Stachybotrys chartarum (or *S. atra* or *S. alternans*) [CAS No. 67892-26-6]

Review of Toxicological Literature

June 2004

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Abstract

Stachybotrys chartarum is a greenish-black mold in the fungal division Deuteromycota, a catch-all group for fungi for which a sexually reproducing stage is unknown. It produces asexual spores (conidia). The morphology and color of conidia and other structures examined microscopically help distinguish the species from other molds found in indoor air that may contaminate materials in buildings that have suffered water intrusion. *S. chartarum* may ultimately overgrow other molds that have also produced colonies on wet cellulosic materials such as drywall (gypsum board, wallboard, sheet rock, etc.). Because of the likelihood that it may produce toxic macrocyclic trichothecenes and hemolytic stachylysin (exposure to which may be associated with idiopathic pulmonary hemorrhage [IPH] in infants), *S. chartarum* exposure is of concern to the members of the general public whose homes and workplaces have been contaminated after water intrusion, to agricultural and textile workers who handle contaminated plant material, and to workers involved in remediation of mold-damaged structures. Dry conidia, hyphae, and other fragments can be mechanically aerosolized as inhalable particulates. Recent work on characterizing the numerous strains or isolates that have been recovered throughout the world has important applications on sampling and analysis for species identification in mold-contaminated buildings and on resolving apparent discrepancies in the environmental and toxicological literature. New assays based on polymerase chain reaction (PCR), immunoassays, and protein translation inhibition assays have improved the accuracy and speed in which *S. chartarum* can be identified and quantified. In addition, PCR-based methods and other genomic work are elucidating the differences among *S. chartarum* strains. Strains can be grouped into two chemotypes (basically those that produce macrocyclic trichothecenes and those that do not), and some strains have now been reassigned to a different species, *Stachybotrys chlorohalonata*. In addition, prior studies of exposure and environmental occurrence may need to be reevaluated with respect to their sampling and laboratory culturing methods. Because *S. chartarum* is slow growing, use of a nutrient-rich medium favors the growth of other mold species that are invariably present, consequently the actual concentrations of *S. chartarum* conidia may be seriously underreported.

Recent epidemiologic investigations have suggested an association between exposure to *S. chartarum* and toxic inflammatory effects in infants, in courthouse workers, and in office workers, as well as between *S. chartarum* and extreme chronic fatigue syndrome in hospital workers. The Centers for Disease Control and Prevention concluded that evidence from current studies does not support a relation between acute PH or hemosiderosis and exposure to *S. chartarum*. Sick building syndrome and the intensification of asthma have also been linked with elevated indoor levels of *Stachybotrys*; however, there is not sufficient evidence to substantiate this association. It is generally accepted that building-related asthma and an increased incidence of upper respiratory disease are associated with living or working in a moldy environment; these associations, however, are with molds that comprise many species.

S. chartarum was reported to induce sensory irritation, inflammatory, and/or pulmonary responses in mice and rats exposed via intranasal instillation, intratracheal instillation, and inhalation. In one reproductive toxicity study, *Stachybotrys* caused a decrease in the percentage of pregnant mice. Additionally, statistically significant differences were seen in the frequency of dead, resorbed, and/or stunted fetuses and in the average litter size of live fetuses in treated animals compared to controls. Cytotoxic effects *in vitro* included induction of tumor necrosis factor-α and interleukin-6 production. Changes in total protein, albumin, pro-inflammatory cytokine, and lactate dehydrogenase in bronchoalveolar lavage fluid were observed in mice exposed to *S. chartarum*.

Executive Summary

Nomination

Mold present in indoor environments was nominated for toxicological studies by a private individual. This nomination reflects a broad public concern regarding the non-infectious adverse health effects of fungal exposures. In May 2004, an Institute of Medicine (IOM) committee concluded that there is sufficient evidence of an association between mold and other factors related to damp conditions and several respiratory ailments: upper respiratory tract symptoms, cough, wheeze, hypersensitivity pneumonitis in susceptible persons, and asthma symptoms in sensitized persons. However, the IOM committee concluded that there was inadequate or insufficient evidence to make conclusions for many other health outcomes, including rheumatologic and other immune diseases, neurological symptoms, cancer, and reproductive effects. Notably, the committee determined that none of these health outcomes met the definitions for the category of "limited or suggestive evidence of no association." The committee also identified numerous research needs. Along with recommendations for more research in exposure assessment and standardization of definitions (e.g., "dampness"), the committee recommended that animal studies be initiated to evaluate the effects of chronic exposures to mycotoxins (i.e., poisons produced by a fungus) via inhalation. Such studies should establish dose-response, lowest observed adverse effect levels, and no observed adverse effect levels since presently available studies are of acute, high-level exposure. Since it is likely that if non-infectious effects are occurring, they are a result of exposure to fungi and not solely to specific mycotoxins, the National Institute of Environmental Health Sciences (NIEHS) Nominations Faculty recommended that toxicological studies be considered for the whole organism.

Although many species of fungus are found in indoor environments, *Stachybotrys chartarum* (also known as *S. atra* and *S. alternans*) was chosen as the representative fungus for toxicological studies because it has received considerable attention in the mass media. Results from some studies suggesting that exposure to water damaged building materials contaminated with *S. chartarum* in the home, school, or workplace may be associated with idiopathic pulmonary hemorrhage (IPH) in infants, sick building syndrome and toxic inflammatory effects in workers has engendered substantial public concern. These concerns, together with the fact that little toxicological data are available from studies evaluating potential systemic toxicity from long-term exposure to this organism under relevant exposure scenarios, support the consideration of *S. chartarum* as a representative fungus for toxicological studies.

Physical Description, Detection and Environmental Occurrence

S. chartarum is a greenish-black mold in the fungal division Deuteromycota, a catch-all group for fungi for which a sexually reproducing stage is unknown. *S. chartarum* is capable of producing mycotoxins that include both macrocyclic tricothecenes (e.g., satratoxins G and H, roridins A and E) and nonmacrocylic tricothecenes (e.g., trichodermol, trichodermin, trichoverrins, and roridin L-2). *Stachybotrys* is easily identified by its morphology, and almost two-thirds of *S. chartarum* isolates produce mycotoxins. Many strains, regardless of geographic origin, produce the same mycotoxins. When dry and physically disturbed, spores produced by *S. chartarum* (referred to as conidia) may become aerosols.

Strain characterization: *S. chartarum* isolates are generally divided into two chemotypes: those that produced macrocyclic trichothecenes (chemotype A) and those that produce atranones (chemotype S), to which no significant biological activity has been ascribed. About 40% are producers of macrocyclic trichothecenes and about 60% of the strains classified as *S. chartarum* are atranone producers. The isolates belonging to the different chemotypes are genetically distinct as well, particularly with regard to the *Tri5* gene, and produce different patterns of volatile organic compounds.

Sampling, Spore Counting, and Culturing: Many sources state that *S. chartarum* is rarely isolated from air because the sticky masses of spores are not easily aerosolized or the spores die rapidly once released from the conidiophore (i.e., a specialized hyphal branch of the fungus that produces asexual spores). However, failure to isolate *S. chartarum* from air samples may be due to poor choice of collection medium, which should be cellulose-rich and nutrient-poor. The use of non-nutrient agar with filter paper for culturing environmental samples helps prevent overgrowth of other fungal species. Microscopic examination alone for species identification is considered to be inferior to examination of cultured samples, but cultured samples also have disadvantages: the turnaround-time is 7-14 days; the proper media is required for collection; only viable parts are counted (hyphal fragments, nonviable spores, and other mold parts that could also produce adverse health effects are missed); and if nutrient-poor culture medium is not used, *S. chartarum* will be underrepresented due to its slower growth. *S. chartarum* spores may be purchased commercially in small freeze-dried or frozen quantities from various type culture collections.

Environmental Occurrence and Persistence: Cereal grains, oil seeds, tree nuts, and dehydrated fruits are susceptible to contamination by fungi and can harbor dangerous concentrations of trichothecenes. Some specific plant hosts of *S. chartarum* include species of maple, walnut, pine, oak, and soybean. Spores may remain viable for years, even decades, in the environment. Because the spores are usually produced in clusters and covered in dried slime, they are not expected to become airborne readily unless they are dry and disturbed or are attached to dust or other particulates.

Indoors, *S. chartarum* has been isolated from gypsum wallboard, pipe insulation, glass fiber wallpaper, and aluminum foil. It typically grows in areas with excessive moisture and temperature variability or on wet material with high cellulose content such as fiberboard, drywall, paper, gypsum board, wood, dust, lint, and wallpaper. Often, the infestation is a result of moisture from excessive humidity, water leaks, condensation, or flooding.

Day-to-day and even hour-to-hour fluctuations in airborne concentrations of fungal spores limit the representativeness of any collected air samples. Often mycotoxin concentrations determined from air samples exceed values expected from mycotoxin concentrations in spores, possibly due to aerosolize dust particles from the contaminated areas that are saturated with mycotoxins. *S. chartarum* was isolated from air in 3% of 68 homes sampled in Southern California (mean concentration 0.3 spore/m^3), 13% of 47 homes examined in London, 4% of homes sampled in central Scotland, and in one of 50 Canadian homes. In one Cleveland, OH, home where an infant developed IPH the initial *S. chartarum* spore concentrations in air were 0.1-9.3 spores/cm^3.

Regulatory Status: *S. chartarum* was included in the initial U.S. EPA Toxic Substances Control Inventory in 1978 as one of the 192 microorganisms reported as "manufactured" in the United States, from such companies as the American Type Culture Collection (ATCC).

No governmental body or industry has established guidelines for acceptable mold levels in normal and problem buildings. Because of data interpretation problems, the Centers for Disease Control and Prevention (CDC) has discouraged proactive monitoring. Since late 2001, according to the American Industrial Hygiene Association (AIHA) some laboratories have been qualified to analyze mold spores and colonies in surface, bulk, and air samples through accreditation by the AIHA Environmental Microbiological Laboratory Accreditation Program. Numerous other laboratories participate in performance-based testing through the Environmental Microbiological Proficiency Analytical Testing Program. When proficient, they may apply for accreditation. The U.S. EPA, NIOSH, and state governments do not offer any approvals, certifications, or licenses for mold analysis.

Human Data
People can be exposed to *S. chartarum* via dermal contact, ingestion, and inhalation. For the general population, the most common reports of exposure involve water-damaged buildings, including homes, office buildings, courthouses, hospitals, a hotel, and schools. Exposures leading to stachybotrytoxicosis have been reported among farmers, workers at facilities processing malt grain or reprocessing moldy grain, textile mill workers using plant fibers, and workers at binder twine factories.

An extensive survey of indoor and outdoor *S. chartarum* air concentrations by geographical regions in the United States found *S. chartarum* in 6% of the 1,717 buildings sampled in the period 1996-1998 (46% offices, 18% schools, 13% hospitals, 4% homes, 0% industrial sites, and 18% other). Reasons for sampling included odors, health complaints, proactive sampling, visual growth, and water damage. When *S. chartarum* was present in 6% of buildings sampled, the median indoor concentration was 12 CFU/m^3 (95% confidence interval [CI], 12 to 118 CFU/m^3). The median outdoor concentration for 1% of buildings studied was also 12 CFU/m^3 (95% CI, 4 to 318 CFU/m^3) when detected.

Recent epidemiologic investigations have suggested there is an association between exposure to *S. chartarum* and toxic inflammatory effects in infants, in courthouse workers, and in office workers, as well as between *S. chartarum* and extreme chronic fatigue syndrome in hospital workers. The case reports of PH and hemosiderosis in infants living in the Cleveland, OH, area indicated that several factors contributed to this outbreak, including potential exposure to *S. chartarum*. However, the CDC concluded that evidence from current studies does not support a causative relationship between exposure to *S. chartarum* and PH in infants. The IOM committee concluded similarly in their recent report. Sick building syndrome and the intensification of asthma have also been linked with elevated indoor levels of *Stachybotrys*; however, there is not sufficient evidence to substantiate this association. It is generally accepted that building-related asthma and an increased incidence of upper respiratory disease are associated with living or working in a moldy environment; these associations, however, are with molds that comprise many species.

Toxicological Data
No data regarding chronic exposure, initiation/promotion, carcinogenicity, or genotoxicity studies or synergistic/antagonistic effects were available.

Chemical Disposition, Metabolism, and Toxicokinetics
S. chartarum is capable of producing at least six chemical classes of toxins: trichothecenes, phenylspirodrimanes, stachybocins, cyclosporins, atranones, and stachylysin. When conidia of *S. chartarum* were instilled into the lungs of rat pups, clearance was rapid, occurring within the first 0.25 hour. In lung homogenates of four-day-old rat pups, viable conidia of the fungus decreased rapidly and fungal DNA decreased exponentially. A half-life of ~1.5 days was calculated for fungal DNA in the lungs. The fungal load decreased even more rapidly in fourteen-day-old rat pups.

