ohff et al. 1985, Schiefer et al. 1986, Thompson and Wannemacher 1986). Also, particulate association of mycotoxins may amplify adverse effects in the animal lungs, possibly as the result of an increase in respiratory tract retention of the mycotoxin (Coulombe et al. 1991).

Doses of mycotoxins that cause specific toxic effects vary with the mycotoxin, the experimental animal species, and the route of administration. Some of the trichothecene toxins (produced by species of *Fusarium, Acremonium, Trichoderma, Myrothecium, Stachybotrys*) are characterized by ingestion LD_{50} levels (lethal dose for 50 percent of exposed animals) well below 1 mg/kg body weight (Schiefer et al. 1989). Health effects of human exposure to nonmacrocyclic trichothecenes have been documented in clinical cancer trials. Intravenous injection of anguidine or diacetoxyscirpenol in doses ranging from 0.2 to 6.0 mg/m^2 body surface area (0.005 to 0.154 mg/kg body weight) commonly resulted in nausea and vomiting. Central nervous system and gastrointestinal tract disturbances were less commonly observed (Goodwin et al. 1978).

46.5 STACHYBOTRYS CHARTARUM

The Fungus *Stachybotrys chartarum*

Species Description. The genus *Stachybotrys* is in the anamorph class Hyphomycetes and was first described by Corda in 1837 (Eppley 1977, Kendrick 1992). The genus is defined by the production of black phialospores produced successively and collecting in a mucilaginous mass at the apex of clustered, inflated phialides. There are approximately 50

species in the genus. *Stachybotrys chartarum* (syn. *S. atra*) is characterized by relatively large ovate spores with smooth to irregularly warty walls. The reported spore size range is 3 to 4 × 7 to 10 μm with extensive variation among different isolates (Korpinen and Uoti 1974, Malloch 1981, Moreau 1974).

Ecology. *Stachybotrys chartarum* is a cellulose-decaying fungus with a worldwide distribution. It has been found on soil, paper, vegetable debris, straw, grains, and wet building materials (Croft et al. 1986, Grant et al. 1989, Hunter et al. 1988, Moreau 1974). The fungus grows optimally at room temperatures when the relative humidity is RH >93% (Forgacs 1972, Grant et al. 1989). Sunlight suppresses mycelial formation but increases spore formation (Bakai 1960).

Recovery in the Indoor Environment. *Stachybotrys* species grow slowly when cultured with other fungi. Although able to inhibit the growth of other fungi, *Actinomycetes* and other bacteria, *S. chartarum* can compete with fungi such as *Penicillium* and *Aspergillus* only on specialized culture media (e.g., high cellulose, low sugar and nitrogen) (Butt and Ghaffar 1972, Hunter et al. 1988, Jarvis 1990, Tobin et al. 1987). In bulk samples, the number of *S. chartarum* colonies detected and the frequency of recovery (*S. chartarum* colonies/total fungal colonies recovered) on cellulose agar can be up to two orders of magnitude higher than on glucose agar (Abdel-Hafez and Shoreit 1985, Abdel-Hafez et al. 1986).

S. chartarum spores are hardy and can survive for long periods at temperatures as low as −40°C. It has been estimated that up to 90 percent of field-collected spores may not be culturable (Miller 1992). However, unpublished data from our laboratory indicate >90 percent germination of spores from fresh cultures.

The Mycotoxins of *Stachybotrys chartarum*

Background. *Stachybotrys* toxins are macrocyclic trichothecenes, a group of chemically related fungal metabolites produced by various species of *Fusarium, Trichothecium, Trichoderma, Acremonium (= Cephalosporium), Cylindrocarpon,* and *Myrothecium,* as well as *Stachybotrys* (Grove 1993; Jarvis et al. 1986, 1995; National Academy of Science 1983).