When instilled in mouse and rat lung tissues, the spores are segregated in granulomas. Stachylysin, a proteinaceous hemolytic agent, is distributed primarily in the inner wall of spores and mycelia of the fungus. Stachylysin diffuses out from the spores slowly. Like stachylysin, satratoxin G is predominately localized in the inner wall layer of spores, primarily along the outer plasmalemma surface. In an *in vivo* mouse model, it was distributed in lysosomes.

Short-term Exposure Studies
Intranasal Instillation: Adult mice intranasally instilled with up to 10^6 spores of *S. chartarum* strain s.72 (satratoxins producer) exhibited severe alveolar, bronchiolar, and interstitial inflammation with luminal hemorrhagic exudates. There was a significant decrease in platelet counts and a significant increase in leukocyte and erythrocyte counts, hematocrits, and hemoglobin concentrations. In contrast, the same

amount of *S. chartarum* strain s.29 (non-satratoxin producer) caused no toxicity or mortality, and the inflammatory response was markedly less than that produced with strain s.72. However, in a separate, similar study using both strains, a dose-dependent increase in monocytes, neutrophils, and lymphocytes in bronchoalveolar lavage fluid (BALF) was observed with no difference between exposure to either strain. In guinea pigs, pulmonary challenge with *S. chartarum* (strain not specified) produced catarrhal-desquamative inflammation in the tracheal and bronchial mucosa, epitheloid degeneration, focal bronchopneumonia, areas of serous hemorrhagic interstitial inflammation, atelectasis, and compensatory emphysema. Degenerative changes in the heart, liver, and kidneys were also observed.

Intratracheal Instillation: The lungs of juvenile mice exposed to spores of *S. chartarum* strain 58-17 ($1.4x10^6$ conidia/mL or 70,000 spores per animal) via a single intratracheal (i.t.) inoculation showed signs of typical granulomatous inflammation reactions. In a separate study, *S. chartarum* spores from isolate DAOM 225489 were found capable of significantly altering convertase activity in both the heavy aggregate form of alveolar surfactant and lamellar bodies in juvenile mice. Experiments in rats with up to $3x10^4$ toxic spores/g also resulted in a strong inflammatory response and pulmonary injury in the animals.

In mice, i.t. exposure to *S. chartarum* strains JS58-17 (trichothecene-producing) and JS58-06 (atranone-producing) spores (30, 300, or 3000 spores/g bw) produced changes in BALF total protein, albumin, pro-inflammatory cytokine, and lactate dehydrogenase (LDH) concentrations that were significantly strain-, dose-, and/or time-dependent. At the low dose, BALF composition was similar for both strains.

Inhalation: Mice exposed to airborne emissions from *S. chartarum* (i.e., vapors and then a combination of vapors, spores, and mycelial fragments and particles containing solid metabolites) (loading factor, 5.12 m^2/m^3, corresponding to 2.8x the loading in a normal room with all surfaces covered by mold) exhibited weak sensory and pulmonary irritation effects. In naive and *S. chartarum*-immunized mice exposed to aerosolized nontoxic *S. chartarum* extract (single exposure of 125 mg/m^3 or 44-83 mg/m^3 twice a week for three weeks), a sensory irritation response in the airways was also observed.

Cytotoxicity

In an *in vitro* study in mouse RAW264.7 macrophages, several strains of *Stachybotrys* species stimulated an immediate increase in the production of reactive oxygen species (ROS) and caused the release of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) from the cells. Treatment of the mouse macrophage cell line with spores/microbes from *S. chartarum* (10^4-10^6 spores/10^6 cells or 10^5-10^7 microbes/mL) induced an increase in TNF-α production and dose-dependent cytotoxicity. In rat alveolar macrophages, *S. chartarum* strains Budapest 1 (0.052-5.2 μg/mL) and Debrecen 1132 (0.0014-1.38 μg/mL) strongly inhibited protein synthesis. In human macrophage cell line 28SC and human lung epithelial cell line A549, *S. chartarum* (10^5-10^7 microbes/mL) induced slight IL-6 production, as well as NO production; both, however, failed to produce significant amounts of IL-1β. Cytotoxicity was dose-dependently induced.

Reproductive and Teratological Effects

Mice were orally administered low doses (3-4000 tissue culture units [TCU]) of *Stachybotrys* toxin from toxicogenic strains 71, 76, and 82 and nontoxicogenic strains 83 and 86 as infected grain, liquid growth medium, or partly purified toxin preparation as a single dose on day 3 or 5 of pregnancy or during a five-day period in the feed. The percentage of pregnancy for all treated animals was statistically lower than that for the controls (70.7% versus 90.5%, respectively). At 100-4000 TCU, a statistically significant increase in the frequency of dead, resorbed, or stunted fetuses and a decrease in the average litter size of live fetuses were seen in treated animals compared to controls.

Immunotoxicity

Inflammatory responses (e.g., increases in BALF total protein immunoglobulin E [IgE] levels) were seen in mice when exposed to an extract composed of five isolates of *S. chartarum* over a four-week period.

Other Data

Five strains of *S. chartarum* isolated from PH case homes in Cleveland (51-08, 51-11, 58-02, 58-06, and 63-07) and the strain isolated from the lungs of a child with PH living in Houston produced significantly more hemolytic activity than the controls. When tested in sheep red blood cells (RBC), consistent production of the hemolysin [identified as stachylysin] was observed in the conidia from the Houston strain.

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1.0 Basis for Nomination

Mold present in indoor environments was nominated for toxicological studies by a private individual. This nomination reflects a broad public concern regarding the non-infectious adverse health effects of fungal exposures. Since it is likely that if such effects are occurring, they are a result of exposure to fungi and not solely to specific fungal mycotoxins, the National Institute of Environmental Health Sciences (NIEHS) Nominations Faculty recommended that toxicological studies be considered for the whole organism. The nomination is also supported by the conclusions in a May 2004 Institute of Medicine (IOM) report on Damp Indoor Spaces and Health (http://www.nap.edu/catalog/11011.html). The IOM committee concluded that there is sufficient evidence of an association between mold and other factors related to damp conditions and several respiratory ailments, including upper respiratory tract symptoms, cough, wheeze, hypersensitivity pneumonitis in susceptible persons, and asthma symptoms in sensitized persons. However, the IOM committee concluded that there was inadequate or insufficient evidence to make conclusions for many other health outcomes, including rheumatologic and other immune diseases, neurological symptoms, cancer, and reproductive effects. Notably, the committee determined that none of these health outcomes met the definitions for the category of "limited or suggestive evidence of no association." The committee also identified numerous research needs. Along with recommendations for more research in exposure assessment and standardization of definitions (e.g., "dampness"), the committee recommended that animal studies be initiated to evaluate the effects of chronic exposures to mycotoxins via inhalation. Such studies should establish dose-response, lowest observed adverse effect levels, and no observed adverse effect levels since presently available studies are of acute, high-level exposure.

Although many species of fungus can be found in indoor environments, *Stachybotrys chartarum* (also known as *S. atra* and *S. alternans*) was chosen as the representative fungus for toxicological studies because it has received considerable attention in the mass media. Results from studies that indicate exposure to water damaged building materials that are contaminated with *S. chartarum* in the home, school, or workplace may be associated with idiopathic pulmonary hemorrhage (IPH) in infants, sick building syndrome, and toxic inflammatory effects in workers has engendered substantial public concern. This concern, together with the fact that little toxicological data are available evaluating potential systemic toxicity from long-term exposure to this organism under relevant exposure scenarios supports the consideration of *S. chartarum* as a representative fungus for toxicological studies.

2.0 Introduction

This report focuses on *Stachybotrys chartarum*, the whole organism or fragments thereof, its spores, or extracts of fungal parts as the environmental agent to which humans are exposed and for which further toxicological studies may lead to a better characterization of the health hazards resulting from such exposures. Included where appropriate is some discussion of the trichothecenes and other metabolites associated with different strains of this organism, which are separated into two chemotypes for the nonvolatile metabolites. [See Appendix B for information on specific mycotoxins.]

2.1 Identification

2.1.1 *Stachybotrys chartarum*

Stachybotrys chartarum (also called *S. atra* and *S. alternans*) is a greenish-black mold assigned to the fungal division Deuteromycota and its single class Deuteromycetes and is a member of the order Moniliales and the family Dematiaceae (Nelson, 2001). The short discussion below introduces some of the basic terminology used in mycology or the study of fungi.

Fungi constitute an independent group (Kingdom) of eukaryotic organisms (Domain Eukaryota, organisms with true nuclei) equal in rank to plants and animals in the modern three-domain classification of all living things (the other two domains Bacteria and Archaea.) Fungal organisms include mushrooms, rusts, smuts, puffballs, truffles, morels, molds, and yeasts (Blackwell et al., 1996). Almost all fungi are heterotrophic, deriving their energy from other organisms (Farabee, undated). The free-living saprobes or saprophytes, producing enzymes that degrade biopolymers, use carbon fixed by other organisms; they live and grow on woody substrates and in soils, leaf litter, dead animals, and animal exudates. Fungi are generally the primary decomposer organisms within their natural habitat. Some fungi, the biotrophs, form symbiotic associations with plants, animals, and prokaryotes. Fungi are the most important plant pathogens, and some are fungal parasites of humans and other animals (Blackwell et al., 1996). About 300 fungal species are human pathogens (DiSalvo, 2004).

The nonmotile bodies (thalli) of fungi are composed of apically elongating walled filaments called hyphae. [Hyphae resemble tubes and are the main growth forms of filamentous fungi (Forensic Analytical, 2004).] A common thallus may reproduce both sexually and asexually. Sporulation is triggered when food becomes depleted. Fungal cell wall components are chitin (microcrystalline homopolymers of *N*-acetylglucosamine and glucans) (Blackwell et al., 1996).

Fungi known to reproduce sexually and asexually are assigned to the fungal phyla (also called divisions) Chytridiomycota (chytrids), Zygomycota (bread molds), Ascomycota (yeasts and sac fungi), and Basidiomycota (club fungi). Such fungi are called pleomorphic and each morph may be named. The teleomorph is a sexual or meiotic state of the fungus, and the anamorph is the asexual or mitotic state (Sigler and Flis, 1998). Fungal classification in these phyla is based on the appearance of the reproductive spores, the method of reproduction, and the nature of the hyphae (Farabee, undated). Zygotic meiosis produces haploid thalli (Blackwell et al., 1996). The sexually reproduced spores (meiospores) are produced in/on the basidia (Basidiomycota), asci (Ascomycota), hyphae (directly) (Zygoomycota), and zoosporangia (Chytridiomycota), and the corresponding names for their spores are basidiospores, ascospores, zygospores, and zoospores (Petersen, undated).

Fungal species for which a sexual stage is unknown or absent (anamorphs) such as *S. chartarum* are placed in another Division called Deuteromycota (also known as Fungi Imperfecti) and in its single class Deuteromycetes. These anamorphs are characterized by production of septate mycelium and/or types of yeasts (Wong, undated). [The mycelium is the network of hyphae that makes up the nonreproductive (vegetative) body of the fungus (Forensic Analytical, 2004). A septate mycelium has a regular cellular structure with cell walls called septa (Wong, undated). In another definition: The cellular cross-walls partitioning the hyphae are called septa (University of California, Berkeley, undated).] The name Deuteromycota does not represent a

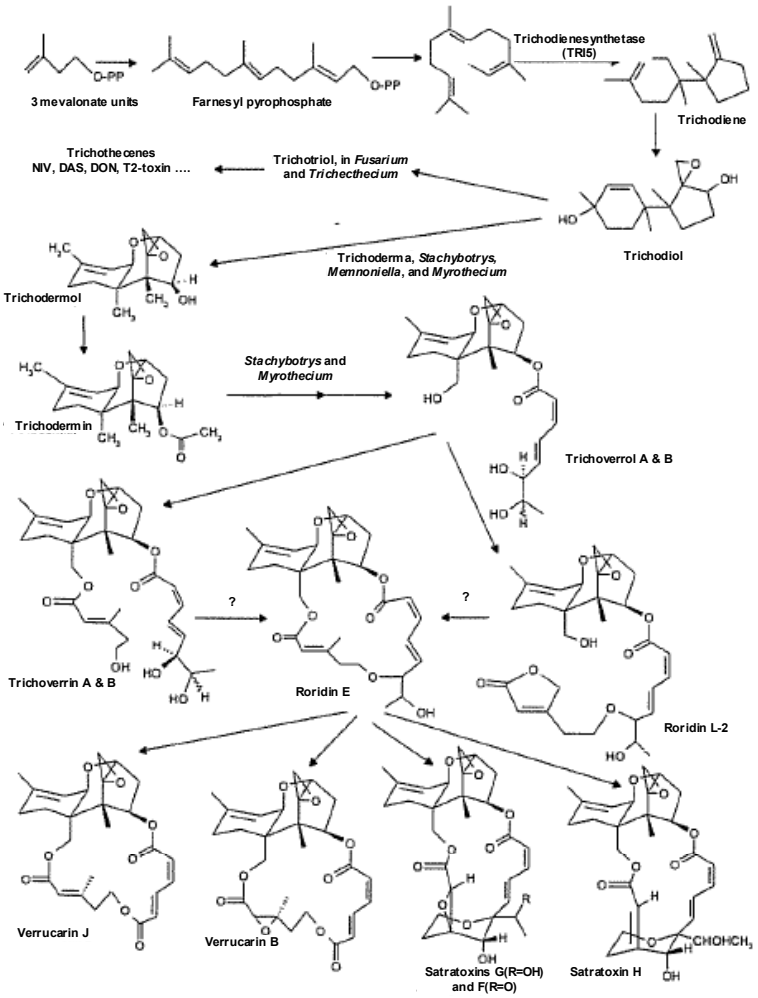
formal taxonomic group because the organisms are polyphyletic. The four orders of Deuteromycetes are Moniliales, Sphaeopsidales, Melanconiales, and Mycelia Sterlia (University of California, Berkeley, undated). In the order Moniliales, conidia (asexual spores produced by mitosis; also called mitospores) and conidiophores (fertile hyphae that bear cells producing conidia) are produced on the mycelium (Wong, undated). The conidium-producing cells (phialides) are often bottle-shaped and produce conidia in basipetal succession. In basipetal conidium production, the base of the chain is comprised of the most recently produced conidium and the chain tip contains the oldest conidia. The base of the chain is the apex of the conidiogenous cell. (Acropetal refers to the reverse situation) (Forensic Analytical, 2004).

2.1.2 Chemicals Produced by *S. chartarum*

2.1.2.1 Mycotoxins

Mycotoxins (i.e., poisonous substances produced by a fungus) produced by *S. chartarum* include the following: satratoxins, roridins, verrucarins, and stachybocins (Albright, 2001). Abbott (2002) included trichoverrins and trichoverrols as mycotoxins that are primarily associated with *S. chartarum*. However, only two MEDLINE abstracts were found that mentioned determination of trichoverrols (A and B) in *S. chartarum* (Jarvis et al., 1986; el-Maghraby et al., 1991). None were found for trichoverrins. Most available toxicological studies have focused on the macrocyclic trichothecenes satratoxins G and H and roridins A and E. The macrocyclic trichothecenes include the satratoxins, the verrucarrins, and roridin E. Nonmacrocyclic trichothecenes include trichodermol (roridin C), trichodermin, trichoverrins, and roridin L-2. See Appendix B for more information on some of the trichothecenes and atranone B. Trichodermol and/or trichodermin are mentioned below in connection with chemotype identification of *S. chartarum* strains.

A better understanding of the discussion on chemotypes might be had by consideration of the biosynthetic pathway for trichothecenes proposed by Nielsen (2002) [see scheme on next page]: Farnesyl pyrophosphate is converted via an intermediate to trichodiene; the step from intermediate to trichodiene is catalyzed by trichodiene synthase (expressed by the gene *Tri5*, which differs by one nucleotide between strains that produce macrocyclic trichothecenes and those that do not). Trichodiene is converted to trichodiol, which in *Stachybotrys*, *Memnoniella*, *Trichoderma*, and *Myrothecium* species is converted to trichodermol. In *Stachybotrys* and *Memnoniella*, trichodermin is converted to trichoverrol A and B. Trichoverrol A and B is converted to roridin L-2 and trichoverrin A and B, which may be cyclized to macrocyclic roridin E. Roridin E is the precursor of verrucarin B and J and satratoxin F, G, and H. All of the trichothecenes are highly cytotoxic except trichodermol and trichodermin.



Biosynthetic Pathway for Trichothecenes

2.1.2.2 Stachylysin

The proteinaceous hemolytic agent produced by *S. chartarum*, stachylysin, is localized primarily in the inner wall of spores and mycelia (as determined by immunohistochemical and immunocytochemical methods) (Gregory et al., 2003). Vesper et al. (2001) characterized stachylysin, which in its monomeric form has a molecular weight of 11,920 Da and is about 40% nonpolar amino acids and contains two cysteine residues. The active hemolytic form is uncertain because of the formation of polydisperse aggregates. Stachylysin is inactivated by heating to 60 °C for 0.5 hour and by dialyzing exhaustively.

2.1.2.3 Other Constituents

Other *S. chartarum* constituents include phenylspirodrimanes (spirolactones and spirolactams), a cyclosporin, atranones A-G, trichodiene, and dolabellane diterpenes (Albright, 2001). Among *S. chartarum* metabolites, the spirocyclic drimanes have received little attention, and no structure assignments have been made for the most abundant ones (Nielsen, 2003c). Nielsen (2002) discussed available toxicity information for other *S. chartarum* metabolites.

2.1.3 Physical-Chemical Properties of *S. chartarum*

2.1.3.1 Morphology

S. chartarum is a greenish black mold. Although it typically appears wet and slimy, it may appear sooty or may be observed as gray-white strands. The slimy mycelial mats are dark olive-gray with smooth margins and ridged or smooth surfaces. Spores are brownish, and the colony may have a powdery surface during sporulation. Cladosporium, Aspergillus, Alternaria, and Drechslera are similar in appearance to the untrained eye (Mold-Help, 2003). The septate spores of the nearly identical *Pithomyces* differentiate this genus from *Stachybotrys* (Nelson, 2001).

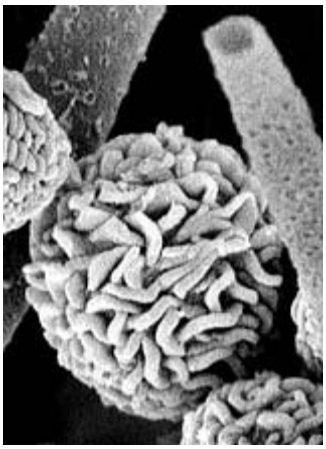
Members of the genus *Stachybotrys* are relatively easy to identify based on microscopic morphology: Conidiophores are determinate, macronematous, solitary or in groups, erect, irregularly branched or simple, septate, and dark olivaceous; the upper parts are often rough walled. The phialides (conidia-producing cells) are 9-14 μm in length, ellipsoid, and olivaceous; occur in whorls; often have conspicuous collarettes; and produce conidia singly and successively into a slime droplet that covers the phialides. The conidia are ellipsoidal, unicellular, 7-12 μm by 4-6 μm, dark brown to black, and covered with slime residue. They remain attached in a mass to the conidiophore. When dry and physically disturbed, conidia may become aerosols. The mature conidia often have a ridged topography seen by scanning electron microscopy (Nelson, 2001). The Mycology Proficiency Testing Program (2002) description of *Stachybotrys* species gives additional information: *Stachybotrys* species have hyaline (transparent or colorless) hyphae. Simple conidiophores are hyaline to black and end in phialides in groups of 3 to 10. The black, unicellular conidia form in slimy masses at the tip of the phialides. Distinguishing characteristics from other fungi include moderately rapid growth (colonies measure up to 3 cm within four days), color pattern (both surface and reverse of colonies are black), and the slimy conidia masses.

Memnoniella fungi may be relegated to the genus *Stachybotrys* because of similar morphology and comparative sequence analysis of the nuclear ribosomal RNA operon (Nelson, 2001). Morphologically, the principal difference is in spore morphology. For example, *M. echinata* spores (phialoconidia) usually appear in dry chains, whereas *S. chartarum* spores are clumped and encased in a polysaccharide coating. Some authors are already relegating *M. echinata* to the genus *Stachybotrys* (Jarvis, 2003).

The morphology of a new species whose isolates have frequently been identified in the literature and type culture collections as *S. chartarum* is described in subsection 2.1.3.3.

Dried *S. chartarum* particles derived by growth on sterile rice, autoclaving, drying, and aerosolizing by acoustic vibration were all less than 15 μm in aerodynamic diameter. The mass median aerodynamic diameter was 5 μm. The particles comprised about 85% conidia and about 6% fragments of hyphae. The rest of the particles were not identifiable (Sorensen et al., 1987).

The mass of conidia of *S. chartarum* at the tip of the conidiophore. Parts of the whorl of phialides are visible. The slime was mostly removed by the fixative. Scanning electron micrograph (Nelson, 2001).



A conidium of *S. chartarum* showing the ridged surface of a mature spore. Scanning electron micrograph (Nelson, 2001).

2.1.3.2 Chemotypes and Other Metabolite Patterns

Almost two-thirds of *S. chartarum* isolates produce mycotoxins. Many strains, regardless of geographic origin, produce the same mycotoxins. Some studies have found that culture medium enrichment (e.g., with glucose) for a nontoxic strain may convert the nontoxic strain to a toxic strain (Pieckova and Jesenská, 1999). For example, el-Maghraby et al. (1991) reported that different mycotoxins were produced by *S. chartarum* and other *Stachybotrys* species when grown on different media. Another report that suggested metabolites produced by a particular isolate could be altered was based on the change in hemolytic activity reported by Vesper and Vesper (2002). In that study, seven of eight strains from homes of infants with pulmonary hemorrhage (PH) in Cleveland and the strain from the lungs of a Texas infant with PH produced stachylysin when grown on tryptic soy broth. Only one of eight strains from control homes produced stachylysin. Yet when 0.7% sheep's blood was added, all strains produced stachylysin.

Vesper et al. (1999) compared the conidial toxicity (protein synthesis inhibition), hemolytic activity, and randomly amplified polymorphic DNA (RAPD) banding patterns of *S. chartarum* strains isolated from Cleveland, OH, homes (IPH case with those of control homes [eight of each]) and with 12 non-Cleveland strains from type culture collections [strains from Canada (six), Finland (two), California (one), U.K. (one), Egypt (one), and Namibia (one)]. Strains were grown on wet wallboard pieces for an eight-week period at 23 ºC. Amounts of *S. chartarum* toxins in strains from the IPH case homes did not differ from amounts in strains from control homes. Conidia of five Cleveland strains (three from case homes and two from non-case homes) and two non-Cleveland strains were highly toxic. Eight strains were consistently hemolytic in a cultured sheep's blood assay (five from Cleveland PH case homes and three from non-Cleveland strains). All 28 strains showed some hemolytic activity after incubation for five weeks. Only three strains—all from Cleveland IPH case homes—were consistently toxic and hemolytic. They were designated 51-06, 51-11, and 58-02. Based on the RAPD analysis, strains 51-06 and 58-02 were statistically different from the other three highly toxic strains from IPH case homes. Data indicated that the Cleveland strains were not much more likely to be highly toxic or highly hemolytic than the non-Cleveland strains and suggested that mycotoxins and hemolysins are both

necessary for IPH induction. [One of the strains used, 58-06 (ATCC 201863), has been reassigned to the species *S. chlorohalonata* (Andersen et al., 2003).]

Nielsen (2001) observed that 80% of isolates causing stachybotrytoxicosis in farm animals in Eastern Europe produced macrocyclic trichothecenes, whereas only 30-40% of isolates from U.S. homes with IPH cases produced macrocyclic trichothecenes.

Jarvis (2003) reviewed studies on *S. chartarum* chemotypes for the nonvolatile metabolites. About one-third of *S. chartarum* isolates produce macrocyclic trichothecenes and about two-thirds produce the less toxic simple trichothecenes—trichodermol and trichodermin. *S. chartarum* isolates are divided into two chemotypes: those that produce macrocyclic trichothecenes and those that produce atranones, to which no significant biological activity has been ascribed. If any strain produces both macrocyclic trichothecenes and atranones, analytical methods have not been sufficiently sensitive to detect them. The recent reassignment of some isolates to a different species (see subsection 2.1.3.3) does not correspond to the two chemotypes. All isolates of the new species so far examined are atranone producers. About 60% of those strains still classified as *S. chartarum* are atranone producers and about 40% are producers of macrocyclic trichothecenes.

Andersen et al. (2002, 2003) designated those *S. chartarum* isolates that produced atranones and dolabellanes as chemotype A and those that produced macrocyclic trichothecenes (satratoxins and roridins) as chemotype S. Both chemotypes were identical in the *Tub1* gene fragment, but the *Tri5* (trichodiene synthase) and *Chs1* gene fragments each showed one nucleotide difference.

Peltola et al. (2002) reported that only 22 of 31 *S. chartarum* isolates were positive for the *Tri5* gene as determined by gene-specific polymerase-chain reaction (PCR). Five of the 22 contained satratoxins and 11 contained trichodermol, a simple trichothecene. Cluster analysis of randomly amplified DNA banding pattern (RAPD) fragments also showed the same two distinct groups of isolates. The results indicated "that the *S. chartarum* isolates belonging to different groups [chemotypes] were genetically distinct in a much wider area than just the *Tri5* gene."

The closely related mold *Memnoniella echinata* (also known as *Stachybotrys echinata*), like *S. chartarum*, produces spirocyclic drimanes, triprenylphenols, and trichothecenes (but only simple ones).

Other groups of researchers are investigating whether unique patterns of microbial volatile organic compounds (MVOCs) might be used to identify the specific fungi present at contaminated sites. Grown on rice cultures, *S. chartarum* uniquely produced 1-butanol, 3-methyl-1-butanol (isoamyl alcohol), 3-methyl-2-butanol, and thujopsene, but the only MVOC produced when grown on gypsum board was 1-butanol. The strains used, 31-22-03, 31-22-06, and 31-22-16, were supplied by the Analytical Services Branch of NIOSH. They had been isolated from water-damaged homes in Cleveland (Gao and Martin, 2002).