The Nature of Trichothecenes. *Trichothecenes* are sesquiterpene alcohols or esters, derived from a common tricyclic skeleton (trichothecane). The colorless, crystalline, optically active solids are sparingly soluble in water and soluble in all common organic solvents at room temperature (Nummi and Niku-Paavola 1977). The skeletal structure includes a six-member oxygen-containing ring, an epoxide group and an olefinic bond. Trichothecenes are classified into four types (A to D) according to their structural characteristics (Grove 1993, National Academy of Science 1983). Macrocyclic trichothecenes (type D) contain a macrocyclic ester or an ester-ether bridge. As of 1991, 172 trichothecenes have been isolated from natural sources, 67 of which are macrocyclic (type D) (Grove 1993).

***The Nature of Trichothecenes Produced by* S. chartarum.** At least eight major macrocyclic trichothecenes have been identified from *S. chartarum* isolates: satratoxins F, G, and H; roridin E; verrucarins B and J; and trichoverrols A and B (Croft et al. 1986; Grove 1993; Harrach et al. 1982; Jarvis et al. 1986, 1995; Pohland 1977; Stack and Eppley 1980). However, it is likely that there are others yet undiscovered. For example, 5 g of *S. chartarum*-contaminated rice culture was lethal to a 40-kg ram. However, the rice culture contained less than 1 mg of total macrocyclic trichothecenes (Jarvis 1991). Given the much

higher LD_{50} values reported for satratoxins H and G (Glavits 1988, Yoshizawa et al. 1986), it is likely that additional toxins produced by *S. chartarum* were present in the culture.

Not all strains of *S. chartarum* can produce mycotoxins in detectable amounts, at least in a laboratory setting (Korpinen and Uoti 1974, Ohff et al. 1985, Sorenson et al. 1987). In surveys of *S. chartarum* strains implicated in mycotoxicoses, not all strains were toxic in tissue culture assays (Korpinen and Uoti 1974) or produced detectable trichothecenes (Bata et al. 1988, Sorenson et al. 1987). Therefore, the presence of *S. chartarum* growth is not proof of toxin presence (Jarvis et al. 1986, Korpinen and Uoti 1974, Pasanen et al. 1993). Only rarely are other *Stachybotrys* species (*S. cylindrospora, S. albipes, S. kampalensis,* and *S. microspora*) reported to produce trichothecenes (Grove 1993, Jarvis et al. 1995).

Experimental Evidence of the Effects of* S. chartarum *Mycotoxins. Table 46.3 summarizes the experimental data on health effects of macrocyclic trichothecenes. Trichothecenes are potent inhibitors of protein and DNA synthesis. They interact with ribosomes (binding the ribosomal peptidyl transferase) and interfere with the initiation and elongation events (Murty et al. 1985, Ueno 1983). The biologically active structure is the epoxide group (Jarvis and Mazzola 1982, Ong 1982). All trichothecene mycotoxins are capable of inducing immunosuppression, skin necrotization, vomiting, leucocytosis, and leukopenia in experimental animals (Ueno 1983, Uraguchi and Yamazaki 1983). Macrocyclic trichothecenes are more toxic (more potent) than the type A or B trichothecenes (e.g., T-2, verrucarol, deoxynivalenol) (Jarvis and Mazzola 1982). The macrocyclic trichothecene esters (verrucarins and roridin derivatives) are the most toxic nonnitrogen natural products known (Bata et al. 1988, Bergmann et al. 1989, Eppley 1977, Glavits 1988, Jarvis and Mazzola 1982, Ong 1982, Pestka and Forsell 1988).

Aerosolization of Mycotoxin-Containing* S. chartarum *Spores. *S. chartarum* toxins can accumulate in spores, mycelia, and in the growth substrate. Because the spores are hydrophilic, they tend to agglomerate and are aerosolized most readily under dry conditions with physical agitation. In a chamber study, *S. chartarum* particles (aerosolized by acoustic vibration) consisted of 85 percent spores and 6 percent hyphal fragments (mass median aerodynamic diameter <5 μm). Satratoxin H was found in concentrations ranging from 6.8 to 12.7 ng/mg dust. Satratoxin G and trichoverrols A and B ranged from none detected (limit of detection = 50 ng) to 6.9 and 4.5 ng/mg dust, respectively (Pasanen et al. 1993, Sorenson et al. 1987). However, in situ studies have shown that few spores are released from surfaces under environmental conditions which are likely to occur under normal occupancy conditions (Wilkins et al. 1998).