Danish isolates of several mold species grown on different substrates produced unique patterns of MVOCs such as sesquiterpene hydrocarbons ($C_{15}H_{24}$) as determined by thermal desorption/HRGC/MS (high resolution gas chromatography/mass spectrometry). *S. chartarum*,

as might be expected from its different chemotypes for nonvolatile metabolites, produced different MVOC patterns (Wilkins [Danish National Institute of Occupational Health], ca. 2000). *S. chartarum* isolates that produced macrocyclic trichothecenes (chemotype S) produced greater amounts of volatile trichodiene, a simple trichothecene derivative, and other sesquiterpenes than isolates that did not produce macrocyclic trichothecenes (chemotype A). The MVOCs were determined by GC/MS, and the nonvolatile compounds were also determined by GC/MS after separation by high performance liquid chromatography (HPLC) and derivatization. Because of the small amounts of trichodiene produced, this metabolite will probably not be useful in detecting *S. chartarum* (Wilkins and Nielsen, 2003). Wady et al. (2001) used GC/MS with solid phase microextraction (SPME) to identify MVOCs from *S. chartarum*-infested gypsum board, paper, mineral wool, and medium-density fiberboard.

2.1.3.3 Reassignment of Some Isolates to a New Species, *Stachybotrys chlorohalonata*

In studies with 25 isolates, Cruse et al. [University of California, Berkeley] (2002) concluded that two distinct phylogenetic species exist within the "single described morphological species" *S. chartarum* based on markers that the authors developed for three polymorphic coding loci. Isolates (23) from the San Francisco Bay Area and seven from other U.S. sites were examined. The same conclusion was reached by Koster et al. [University of Toronto] (2003), who examined 52 indoor and outdoor isolates that had been collected from geographically diverse locations. The authors used a different set of molecular markers to support the existence of two distinct phylogenetic species in samples previously identified as *S. chartarum*.

Andersen et al. [Mycology Group, Bio-Centrum-DTU, Denmark] (2002, 2003) identified an undescribed species within a group of 122 *Stachybotrys* isolates from buildings in Denmark, Finland, and the United States. In an extensive analysis by morphological, chemical, and phylogenetic methods, the authors assigned 10 of 25 isolates previously identified as *S. chartarum* to the new species *Stachybotrys chlorohalonata*. Two of the 10 species-reassigned isolates (CBS 109284 and CBS 109285) were from buildings in Denmark and were isolates used in the Andersen et al. (2002) studies. One reassigned strain had been isolated from water in Belgium (IHEM 9905) and had been used in a study by Peltola et al. (2002). The six U.S. isolates of *S. chlorohalonata* had been used in studies by Jarvis et al. (1998) and Vesper et al. (1999, 2000a) (ATCC 201863 = JS58-06) and Cruse et al. (2002) (IBT 40285, IBT 40287, IBT 40290, IBT 40292, IBT 40294). [The JS refers to the culture collection at the laboratory of Dr. Bruce B. Jarvis. The other identifiers are explained in section 3.0.]

While both *S. chartarum* and *S. chlorohalonata* grown on cornmeal agar (CMA) develop obovoid phialides and ellipsoidal conidia of approximately the same maximum size, the conidia of *S. chlorohalonata* are smooth. The colony of *S. chartarum* is unpigmented and grows to 23 mm while the *S. chlorohalonata* colony is pigmented green [produces a green extracellular pigment on CYA (Czapeck yeast agar) medium] and grows to 14 mm. *S. chlorohalonata* isolates were also subgrouped by chemotype. Three of the isolates, including the one corresponding to JS58-06, produced trichodermin as well as atranones and dolabellanes (chemotype A) on potato starch agar; one isolate did not produce detectable atranones and dolabellanes. In the *S. chlorohalonata* isolates that produce the simple trichothecenes trichodermol and trichodermin, it was possible to amplify the *Tri5* gene (Andersen et al., 2003). Appendix C provides designations for several *S. chartarum* strains.

2.2 Sampling and Analysis

2.2.1 *S. chartarum*

2.2.1.1 Sampling, Spore Counting, and Culturing

A comprehensive review of indoor air sampling for fungal contamination was recently published by Portnoy et al. (2004). Assessment of airborne mold may involve counting agar strips or plates that have been exposed by Andersen air sampling methods or by the Reuter centrifugal sampler (RCS) (Sigler, 2001). Collection of *S. chartarum*-contaminated materials and media generally combines surface sampling (wipes by sponges or swabs and collection by tape lifting) and air sampling. Bulk samples may be preserved in zip-lock plastic bags (Pinto, undated).

Spore trap analysis may be done by commercial spore trap methodologies such as Air-O-Cell, LARO-100, Cyclex, Cyclex-D, and Burkhard). They are fast methods of counting and identifying the genus of spores, but identification is subjective, depending on analyst expertise. Both viable and nonviable spores are counted (EMSL Analytical Inc., undated).

Precise fungal species identification in mold-contaminated samples may be done by microscopic examination by laboratory microbiologists and mycologists. This method can be expedited by field workers who take photomicrographs on site via a digital imaging system (Digital Diagnostic Laboratories, 2001).

Frequently, collected dust and air samples are sent to testing laboratories to be cultured. Microscopic examination alone is considered to be inferior to examination of cultured samples, but cultured samples have disadvantages, too (Pinto, undated; EMSL Analytical Inc., undated) such as

- Several day turnaround time (7-14 days)
- Subjective
- Requires proper media for collection
- For *Stachybotrys*, only viable (living) parts are counted, which omits hyphal fragments, nonviable spores, and other mold parts that could also produce adverse health effects
- Unless a nutrient-poor culture medium is used, *S. chartarum* will be underrepresented due to its slower growth (See discussion in subsection 3.1.)

Whereas many sources have stated that *S. chartarum* is rarely isolated from air because the sticky masses of spores are not easily aerosolized or the spores die rapidly once released from the conidiophore, Harrington (2003 abstr.) showed that the failure to isolate *S. chartarum* from air samples is probably due to poor choice of collection medium, which should be cellulose-rich and nutrient-poor. Air samples were collected by an N6 single-stage impactor on eight media in a room with mold-infected ceiling tiles and drywall. For seven of the media, cultured under otherwise identical conditions for 10 days, total fungal counts ranged from 176 to 1,108 CFU/m^3 with the *S. chartarum* percentage of the total ranging from 0% (on Emmon's modification of Sabouraud agar [ESA] to 20% (cornmeal agar) or 21% (potato flake agar) [PFA]). The percentage on the common potato dextrose agar was 13%. The best medium for *S. chartarum* growth was sterile filter paper on non-nutrient agar [FPWA]. While the total fungal count was only 441 CFU/m^3, that for *S. chartarum* was 405 CFU/m^3 (92% of the total).

The Occupational Safety and Hazard Administration (OSHA) air sampling method for *S. chartarum* involves directing the sampled air over a collection medium in an Andersen N-6 sampler. With malt extract agar; a maximum volume (time-weighted average [TWA]) of 141.5 L and a TWA rate of 28.3 L/min are used. When the medium is a 37 MM mixed cellulose ester filter (0.8 micron-open faced), the TWA volume is 120 L and the TWA rate is 1.0 L/min (OSHA, 2000).

2.2.1.2 Immunoassays and Other Methods for Total Trichothecenes

S. chartarum spores that can no longer germinate (and thus cannot be cultured) may still contain trichothecenes. Measurement of total trichothecene toxicity is more reliable than the counting of viable spores as a measure of potential exposure. Rabbit skin tests, which are highly sensitive for trichothecenes, were the first toxicity assays for determination of *S. chartarum* toxins (Adler, 2002). A recently developed cytotoxicity assay used the unicellular cyanobacterium *Synechococcus cedrorum* to test *S. chartarum* strains K15822, K14722, and K14186 (Zaichenko et al., 2002 [Ukrainian]).

Protein translation inhibition assays are frequently used toxicity tests. Older protein translation inhibition assays to measure total trichothecene activity were slow and labor intensive; new assays based on translation of firefly luciferase have reduced the total time to less than two hours. Yikes et al. (1999) compared the results for T-2 toxin, satratoxin G, and deoxynivalenol in the luciferase translation assay with other methods. Although measurement of specific trichothecenes in mold particulates in air require costly methods, the luciferase translation assay is rapid, inexpensive, and practical for detecting and quantifying their toxicity in contaminated indoor environments. Double filtration of extracts excludes most interferences in this assay. When compared to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, there was a 400-fold increase in sensitivity and a higher specificity for trichothecenes. Protein translation inhibition assays have been used in recent studies on the chemotypes of *S. chartarum* strains.

Specific polyclonal and monoclonal antibodies raised against mycotoxin-protein conjugates have been used in sensitive radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs) (Pestka, 1988). Such assays may be the basis of kits for use by persons with limited technical skills. For example, EnviroLogix markets a QuickTox™ kit with lateral flow membrane strips (dipsticks) for *Stachybotrys* and *Aspergillus niger* that detect mycotoxins collected in spores (LOD for *Stachybotrys* is 100,000 spores per milliliter), giving results in the field within five minutes (EnviroLogix 2003a,b,c). EnviroLogix sells an enzyme immunoassay (EIA) for total trichothecenes. "The test is specific for an epitope shared by many trichothecenes and their intermediate metabolites and breakdown products." The test can detect the presence of mycotoxins in most house dust samples (Portnoy et al., 2004).

An ELISA method has also been used to determine stachylysin, which is practically unique to *S. chartarum*, in human and rat sera and environmental samples (Van Emon et al., 2003; Obriant, 2003a,b).

2.2.1.3 Genetic Probes for Species Identification and Other Genomic Methods

There has been considerable interest in the development of PCR methods for mold analysis and identification over the last six years. In repeated cycles of precise heating or cooling of a DNA sample with reagents and primers, PCR produces many identical copies of a particular DNA sequence. The primers are short DNA sequences that are bound by complementary sequences on the target DNA molecules.

Numerous publications by Vesper and Haugland and their co-workers at the U.S. Environmental Protection Agency (U.S. EPA) Office of Research and Development, National Exposure Research Laboratory, Cincinnati, OH, reported the development of the DNA-based system that rapidly identifies and quantifies molds within hours. Up to 96 analyses can be run simultaneously (Casey, 2001). The quantitative real-time PCR method developed by U.S. EPA uses both primers and probes. The probes are fluorescently tagged primers that aid optical monitoring of the PCR products (Kopp, 2003). For example, Haugland et al. (1999) reported on "Quantitative measurement of *S. chartarum* conidia using real time detection of PCR products with the TaqMan™ fluorogenic probe." The primers and probe used for *S. chartarum* in the U.S. EPA PCR technology are the following:

Stachybotrys chartarum (assay name: Stac)
Forward Primer StacF4: 5'-TCCCAAACCCTTATGTGAACC
Reverse Primer StacR5: 5'-GTTTGCCACTCAGAGAATACTGAAA
Probe StacP2: 5'-CTGCGCCCGGATCCAGGC

(U.S. EPA, 2003 [EPA Technology for Mold Identification and Enumeration http://www.epa.gov/nerlcwww/moldtech.htm]).

Although initial EPA focus was on *S. chartarum*, currently more than 130 mold species can be detected and enumerated in samples with from 5 spores to one million spores. Sampling for PCR analysis is similar to methods for microscopic analysis: A known volume of air (typically 1,000 L) is drawn through sterile film in cassettes (Kopp, 2003).

Cruz-Perez et al. (2001) reported a rapid, quantitative PCR method for characterization of *S. chartarum* strains grown in the laboratory. PCR inhibitors were noted in some samples. To detect low levels (LOD: two spores) of *S. chartarum*, a PCR assay using the primer set FF2/FR1 can be used (Zhou et al., 2000).

Specific primers for *S. chartarum* have been based on the *Tri5* gene. Sequencing ribosomal DNA is another method for species identification. The Internal Transcribed Space (ITS) and the 5.8 s rDNA are transcribed (Land et al., 2003).

True group- and species-specific detection can be improved by following selective primer-based PCR amplification with "probe hybridization to a specific target in the PCR products." Wu et al. (2003) selected the nuclear small subunit ribosomal DNA (18S rDNA) to design probes, such as Sta-6, which is specific to the genus *Stachybotrys*. The probe sequence (5′-3′) is GTCCTGGGCTGCACGCGCGTTA.

Although bead milling (also called bead beating) to disrupt spores and release DNA was adequate for DNA extraction from fungal conidia from air and tap and surface water samples, silica adsorption was required for further purification of DNA from dust samples (Haugland et al., 2002). Keswani and Chen [NIOSH] (2002 abstr.) reported that bead beating of *S. chartarum* spores followed by a 10-fold dilution overcame interferences of dusts in indoor air samples. Dean et al. [U.S. EPA, RTP] (2004) developed a rapid and cost effective method for extracting and purifying the DNA of multiple fungal organisms collected by air sampling. The purified DNA is suitable for enzymatic manipulation and PCR analysis.

The genome structure of *S. chartarum* strains isolated from man-made cellulose-containing materials and natural substrates in Russia was recently investigated by PCR using a primer complementary to the core sequence of the short interspersed repetitive element (SINE) retrotransposon. Although some strains showed differences in their toxicity to *Paramecium caudatum*, their susceptibility to fungicides, and their genome structure, PCR analysis found no correlations among the genome structure, strain properties, and the geographic area and substrate from which the strain was isolated.

Birren et al. (2003) of the Fungal Genome Initiative Steering Committee noted reasons why genomic or molecular analysis of *S. chartarum* would be valuable and/or interesting:

- Except for identification of species-specific sequences for detection in environmental samples, little molecular work has been done.
- The closest relatives of *S. chartarum* phylogenetically are haploid ascomycetes, but it is not known whether *S. chartarum* is haploid or diploid.
- Studies of biosynthetic and regulatory genes for *S. chartarum* biosynthesis of trichothecenes would allow comparisons with such knowledge already developed for *Fusarium* species.
- Genomic sequence elucidation would facilitate studies trying to establish the role of *S. chartarum* in disease and provide information needed to develop a vaccine, disease treatments, and diagnostic tools.

2.2.2 Quantitation of Mycotoxins and Other Metabolites

Chromatographic methods for determining *S. chartarum* toxins were reviewed by Hinkley and Jarvis (2001). Adler (2002) reviewed HPLC, TLC, GC-MS, CE, ELISA, and bioassays for molds. Niels and Thran (2001) developed a fast method for screening fungal cultures using gas chromatography with tandem mass spectrometry (GC/MS/MS). Chung et al. (2003) determined satratoxin G in *S. chartarum* grown in rice cultures by ELISA. Nielsen and Smedsgaard (2003) and Nielsen (2002) reported on a micro-scale method ("LC-UV-MS for detection and dereplication") for metabolite screening of more than 400 fungal metabolites. More details are given in Appendix B. Analyses for total trichothecenes were discussed under subsection 2.2.1.2. Analytical methods for MVOCs were mentioned in subsection 2.1.3.2. Measurement of MVOCs may facilitate identification of hidden contamination sources (Portnoy et al., 2004).

2.3 Commercial Availability

S. chartarum spores may be purchased in small freeze-dried or frozen quantities (shipped in ampoules) from type culture collections. (Apparently, researchers rehydrate the spores and

culture them on appropriate media to generate sufficient biomass for experiments.) Materials available from previous studies may be ordered from the following sources:

- American Type Culture Collection (ATCC) (62 isolates) (ATCC, 2004);
- University of Alberta Microfungus Collection and Herbarium (UAMH [formerly called the University of Alberta Mold Herbarium]) (10 isolates) (Sigler and Flis, 1998);
- VTT Culture Collection at the Technical Research Centre of Finland, Espoo (eight isolates, six of which are stated to produce Stachybotrys toxin) (Suihko, 1999).
- IBT Culture Collection at BioCentrum-DTU, Denmark
- CBS Culture Collection, Utrecht, the Netherlands
- IHEM Culture Collection BCCM® in Belgium

Some of the isolates have recently been identified as a different *Stachybotrys* species. See subsection 2.1.3.3. Additional information about the specific isolates is discussed in report section 6.0 below.

If *S. chartarum* is considered as a plant or animal pathogen, permits for importation into the United States must be obtained from appropriate departments of the U.S. Department of Agriculture (USDA). If considered a human pathogen, an import permit would be requested from the U.S. Centers for Disease Control and Prevention (Sigler and Flis, 1998).

3.0 Production Processes

No commercial production processes were identified. Many researchers have studied growth of *S. chartarum* on different laboratory nutrient media and on other substrates such as wet building materials. Some of these studies are discussed here.

3.1 Growth on Laboratory Nutrient Media

Nielsen (2001) showed relative growth of isolates identified as IBT 7711 and IBT 14915 in Petri dishes after 14 days and on six different media. To compete with *Penicillium* and *Aspergillus*, *S. chartarum* requires high moisture and high cellulosic content in a growth medium that has low sugar and low nitrogen content (Chapman, 2003). Ninety percent of field-collected *S. chartarum* spores cannot be cultured (Miller, 1992; cited by Chapman, 2003). Although *S. chartarum* conidia rapidly lose viability when airborne, they are still toxic (Wu et al., 2003). [So-called loss of viability may be due to use of a nutrient-rich growth medium.]

Harrington (2002 abstr.) examined the growth and sporulation of ten pure *S. chartarum* isolates on 18 fungal growth media under constant light, alternate light/dark, and constant dark for 10 days at 23-24 ºC. No sporulation occurred in constant dark, and sporulation, when it occurred at all, was delayed in constant light. Fastest growth and sporulation were observed on inhibitory mold, potato dextrose, cornmeal, rice extract, and 2% oatmeal agars. Non-nutrient agar with filter paper is useful for environmental samples to prevent overgrowth of other fungal species. However, LA Testing Microbiological Laboratory (undated) recommended either cellulose agar incubated at room temperature or potato dextrose incubated at 35 ºC as media for air sampling for viable *Stachybotrys* species. Cornmeal agar is also commonly used in investigations of indoor contamination by *S. chartarum*.

3.2 Growth on Building Materials

Settled mold spores on damp or wet drywall, carpet and pad, wood, insulation, and paper will swell and begin to grow, sending out a network of hyphae. Optimum growth in buildings for most molds occurs at 68 to 86 °F (20 to 30°C) (SERVPRO Industries, 2001).

Water activity is the most important factor for mold growth. An activity of 0.96 (corresponding to 96% relative humidity) at steady state produced less growth of *S. chartarum* than a water activity of 0.98 (Gravesen et al., 1997; cited by Gravesen et al., 1999). At a value of 0.75, mold growth, as well as mycotoxin production, was inhibited (Reddy and Reddy, 1992c).

While moisture is necessary to initiate growth on the "food" surface, the dense mycelial mat retains water, acting as a vapor barrier. Even when the original water source has been removed, *S. chartarum* may continue to propagate. Metabolism provides additional water (Harriman et al., 2001). *S. chartarum* grows best on high-cellulosic substrates at warm to moderately hot temperatures. Although it does not grow as quickly as *Aspergillus*, *Penicillium*, and *Cladosporium*, other molds associated with growth after water intrusion in a building, *S. chartarum* usually becomes dominant within one to two weeks and may crowd out the other molds (Mold-Help, 2003). (*Aspergillus* and *Penicillium* species colonize between two and three days while *S. chartarum* requires eight to twelve days [Harrington et al., 2001].)

Laboratory experiments of *S. chartarum* growth on plasterboard used an isolate collected from a mold-damaged building in Finland. Removal of the starch in both the core and the liner was necessary to limit growth. While slightly decreased, spores from a core containing a biocide were more toxic than those from untreated plasterboards. The gypsum source determined the nutritional value of the core material (Muroniemi et al., 2003).

Reesler et al. (2003) compared growth in nutrient media cultures with laboratory growth on gypsum board. Ergosterol content and activity of β-*N*-acetylhexosaminidase correlated well with biomass density of *S. chartarum* and *Aspergillus versicolor* when each was grown on agar overlaid with cellophane. Conversion factors for estimated biomass based on these parameters were used to estimate biomass density of molds grown on gypsum board. Growth on gypsum board was significantly slower and stationary-phase biomass density was significantly lower than on agar.

Stachybotryotoxins and trichothecenes are produced in phialides, conidia, and conidiophores [of some chemotypes]. When grown on straw, toxins begin accumulating after about four days and reach a maximum after two to three weeks. Although the optimum pH is 5.6-6.0, *S. chartarum* will grow at pH 3.0 to 9.8. Optimum temperatures are 20-25 °C (range 2.5-40 °C). The spores will resist drying but will die at 60 °C within ten minutes. At 84-100% relative humidity, the fungus reproduces rapidly on wallpapers and plasters and produces satratoxins G and H (Pieckova and Jesenská, 1999).

The growth of *S. chartarum* and production of satratoxin can be completely inhibited with acetic acid and propionic acid. The food preservatives sodium metabisulfate and propionic acid were also found to be very effective inhibitors of growth and satratoxin production, followed by

benzoic and boric acids. Formaldehyde, on the other hand, allowed the mycelial growth of *S. chartarum* but inhibited satratoxin production (Reddy and Reddy, 1992a,b).

For the growth and production of stachybotryotoxin from *S. chartarum Bonord.* 13959a, medium with a balanced content of nitrogen and carbon and with a trace elements mixture was found to be most favorable. Although bioadditions such as yeast and corn extract did not increase the yield, high aeration conditions did (0.56 and 0.44 g O_2/1 h) (Andrienko and Zaichenko, 1998; Zaichenko and Dakhnovskii, 1975). The formation on wheat grain and straw was highest when the *S. chartarum* was cultivated at 30 and 37 °C, pH 6.8, and at a humidity between 20 and 25% (Azimov and Ibragimov, 1981).

4.0 Production and Import Volumes

No published information was found. To judge from the size of unit containers shipped from type culture collections, production and import volumes are minimal. Industries that might use *S. chartarum* could produce site-limited quantities, but little information was developed on this topic (see section 5.0).

5.0 Uses

Wang [Genencorp International (USA)] (2001) patented novel phenol-oxidizing enzymes naturally expressed by pure *Stachybotrys* cultures. *S. chartarum* strain MUCL 38898 was mentioned in the patent abstract. The enzymes are useful in detergents for bleaching stains (Wang et al., 2002).

Li et al. (2002) (Chinese) included *Stachybotrys atra* among microorganisms capable of degrading cellulosic and lignin waste products (corn straw powder and distillers grains were the examples) and producing crude protein for animal feeds.

Strain ATCC 16026 is used for fungus resistance testing of adhesives as specified by British Standard BS 3046, Appendix G (ATCC, 2004).

6.0 Environmental Occurrence and Persistence

6.1 Outdoor Environment

Cereal grains, oil seeds, tree nuts, and dehydrated fruits are susceptible to contamination by fungi. Moldy grains, cereals, and other agricultural products can harbor dangerous concentrations of trichothecenes. Molds that produce trichothecenes besides *S. chartarum* are *Fusarium*, *Trichoderma*, *Cephalosporium*, and *Vertici monisporium* (Steyn, 1995). Some specific plant hosts of *S. chartarum* include species of maple, walnut, pine, oak, and soybean (Farr et al. [USDA], undated). A macrocyclic trichothecene-producing isolate of *S. chartarum* was found associated with soybean roots sampled in the United States. PCR analysis of a 198-bp DNA fragment showed that the isolate (presumably ATCC MYA-2106) was completely identical with strain ATCC 9182 (62nd *S. chartarum* entry in online catalog; no source identification) and differed from strain UAMH 7900 (a Canadian indoor air sample collected by RCS strip) by only one nucleotide mismatch (Li [S] et al., 2002).

S. chartarum spores may remain viable for years, even decades, in the environment. Because the spores are usually produced in clusters and covered in dried slime, they are not expected to

become airborne readily unless they are both dry and disturbed or are attached to dust or other particulates (Hossain et al., 2004). In the outdoor environment, the sticky masses of *S. chartarum* spores may be disseminated by insects (Abbott, 2002) and probably other animals.

6.2 Indoor Environment

S. chartarum is generally reported as uncommon in outdoor and indoor air. Strains may be found in soil and may grow on cellulose-rich material such as hay, straw, grain, hemp, plant debris, dead roots, wood pulp, cotton and other cellulosic fabrics [e.g., rayons], paper, book binding glue, and materials from plant fiber processing facilities. Unless appropriate media for trapping and culturing are used, the occurrence of *S. chartarum* may be underreported (Hossain et al., 2004).

In homes, *S. chartarum* has been isolated from gypsum wallboard, pipe insulation, glass fiber wallpaper, and aluminum foil. Soiled surfaces and surfaces covered with susceptible paint or paper may facilitate mold growth in the absence of dampness (Hossain et al., 2004). Dust sampled near mold-contaminated sites in two U.S. homes had more than 1,000 *S. chartarum* conidia per milligram while dusts collected at sites farther from the contaminated site in these homes contained more than 100 conidia/mg. The conidia measurements in these samples were made by the real-time, fluorescence probe-based detection of PCR products (TaqMan^{TM} system) and agreed with values resulting from direct microscopic enumeration (Roe et al., 2001).

Day-to-day and even hour-to-hour fluctuations in airborne concentrations of fungal spores limit the representativeness of any collected air samples. Often mycotoxin concentrations determined from air samples exceed values expected from mycotoxin concentrations in spores. One likely explanation for this phenomenon is that dust particles saturated with mycotoxins are being aerosolized from mycotoxin-rich substrate materials (Hodgson and Lieckfield, 2003).

S. chartarum was isolated from air of homes in Southern California (3% of 68 homes) with a mean concentration of 0.3 spore/m^3 (maximum 52 spores/m^3). In 47 homes examined in London, *S. chartarum* was found in 13%. It was found in 4% of samples from central Scotland and in one of 50 Canadian homes (Pieckova and Jesenská, 1999).