Human/*Stachybotrys* Interactions

Background. *S. chartarum* has been associated with animal intoxication and occasionally with human mycotoxicoses (Forgacs 1972, Hintikka 1978). Stachybotryotoxicosis was first diagnosed as a high-mortality disease of farm horses. Susceptibility to the effects of exposure seemed to be independent of nutritional status and age. The duration of disease, from time of detection of febrile reaction to death of an untreated horse, was 1 to 3 days (Shulyumov et al. 1960). The most common histological features reported in livestock are necrosis, aplastic anemia, and mucosal hemorrhage (Hintikka 1978, Schneider et al. 1979).

Occupational Exposures. Documented cases of inhalation exposure and subsequent health effects are rare. Reactions from concurrent dermal contact are the most commonly reported exposures. Accidental cases of trichothecene poisoning have been documented from laboratory dermal exposures. Symptoms included severe skin irritation, numbness,

TABLE 46.3 Summary of Experimental Data on Effects of Macrocyclic Trichothecenes

Toxin	Assay system	Mode of exposure	Measured outcomes	Results	Ref.
Verrucarin A	Mouse spleen cells	Cell culture	Interleukins	Low doses superinduces IL5; high doses inhibit all ILs	Ouyang et al. (1995)
Verrucarin A, Roridin A	Rat spleen lymphocytes	Cell culture	Protein synthesis inhibition	In vitro response not always accurate predictor of whole-animal lethality	Thompson and Wannemacher (1986)
S. chartarum extracts	Mammalian epithelial kidney cells	Cell culture	RNA concentrations	Reduction in RNA, inhibition of RNA synthesis	Bodon and Palyusik (1970)
Satratoxin G	Mice	Single inter-peritoneal injection	Histology, death	LD_{50}: 1.23 ± 0.08 mg/kg body weight	Yoshizawa et al. (1986)
Satratoxin H	Mice	Single inter-peritoneal injection	Histology, death	LD_{50}: 5.69 ± 0.43 mg/kg body weight	Yoshizawa et al. (1986)
Roridin A	Dogs	Single intra-venous injection	Cardiac measures	2 mg/kg: atrioventricular block, increased heart rate, hypotension	Bubien and Woods Jr. (1987)
S. chartarum spores	Mice	Single intra-nasal injections	Histology after 3 days	1×10^6 spores/ 50 μL PBS: inflammatory lung injury	Nikulin et al. (1997)
S. chartarum spores	Rats	Intratracheal instillation	Bronchoalveolar lavage	Dose-effect relationship, effects mycotoxin-related	Rao et al. (2000)
S. chartarum spores	Guinea pigs	Single intra-nasal spray	Histology	Inflammation of large bronchi, alveoli; cytotoxicity in lung, heart, liver	Samsonov and Samsonov (1960)

loss of sensitivity, and skin peeling. Patients recovered without sequelae (National Academy of Science 1983). Handlers of *Stachybotrys*-contaminated straw have reported cough, rhinitis, burning sensation in the mouth and nasal passages, and cutaneous irritation at the point of toxin contact. Rash, dermatitis, exudate, catarrhal angina with painful pharyngitis, bloody exudate from nose, fever (rare), moderate to severe cough, and leukopenia have been reported in some cases. The disease (clinical symptoms) developed within 2 to 3 days of contact with an average duration of 3 weeks (Hintikka 1978).