In a Cleveland, OH, home where an infant developed IPH, initial *S. chartarum* spore concentrations in air were 0.1-9.3 spores/cm^3. Toxicity of the air particulates based on a protein synthesis inhibition assay was low. House dust contained 400-2,100 spores/mg. Although none of the nine *S. chartarum* isolates from the home produced highly toxic spores after growing for up to 30 days on wet wallboard, three isolates were consistently hemolytic. One of the hemolytic isolates appeared to be related to isolates from homes of other infants based on DNA banding patterns. Spore counts were high during remediation, but *S. chartarum* was not detectable after remediation (Vesper et al., 2000a).

In a field study of 150 wall cavities from 31 U.S. residential condominiums, air samples containing culturable fungi and spores were collected by "aggressive" sampling on 25-mm cassettes containing 0.8-µm mixed cellulose ester filters. Aggressive sampling involved tapping the wall around the probe inserted into the cavity with the palm of the hand or a rubber mallet. Fungi were identified microscopically and by culture. Sixty-six percent of the samples had

detectable, culturable fungi. *Aspergillus* and/or *Penicillium* were the only genera in 69% of the samples. *S. chartarum* spores were detected in 25% of the samples collected on cellulose ester filters, but only 2% of malt extract agar plates and 6% of cornmeal agar plates developed *S. chartarum* colonies (Spurgeon, 2003).

In laboratory experiments with low air velocity flow conditions, *S. chartarum* release of spores (viable and nonviable) and fragments from contaminated gypsum wallboard was directly proportional to airflow. Releases were indirectly [*sic*] proportional to relative humidity (Menestreza and Foarde [U.S. EPA, Research Triangle Park], 2004).

S. chartarum was found on surfaces at five sites of two zoological institutions. The abstract did not specify whether the sampling was in the exhibit area or the night-time holding areas. No association of poor animal health and *S. chartarum* was noted in the abstract, but poor animal health was significantly associated with air samples of *Penicillium chrysogenum* taken at 16 sites of five zoological institutions (Wilson and Straus, 2002).

Numerous fungal species, predominantly *Aspergillus* and *Penicillium*, were identified by culturing samples of biofilms lining potable water distribution pipes in Springfield, MO. Concentrations of yeasts ranged from 0 to 8.9 colony-forming units (CFU) per square centimeter while concentrations of filamentous fungi ranged from 4.0 to 25.2 CFU/m^2. Biofilm deposit thickness ranged from less than 1 to 4 mm. *S. chartarum* was found in amounts ranging from 2.8 to 4.8 CFU/m^2 in biofilms on iron pipes at two of eight sites sampled. Fungi were present primarily as spores, not hyphal growth. Some previous studies have reported fungal concentrations in drinking water, but no water analysis was presented in this publication. The presence of soil-inhabiting fungi in the pipes may have been due to ineffective filter barriers or to soil intrusion from damaged mains. Contamination also may have occurred during maintenance (Doggett, 1999).

S. chartarum isolates in the type culture collections mentioned in subsection 2.3 were often collected from the environment. Information about each isolate may include the substrate from which the culture was obtained, the country of origin, growth conditions (culture medium and temperature), the identity of the depositor, a literature reference, and the identity of the mycotoxins produced. For example, ATCC number 201210, designated as JS5802 or 58-02, was collected in a basement drain pipe in a Cleveland, OH, home (references given: Jarvis et al., 1998, and Vesper et al., 1999, 2000b). The strain produces satratoxin H, roridin L-2, and trichoverrol B. Other isolates include 58-17, which was sampled from dust below a Cleveland bathtub in a PH case home and 58-18, which was sampled from wallboard in a Cleveland PH case home. Many other isolates are simply listed as coming from the home of an infant with IPH such as the stachylysin-producing strain 58-06 or from a house not associated with IPH such as 58-08. A total of 16 Cleveland isolates are from eight case and eight control homes (ATCC, 2004). Note that strain 58-06 (ATCC 201863) has been reassigned to the species *S. chlorohalonata* (Andersen et al., 2003). Other sources in the ATCC collection include balsa wood that had been submerged in the Patuxent River in Maryland; tile and raw flax fiber from the Netherlands; paper from Italy; barley wheat, commercial pig feed, damaged gypsum board, and old cardboard from a dump from Finland; woodchuck dung, wood, paper, and tiles from Canada; soil from India; oats and straw from Hungary (numerous isolates); wheat straw from

Egypt; a sand dune sample from Brazil; and soybean roots from Illinois (ATCC, 2004). Isolates in the UAMH catalog (Sigler and Flis, 1998) are from the United States (feathers from a dead bird in a damp chamber, California); Namibia (desert sand); Canadian province Alberta (skin and hair from a cat, a leaf in soil, indoor air from RCS strips [also an isolate from Saskatchewan], gut contents of an alfalfa leafcutting bee, a trap door for plumbing access in a high-rise apartment); and Ontario (wood, paper, and tile). Environmental samples from the VTT Culture Collection were isolated from a recycled fiber pulp in Spain and from a moldy house in Finland (Suihko, 1999).

When removal and/or disposal is not practical, *S. chartarum*-contaminated materials may be decontaminated by alkaline treatment (e.g., sodium hydroxide or gaseous ammonia). Trichothecenes are stable to sunlight and UV light, X-rays, thermal treatment up to 120 °C, and acids (Hossain et al., 2004).

7.0 Human Exposure

Occupational exposures leading to stachybotrytoxicosis have been reported among farmers, workers at facilities processing malt grain or reprocessing moldy grain, textile mill workers using plant fibers, and workers at binder twine factories (Robbins et al., 2000). Handlers of contaminated hay and straw and remediators of mold-contaminated buildings may inhale spores unless they wear appropriate personal protective equipment (Abbott, 2002).

In a large horticulture facility, three female employees came in contact with black conidia masses of the fungus on decomposable pots made of recycled paper; skin inflammation and scaling occurred. Air sampling found 30-100 *S. chartarum* conidia per cubic meter of air (Dill et al., 1997). Potential exposure to satratoxin H exists with the use of cellulose-containing respirator filters in extremely humid (saturated) conditions (Pasanen et al., 1994).

For the general population, potential exposure to *S. chartarum* is via dermal contact, ingestion, and inhalation. The most common reports of exposure involve water-damaged buildings, including homes, office buildings, courthouses, hospitals, a hotel, and schools (Auger et al., 1994 [cited by Johanning et al., 1996]; Flappan et al., 1999; Hodgson et al., 1998; Trout et al., 2001). In one investigation of water-damaged sites of a building, the dominant colonizers were *S. chartarum* (10^3 to 10^5 visible conidia per cubic meter air); the water-damaged gypsum liner contained satratoxin (17 ng/mg) (Andersson et al., 1997).

Human exposure by inhalation of airborne spores and hyphal fragments in mold-contaminated buildings may occur when contaminated dust and surfaces are mechanically disturbed (HUD, 2001 draft). Air from 39 single-family homes in Cincinnati, OH, was sampled with Andersen two-stage viable microbial particle sizing sampler instruments; collected on different types of agar; and incubated in Petri dishes. The number of viable CFU/m^3 for all microorganisms was calculated based on the number of colonies per plate. *S. chartarum* was identified in samples from 13% of the homes, all of which had total microorganism concentrations >1000 CFU/m^3. The frequency of *S. chartarum* identification was significantly higher than reported in other publications due to differences in materials and methods (e.g., culture medium) and the nonrandom selection of sampling sites (Green et al., 2003).

An extensive survey of indoor and outdoor *S. chartarum* air concentrations by geographical regions in the United States found *S. chartarum* in 6% of the 1,717 buildings sampled in the period 1996-1998 (46% offices, 18% schools, 13% hospitals, 4% homes, 0% industrial sites, and 18% other). Reasons for sampling included odors, health complaints, proactive sampling, visual growth, and water damage. When present (in 6% of buildings sampled), the median indoor concentration of *S. chartarum* was 12 CFU/m^3 (95% confidence interval [CI], 12 to 118 CFU/m^3), and the median outdoor concentration (in 1% of buildings studied) when detected was also 12 CFU/m^3 (95% CI, 4 to 318 CFU/m^3). The median for all fungi in indoor air was less than 1,300 CFU/m^3 and the mean was 80 CFU/m^3. Rose Bengal agar (RBA) was used for 99% of the samples collected by Andersen N6 samplers. Malt extract agar was used for the other 1%. Plates were cultured at 23±3 °C in the laboratory and inspected four to 14 days after primary exposure. Fungal species were identified by macroscopic and/or microscopic examination. The detection limit was 12 CFU/m^3 (Shelton et al., 2002). [Note that these values may underestimate the presence of *S. chartarum*. Harrington (2003 abstr.) found that when an air-collected sample was grown on RBA at 22-23 °C, *S. chartarum* represented only 16% of the total fungal species after 10 days, in contrast to growth of the same air sample on a nutrient-poor medium (FPNA), when *S. chartarum* represented 92% of the total fungal species.] Chapman (2003) stated that an uncontaminated indoor environment is considered to have concentrations of total fungi of up to 100-1000 CFU/m^3. The best way to determine an individual's exposure is to use a Burkard personal air sampler by an experienced pollen and mold counter to screen indoor air.

Hardin et al. (2003) concluded after reviewing the literature that delivery of a toxic dose of mycotoxins in the indoor environment by inhalation is highly unlikely based on considerations of indoor concentrations, animal dose-response data, and dose-rate data.

Although clusters of IPH have occurred in Cleveland, OH (more than forty cases since 1993), Chicago, IL, (1993-1994), Detroit, MI (1992-1995), Ann Arbor, MI (1988-1993), and Milwaukee, WI (1993-1996), the Cleveland cases were apparently the first to be associated with the presence of *S. chartarum* (Vesper et al., 1999).

The Environmental Health Investigations Branch (EHIB) of the California Department of Health Services stated that serology tests for assessing exposure to *S. chartarum* have no clinical use; they cannot prove patient exposure to *S. chartarum* or its toxins. The kinetics of the decline of antibodies is not predictable and so-called-specific antibodies may have formed as a reaction to a different species. At the time this fact sheet was written, no urine or serum biomarkers for *S. chartarum* exposure had been validated (EHIB, 2000). Chapman (2003) noted, "Human response to fungal antigens may induce IgE or IgG antibodies that connote prior exposure but not necessarily a symptomatic state." Stachylysin, however, may provide the basis for a more specific assay. Stachylysin, the hemolytic agent produced by *S. chartarum*, has been quantified by U.S. EPA-developed enzyme-linked immunoassay (ELISA) methods in *S. chartarum* spores and mycelia and in mold-contaminated wallboard and dust and in the serum of five humans exposed to the fungus in water-damaged environments. The mean serum concentration was 371 ng/mL. The concentration of stachylysin per 10,000 *S. chartarum* conidia (said to be strain ATCC 6417 [cannot identify in online catalog]) was 6,071 compared to 0.12 to 3.1 per 10,000 when found in the conidia of five other species. Stachylysin was not detected in the conidia of 83 other common molds tested (Van Emon et al., 2003).

8.0 Regulatory Status

S. chartarum was included in the initial U.S. EPA Toxic Substances Control Inventory in 1978 as one of the 192 microorganisms reported as "manufactured" in the United States (U.S. EPA, 1994). The U.S. EPA, NIOSH, and state governments do not offer any approvals, certifications, or licenses for mold analysis.

A document by the State of North Carolina (2001) stated that no governmental body or industry has established guidelines for acceptable mold levels in normal and problem buildings. Because of data interpretation problems, the Centers for Disease Control and Prevention (CDC) has discouraged proactive monitoring. Analytical methodology differed among laboratories and a program for accreditation of analytical laboratories was needed. Since late 2001, some 49 laboratories have received accrediation by the American Industrial Hygiene Association (AIHA) Environmental Microbiological Laboratory Accreditation Program (EMLAP) to analyze mold spores and colonies in surface, bulk, and air samples. Numerous other laboratories participate in performance-based testing through the Environmental Microbiological Proficiency Analytical Testing Program (EMPAT) (AIHA, 2003).

The Institute of Inspection, Cleaning, and Restoration Certification (IICRC), a nonprofit certifying body, has a standard for mold remediation and recommends sampling before, during, and after remediation (SERVPRO Industries, 2001). The latest standard, IICRC S520, approved in late 2003, "provides a philosophical shift away from setting numerical mold contamination action levels. Instead, it establishes mold contamination definitions, descriptions and conditions (1, 2, 3), and general guidance, which, when properly applied, can assist remediators and others in determining criteria that trigger remediation activities or confirm remediation success." When a building has been contaminated at condition 3, requiring remediation, a full fungal ecological examination by qualified experts is recommended (Bishop, 2004a,b).

9.0 Toxicological Data

9.1 General Toxicology

Stachybotryotoxicosis, characterized by symptoms such as irritation of the mouth, throat, and nose, shock, dermal necrosis, hemorrhage, leukopenia, nervous disorder, and death, was first reported in horses in Russia during the 1930s. In the United States, the toxicosis was observed not only in horses but also in pigs, sheep, and calves. The first cases of human affliction were also reported by Russian investigators; people affected included workers in cottonseed oil processing plants, grain processing plants, grain elevators, and textile mills. Common symptoms included rash, conjunctivitis, bloody rhinitis, fever, headache, fatigue, and a burning sensation of the eyes and nasal passage. The history of this disease is presented in several reviews (e.g., CDC [1999]; Flappan et al. [1999], Nelson [2001], and Newberne [1974]).

The possible health effects of *S. chartarum* mycotoxins derive from their function as inflammatory agents, their ability to produce dermatitis, and their capability to be immunosuppressive, hemotoxic, and hemorrhagic (Albright, 2001). [See Appendix B for information on specific mycotoxins.] Animals (not otherwise specified) injected with *Stachybotrys* spores experienced bleeding in the brain, thymus, spleen, intestine, lung, heart, lymph node, liver, and kidney and in some cases death (Aerias, 2001b).

9.1.1 Human Data

Although numerous reports evaluating *S. chartarum* and potential occupation exposure have been published since the outbreak of stachybotryotoxicosis in Russia, the occurrence of trichothecene toxicosis in a Chicago home was the first to report a potential problem with the fungus in homes and other buildings. Over a five-year period, residents complained of headaches, sore throats, flu-like symptoms, recurring colds, diarrhea, fatigue, dermatitis, and general depression. Air sampling of this home revealed spores of *S. chartarum* (Croft et al., 1986; cited by Nelson, 2001). Recent epidemiologic investigations have suggested an association between high exposure to *S. chartarum* and toxic inflammatory effects in infants, in courthouse workers, and in office workers, as well as between *S. chartarum* and extreme chronic fatigue syndrome in hospital workers (Auger et al., 1994 [cited by Johanning et al., 1996]; Johanning, 1998; Montaña et al., 1997 [both cited by Johanning et al., 1999]; Etzel et al., 1998; Hodgson et al., 1998; Sorenson et al., 1996). In the office environment, the toxins satratoxin H and spirocyclic lactones were detected in samples of water-damaged sheetrock and in air samples. The prolonged and intense exposure to toxigenic *S. chartarum* and other atypical fungi was associated with reported disorders of the respiratory and central nervous systems, the mucous membranes, and with a few parameters of the cellular and humoral immune system (Johanning et al., 1996). [See **Table 1**.]

Table 1. Human Studies of *Stachybotrys* Mycotoxins

Study Subject(s)	Notes	Reference
health hazard evaluation at former Cowlitz County Health Department Building in Longview, WA	Employees reported upper respiratory problems, aches and pains in joints and muscles, and skin rashes. A decrease in all symptoms occurred after leaving the building. Inspection revealed the presence of *Stachybotrys*, *Aspergillus*, and *Penicillium*.	Boudrea and Perkner (1997)[a]
schoolchildren	*Stachybotrys* was isolated from 11 of 48 schools. Propagules of *Stachybotrys* and *Penicillium* species may be related to sick building syndrome.	Cooley et al. (1998)
infants with pulmonary hemorrhage or hemosiderosis	"Increased Incidence of Pulmonary Hemorrhage/Hemosiderosis in Infants"—an investigation of the possible causal mechanisms associated with the increased incidence in Cleveland, OH.	Dearborn (1999)
idiopathic pulmonary hemorrhage diagnosed in 37 infants in Cleveland, OH, area between 1993-1998	CDCP found an association with household exposure to *S. chartarum* and other fungi. (An additional 101 cases of acute idiopathic pulmonary hemorrhage have been reported in infants in the United States over the past five years.)	Dearborn et al. (1999)
health hazard evaluation of Kaiser Permanente at the Northlake Atrium in Atlanta, GA	Employees reported cough, sneezing, urticaria, shortness of breath, otitris, sinusitis, asthma, viral meningitis, and pseudotumor cerebri. Consultant reported mycotoxin association with *Stachybotrys* mold in sampling. NIOSH collected samples of fungal contamination and identified *Penicillium*, *Aspergillus*, *Alternaria*, and unidentified yeasts (no *Stachybotrys*).	Deitchman et al. (1994)[b]

Table 1. Human Studies of *Stachybotrys* Mycotoxins (Continued)

Study Subject(s)	Notes	Reference
child with pulmonary hemorrhage	*Stachybotrys* was isolated from the bronchoalveolar lavage fluid (BALF), as well as from his water-damaged home. Patient recovered completely upon removal from the home and subsequent cleaning of the house.	Elidemir et al. (1999)
pulmonary hemosiderosis in infants in Cleveland, OH	Many isolates of *S. atra* (not specified) and two isolates of a related toxigenic fungus *M. echinata* were isolated. The latter produced trichodermol and trichodermin	Jarvis et al. (1996)
39F and 14M (mean age, 34.8 yr) working for a mean of 3.1 yr at a water-damaged office building	Strong association with exposure indicators and significant difference between employees and controls (n=21) was found for lower respiratory symptoms, dermatological symptoms, eye symptoms, constitutional symptoms, chronic fatigue symptoms, and several enumeration and function laboratory tests, mainly of the white blood cell system. The proportion of mature T-lymphocyte cells was lower in employees than in controls. [satratoxin H and spirocyclic lactones identified in samples]	Johanning et al. (1996)
child with pulmonary hemorrhage	Strain of *S. chartarum* was isolated from the lung. Purification of stachyrase A was reported. [specific toxin: stachyrase A]	Kordula et al. (2002)
assessment for presence of *S. atra* fungi in homes in Cleveland, OH	*S. atra* fungi were more abundant in homes with cases of hemosiderosis compared to *Aspergillus* and *Cladosporium* fungi (mean concentration in surface samples significantly higher in homes with cases compared with those without).	Kullman and Sorenson (1996)
humans	Review evaluating epidemiological studies regarding *Stachybotrys*	Sudakin (2000)
adults with human health concerns such as nasal bleeding and infants with pulmonary hemosiderosis	Results support suggestion that stachylysin may be responsible for hemorrhaging in humans. Most *Stachybotrys* strains produced stachylysin in tryptic soy broth (TSB); all strains produced stachylysin when grown on TSB with 0.7% sheep's blood. [specific toxin: stachylysin]	Vesper and Vesper (2002)
health hazard evaluation of the Martin County Courthouse Complex in Stuart, FL	Severe contamination consisted primarily of *Stachybotrys*, as well as *Aspergillus* and *Penicillium*. Complaints (not specified in abstract) led to evacuation of the building.	Weber and Martinez (1996)[c]
health hazard evaluation at the Ronald McDonald House in Durham, NC	Employees reported diarrhea, stomach cramps, vomiting, dizziness, weakness, and fatigue. They attributed symptoms to exposure to fungi and their toxins while performing renovations on contamined building materials at the McDonald House.	Weber and Page (1999)[d]
acute pulmonary hemorrhage in an infant in Delaware	Delaware Division of Public Health obtained cultures from the infant's bedroom. DNA evidence of *S. chartarum* and growth of other molds associated with pulmonary hemorrhage (*Penicillium* and *Aspergillus*) were found.	Weiss et al. (2002)

Note: Data were extracted from search results via STN Int. biomedical files, PubMed, and TOXNET TOXLINE.

NIOSH Health Hazard Evaluation Reports No. [a]HETA 97-0048-2641, [b]HETA-92-0244-2373, [c]HETA 93-1110-2575, [d]HETA 98-0026-2745

In the mid-1990s, the case reports of PH and hemosiderosis in infants living in the Cleveland, OH, area generated an intensive study into the cause of the problem. Several factors were linked with this outbreak, including the presence of *S. chartarum* (Dearborn et al., 1999; Etzel et al., 1998). There is, however, considerable controversy about the role of *S. chartarum* in PH in the Cleveland incident and in human health in the indoor environment (e.g., see Ammann [undated]; Fung et al. [1998], Kuhn and Ghannoum [2003], Nelson [2001], and Sudakin [1998, 2000]).

From their investigations, the Centers for Disease Control and Prevention (CDC) concluded that evidence from currently available studies does not support a relation between acute PH or hemosiderosis and exposure to *S. chartarum* (CDC, 1999, 2000, 2002). [Findings on specific topics, such as study design and analytical methods, fungal sampling, as well as those on water damage assessment, can be found in detail at URL http://www.cdc.gov/od/ads/ref29.pdf.]

More recently, a case of PH in a one-month-old infant residing in a suburban home with water damage and the toxigenic mold *S. chartarum* was reported. Spores of the fungi were observed in the air collected from the infant's bedroom and in water-damaged building materials. Mycotoxin analysis of a contaminated closet ceiling found levels of the *S. chartarum* mycotoxins roridin L-2 (0.5 ng/cm^2), roridin E (0.7 ng/cm^2), and satratoxin H (3.2 ng/cm^2) (Flappan et al., 1999). An employee of a water-damaged hotel where *Stachybotrys* was one of the most predominant fungi exhibited bioaerosol lung damage; samples analyzed showed the presence of satratoxin, roridin, atranones, and phenylspirodrimanes (Trout et al., 2001). The first isolation of the fungus from human body fluids—i.e., the bronchoalveolar lavage fluid (BALF) of a child with PH—has also been described (Elidemir et al., 1999).

Sick building syndrome has been linked with elevated indoor levels of *Stachybotrys* (e.g., Cooley et al., 1998). In a 22-month study of 48 schools located along the Gulf of Mexico and the U.S. Atlantic seaboard, approximately 30% of staff (note: children were not given the surveys) reported symptoms only when in the building; most common were nasal drainage, congestion, and itchy, watery eyes. Over half of the staff also had increased incidences of respiratory infections such as tonsillitis, bronchitis, and pneumonia. Cleanup of the schools resulted in the decrease of health complaints (EHP Forum, 1999). Additionally, exposure to *Stachybotrys* in indoor air has been linked with the intensification of asthma (Flannigan et al., 1991; Kozak et al., 1979; both cited by Johanning et al., 1996). As with the conclusions regarding acute PH or hemosiderosis, associations between exposure to *Stachybotrys* and ill effects have been reported, but there is no proof of causation. For example, in a study of 50 Canadian homes in which occupants complained of respiratory or allergic symptoms, occupants of only six houses had building-related illness. Results from analyses of air and dust for fungus and its products in 37 of the homes showed *S. chartarum* was detected in only one house. In another example, the examination of water-damaged buildings in the Pacific Northwest as a result of neurobehavioral and upper respiratory health complaints found *S. chartarum* in only one of 19 cultures from building materials (Kuhn and Ghannoum, 2003). In a population-based incident case-control study to assess the risk of asthma in relation to specfic immunoglobulin G (IgG) antibodies to dampness-related microbes, no association was found to *S. chartarum* (Jaakkola et al., 2002). It is generally accepted that building-related asthma and an increased incidence of upper respiratory disease are associated with living or working in a moldy environment; these associations, however, are with molds that comprise many species and are not specific to *Stachybotrys* (Miller et al., 2003).

9.1.2 Chemical Disposition, Metabolism, and Toxicokinetics

S. chartarum is capable of producing at least six chemical classes of toxins: trichothecenes, phenylspirodrimanes, stachybocins, cyclosporins, atranones, and stachylysin. Conidia of *S. chartarum* instilled into the lungs of rat pups were rapidly cleared within the first 15 minutes (determined by dilution plate counting and quantitative PCR analysis). In lung homogenates of

four-day-old rat pups, viable conidia of the fungus decreased rapidly; only ~0.7% of viable fungal elements and 5% of fungal DNA were recovered after one week. Fungal DNA decreased exponentially and was only 0.03% by the fourteenth day after exposure. A half-life of ~1.5 days was calculated for fungal DNA in the lungs. The fungal load decreased even more rapidly in fourteen-day-old rat pups (Yike et al., 2003).

Stachylysin, a proteinaceous hemolytic agent, is distributed primarily in the inner wall of spores and mycelia of the fungus. When instilled in mouse and rat lung tissues, the spores (strains 58-06 and 58-17, respectively) are segregated in granulomas. Stachylysin diffuses out from the spores slowly indicating that spore production, release, or both are relatively slow (Gregory et al., 2003). Like stachylysin, satratoxin G, the major macrocyclic trichothecene mycotoxin produced by *S. chartarum*, is predominately localized in the inner wall layer of spores, primarily along the outer plasmalemma surface. In an *in vivo* mouse model, spores of *S. chartarum* strain 58-17 were inoculated intratracheally (i.t.). Satratoxin G was distributed in lysosomes, along the inside of the nuclear membrane in nuclear heterochromatin and rough endoplasmic reticulum (RER) within alveolar macrophages. Alveolar type II cells also showed modest labeling of the nuclear heterochromatin and RER (Gregory et al., 2004).

9.1.3 Short-Term Exposure Studies

The details of the following studies, except where noted, are presented in **Table 2**.