Indoor Exposures. *S. chartarum* is considered by some to present a serious health threat for individuals in contaminated indoor environments (Eastern New York Occupational Health Program 1995, Etzel et al. 1998, Miller 1992). One relatively well-defined case involved a home with heavy infestation of *S. chartarum* (Croft et al. 1986). Water damage had occurred in the house over a period of several years. Extensive fungal growth was evident on the ceiling of an upstairs bedroom and in the air ducts, and *S. chartarum* spores were collected from room air samples. The variety of symptoms reported by the occupants of this house (headaches, sore throats, hair loss, flu symptoms, diarrhea, fatigue, dermatitis, and generalized malaise) was generally consistent with the nonspecific nature of indoor air quality complaints (Tobin et al. 1987). Cleanup workers experienced skin and respiratory irritation. Verrucarins B and J, satratoxin H, and trichoverrins A and B were isolated from bulk samples of contaminated ceiling tile and airduct dust. Extracts of these samples were injected per os (orally) into rats and mice. Within 24 hours, all animals had died. Histology demonstrated degeneration, necrosis, and hemorrhage within the brain, thymus, spleen, intestine, lung, heart, lymph nodes, liver, and kidney. After the home was thoroughly cleaned, the occupants no longer suffered symptoms.

A highly publicized outbreak of pulmonary hemosiderosis occurred in a cluster of cases involving newborn babies living in damp, moldy homes in Cleveland (CDC 1997). A case-control study indicated that residing in water-damaged buildings positively correlated with the case patients (Montana et al. 1997) and that cases were more likely to live in houses with *S. chartarum* in the air (Etzel et al. 1998). The presence of toxins in the air was not confirmed. However, in a reanalysis of the medical and sampling data, the Centers for Disease Control have concluded "the evidence...was not of sufficient quality to support an association between *S. chartarum* and acute idiopathic pulmonary hemosiderosis" (CDC 2000).

S. chartarum has also been blamed for nonspecific symptoms often associated with complaints of poor indoor air quality (Cooley et al. 1998, Johanning et al. 1996, Sudakin 1998). However, as in the Cleveland outbreak, the presence of mycotoxins has seldom been confirmed, and exposure has been inferred from the presence of *S. chartarum* in bulk samples. The causal link between detection and identification of a toxigenic species of fungus in nonagricultural indoor environments and adverse health effects has yet to be definitively proved.

46.6 RISK ASSESSMENT

The process of risk assessment entails four major steps: hazard identification, exposure assessment, dose-response analysis, and risk characterization. Risk assessment is a tool that enables the public health community to determine what substances are risks, to determine at what levels the substances become risks, and to rank the importance of such risks. One specific regulatory outcome of the risk assessment process is the establishment of standards and guidelines of safe human exposure levels (Calabrese 1996, Malsh et al. 1994, U.S. Environmental Protection Agency 1995).

Standards for Mycotoxin Exposures

Official exposure limits exist only for mycotoxin concentrations in food and only for the best-known toxins such as aflatoxin, ochratoxin, and deoxynivalenol (Stoloff et al. 1991, van Egmond 1995). Some guidelines set limits for toxigenic fungi recovered in air (cfu/m^3), but the definition of "toxigenic" in these guidelines is ambiguous (Eastern New York Occupational Health Program 1995, Rao et al. 1996). Also, basing guidelines on culturable fungi may not be the best method of limiting risk of mycotoxin exposures since toxins can be found in nonviable particles (Miller 1992). For *S. chartarum* exposure, no official standards exist, but several different kinds of guidelines have been proposed. The presence of 10^3 to 10^4 *S. chartarum* spores/m^3 has been proposed as a level to initiate building evacuation (Eastern New York Occupational Health Program 1995). The presence in air of 10^3 *S. chartarum* spores/m^3 of air was proposed as a guideline by Miller (1992) on the basis of extrapolation from the Canadian Acceptable Daily Intake of anguidine (a non–*S. chartarum* trichothecene). Using published data (Sorenson et al. 1987) on the amount of toxin in bulk samples of *S. chartarum* spores, Burge (1996) estimated periods of time (ranging from 0.1 to 1100 days) necessary to accumulate 1 ng satratoxin at different spore concentrations in air (Burge 1996). Le Bars and Le Bars (1985) estimated that inhalation of 3 to 5 mg of spores could cause symptoms if the fungal strain is highly toxigenic. None of these guidelines is based on a step-by-step risk assessment process. Such a process entails collection of data regarding prevalence of the fungus, production of toxins, source strength, aerosolization parameters, and inhalational health effects. These data are currently not available for *S. chartarum* or any other fungal species.

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