Intranasal Instillation

Adult mice intranasally instilled with up to 10^6 spores of *S. chartarum* strain s.72 (containing satratoxins G and H) exhibited severe alveolar, bronchiolar, and interstitial inflammation with luminal hemorrhagic exudates. Animals were lethargic, showed weight loss, and two of four mice died within three days. There was a significant decrease in platelet counts and a significant increase in leukocyte and erythrocyte counts, hematocrits, and hemoglobin concentrations. In contrast, *S. chartarum* strain s.29 caused no toxicity or mortality, and the inflammatory response was markedly less than that produced with strain s.72 (Nikulin et al., 1996 [cited by Dearborn et al., 1999 and Wilkins et al., 1998]; Nikulin et al., 1997). In a separate, similar study using both strains, a dose-dependent increase in monocytes, neutrophils, and lymphocytes in BALF was observed with no difference between exposure to either strain. Additionally, significant inductions in pro-inflammatoric cytokine mRNA expression and chemokine expression in the lungs were reported at the higher concentration (Leino et al., 2003).

In guinea pigs, pulmonary challenge with *S. chartarum* (study details not provided) produced catarrhal-desquamative inflammation in the tracheal and bronchial mucosa, epitheloid degeneration, focal bronchopneumonia, areas of serous hemorrhagic interstitial inflammation, atelectasis, and compensatory emphysema. Degenerative changes in the heart, liver, and kidneys were also observed (Samsonov and Samsonov, 1960; cited by CDC, 1999).

Table 2. Short-Term Exposure Studies with *S. chartarum*

Test Animal, Strain, Age, Number, and Sex	Strain Identification	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Intranasal Instillation				
Mice, strain n.p., 5-wk-old, number and sex n.p.	*S. chartarum* strains s.29 and s.72	intranasally instilled; 10^6 spores	With *S. chartarum* strain s.72, severe alveolar, bronchiolar, and interstitial inflammation (i.e., neutrophils, macrophages, lymphocytes) with luminal hemorrhagic exudates were observed. Animals were lethargic, showed weight loss, and 2 of 4 mice died within 3 days. With *S. chartarum* strain s.29, no toxicity or mortality was seen, and the inflammatory response was significantly less.	Nikulin et al. (1996; cited by Dearborn et al., 1999 and Wilkins et al., 1998)
Mice, BALB/c, 6- to 8-wk old, 8F per dose group	*S. chartarum* strains s.29 (nonsatratoxin-producing) and s.72 (satratoxin-producing)	intranasally instilled; 10^3 or 10^5 spores in PBS instilled 2x/wk for 3 wk; animals killed 24 h after last spore injection	*With both strains*, an influx of inflammatory cells into the BALF was observed, and the higher concentration caused a significant increase in monocytes, neutrophils, and lymphocytes into the lungs. Pro-inflammatory cytokine expression: The higher dose induced a significant increase in IL-1β (3-fold), IL-6 (6-fold), and TNF-α (4-fold) mRNA expression compared to controls. Chemokine expression: Macrophage-inflammatory protein (MIP)-1α/CCL3 showed ~10x higher mRNA expression in the lungs of mice that had received the higher dose, while both MIP-1β/CCL4 and monocyte-chemoattractant protein (MCP)-1/CCL2 showed ~6x higher expression levels. Lipopolysaccharide-induced chemokine (LIX)/CXCL5 was dose-dependently increased; a significant increase was seen with exposure to strain s.72 (16-fold) versus strain s.29 (threefold). The lower spore dose did not induce infiltration of inflammatory cells into the lungs. Additionally, it did not produce a significant increase in the expression of major Th1 (IFN-γ and IL-12 p40) or Th2 cytokines (IL-4 IL-5, and IL-10). No changes occurred in the bronchial responsiveness to inhaled methacoline between the different groups. No significant increase in the levels of total IgE and no detectable levels of specific IgE, IgG_{2a}, and IgG_1 were found.	Leino et al. (2003)
Mice, NMRI, 5-wk-old, 5M and 5F per dose group	*S. chartarum* strains s.29 (nonsatratoxin-producing) and s.72 (satratoxin-producing)	intranasally instilled; 10^3 to 10^5 spores instilled 2x/wk for 3 wk	Severe alveolar, bronchiolar, and interstitial inflammation with luminal hemorrhagic exudates were observed. The severity of the inflammation was dose-dependent. A significant decrease in platelet counts and a significant increase in leukocyte and erythrocyte counts, hematocrits, and hemoglobin concentrations were also seen.	Nikulin et al. (1997)

Table 2. Short-Term Exposure Studies with *S. chartarum* (Continued)

Test Animal, Strain, Age, Number, and Sex	Strain Identification	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Intratracheal Instillation				
Mice, Carworth Farms White (CFW), 21- to 28-days-old, 5-6M per dose group	*S. chartarum* strain 58-17 (isolated from a home in Cleveland, OH)	i.t.; $1.4x10^6$ conidia/mL = 70,000 spores per animal); mice killed at 24 or 48 h post-inoculation	No apparent clinical signs of respiratory distress or sickness were observed. Alveolar type II cells had significant ultrastructural changes compared to controls. Mitochondria were condensed and electron dense with separated cristae; nuclei were swollen and had scattered chromatin and a poorly defined nucleolus; and cytoplasm frequently had rarefaction and clustered electron dense granules. Plasmalemma of some mice had significant distension while some did not have microvilli. Lamellar bodies, increased in mice exposed for 48 h, were generally swollen and contained lamellae with irregular profiles. Vesicles and membranal figures were found in the alveolar space.	Rand et al. (2002)
Mice, CFW, 21- to 28-days-old, 5-6M per dose group	*S. chartarum* isolate ATCC 201211 (producing both satratoxin H and isosatratoxin F)	i.t.; $1.4x10^6$ conidia/mL = 70,000 spores per animal); mice killed at 12, 24, 48, 72, or 96 h post-inoculation	No apparent clinical signs of respiratory distress or sickness were observed. Weight gain for all animals anesthetized was significantly decreased from 12-24 h post-inoculation. Lungs had granulomatous inflammation reactions. At 12-24 h post-inoculation, granulomas had pleomorphic inflammatory cell infiltrate comprising fibroblasts, macrophages, and the occasional polymorphonucleocyte. At 48 h, polymorphonucleocyte abundance in granuloma increased. Alveolar air spaces, the area of which was significantly depressed, had cellular debris, macrophages with and without ingested spores, and the occasional lymphocyte. At 24-48 h, alveolar capillaries in some animals were dilated and engorged with erythrocytes. At 24-72 h, hemosiderin was detected in lung tissues.	Rand et al. (2003)

Table 2. Short-Term Exposure Studies with *S. chartarum* (Continued)

Test Animal, Strain, Age, Number, and Sex	Strain Identification	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, CFW, Swiss Webster, 3- to 4-wk old, 4-5M per dose group	*S. chartarum* isolate DAOM 225489 (from a Hawaiian hotel)	i.t.; $1.4x10^6$ conidia/mL; mice killed at 24 h post-inoculation	No apparent clinical signs of respiratory distress or sickness were observed. Conversion from the biologically active, heavy aggregate form of alveolar surfactant (H) to the light biologically inactive alveolar surfact form (L_{vivo}) was significantly increased compared to controls. Conversion of lamellar bodies (LB) to L_{vivo} was significantly decreased compared to controls but was significantly less than that for H to L_{vivo} conversion. Protein concentrations in raw BALF (RL), H, L_{vivo}, and LB were significantly elevated. The phospholipid concentrations of H and LB were significantly higher than controls, while in L_{vivo}, the mean phospholipid concentration was significantly lower.	Mason et al. (2001)
Mice, CFW, Swiss Webster, 21- to 28-days old, 5M per dose group	*S. chartarum* strains JS58-17 (trichothecene-producing) and JS58-06 (atranone-producing)	i.t.; 30, 300, or 3000 spores/g bw; mice killed at 3, 6, 24, 48, and 96 h post instillation	At the MD and HD, BALF composition reflected differences in strain toxicity, while at the LD, it was similar between the two. In BALF, total protein was significantly increased in lungs given the HD of strain 58-17. Albumin concentrations were significantly increased in lungs exposed to the HD of strain 58-06 and the MD and HD of strain 58-17. IL-1β, TNF-α, IL-6, and LDH levels were increased at all levels for both strains. Dose-dependent-like responses were seen for total protein, albumin, and IL-6 for both strains but were lacking for IL-1β and TNF-α levels in the 58-17 mice and for IL-1β in the 58-06 mice. No mice showed apparent clinical signs of illness or respiratory distress. The NOAEL was <30 spores/g bw for both strains.	Flemming et al. (2004)

Table 2. Short-Term Exposure Studies with *S. chartarum* (Continued)

Test Animal, Strain, Age, Number, and Sex	Strain Identification	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Rats, Sprague-Dawley, 4-days-old, 36 (not broken down by sex)	*S. chartarum* strain JS5817 (isolated from a home in Cleveland, OH)	i.t.; 1.0-8.0 x 10^5 spores/g bw; rats observed for up to 14 days	LD_{50} = 2.7x10^5 spores/g bw was calculated on day 11 of life. The lungs of all dead rats were significantly hemorrhagic; this was seen with high doses (4-8x10^5/g). The growth of survivors was dose-dependently impaired. One day after treatment, 28% of rats exhibited apneas. Four days later, pups had a significant increase in minute ventilation. At seven days, maximum alteration in pulmonary function was observed; treated mice had decreased respiratory rate, higher tidal volume, and increased Penh. At 1.1x10^5 spores/g, an increased respiratory resistance was observed. Lungs showed fresh hemorrhage, sparse hemosiderin-laden macrophages, and evidence of inflammation (thickened alveolar septa infiltrated by lymphocytes and mononuclear cells) and intra-alveolar macrophages. In all exposure groups, an increase in interstitial cellularity, consistent with mild chronic interstitial pneumonia, was seen. No histological changes were seen in the spleen, thymus, intestines, kidneys and brains of treated animals. With higher doses, mild focal necrosis occurred in the liver occasionally. In BALF, significant increases were seen in the numbers of macrophages (2-fold), lymphocytes (5-fold), and neutrophils (7-fold). Additionally, hemoglobin levels were increased 2-fold, while IL-1β increased by more than 6-fold and TNF-α by 30-fold. At day 12, no significant differences in these latter concentrations were seen.	Yike et al. (2002)
Rats, strain n.p., 11-wk-old, number and sex n.p.	*S. chartarum* (toxic strain)	i.t.; up to ~3x10^4 spores/g	A strong inflammatory response and pulmonary injury were observed. In BALF, LDH, hemoglobin, albumin, and leukocyte levels were increased.	Rao et al. (2000a,b; cited by Yike et al., 2002)
Inhalation				
Mice, BALB/c, age n.p., 4M per dose group	*S. chartarum* airborne emissions: E1=volatile chemicals and E2=volatile chemicals, spores, and mycelial fragments)	inhalation; loading factor of 5.12 m^2/m^3 for 30 minutes with covered dishes (Group E1) and 30 minutes with open dishes and a fan turned on (Group E2)	Respiratory rate was decreased ~15% in the E1 group and 7% in the E2 group. The time of pause (measurement of pulmonary irritation) was statistically significantly increased to ~10% in both groups, while an increases of ~10% in the time of break (sensory irritation) was observed only in the E2 group. [The detected VOCs were mainly siloxanes from the sealant for the window of the exposure chamber. Total satratoxins calculated as satratoxin H was ≥100 µg/aluminum dish.]	Wilkins et al. (1998)

Table 2. Short-Term Exposure Studies with *S. chartarum* (Continued)

Test Animal, Strain, Age, Number, and Sex	Strain Identification	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
Mice, (Hsd/Ola):NIH/S, 4-wk-old, 4M	aerosolized extract from nontoxic strain of *S. chartarum* ATCC 208877 [Diameter of the majority of aerosols from test solutions ranged from 0.3-3.0 µm.]	inhalation; single aerosol exposure of 125 mg/m^3; animals sacrificed on day 44	The time of break was increased by over 20%; the sensory irritation response occurred during the first aerosol exposure. During the control and recovery period, no sensory irritation was observed. Bronchoconstriction was not seen. No inflammation or tissue damage was seen in the nasal cavity.	Korpi et al. (2002)
Mice, BALB/cJBom, 6-wk-old, 8F per dose group	aerosolized extract from nontoxic strain of *S. chartarum* ATCC 208877 [Diameter of the majority of aerosols from test solutions ranged from 0.3-3.0 µm.]	inhalation; aerosol exposures of 44-79 mg/m^3 (Sc-immunized) or 44-83 mg/m^3 (nonimmunized) on days 26, 27, 33, 34, 40, and 41; animals sacrificed on day 44	The time of break was increased by over 20% in all groups receiving Sc aerosols; responses from Sc-immunized and nonimmunized mice were similar and occurred during the first aerosol exposure for both groups. During the control and recovery period, no sensory irritation was observed. Broncho-constriction was not seen, but slight pulmonary irritation in phase 1 (rapid shallow breathing) was observed in a few cases, similar to controls. In Sc-immunized and nonimmunized mice, no inflammation or tissue damage was seen in the nasal cavity, but 2 of 3 and 1 of 4 mice, respectively, had few macrophages in the small hilar lymph nodes of the lungs. In few nonimmunized mice, some granuloctyes (neutrophils, eosinophils) in the alveoli (2 of 4) and slight hyperplasia of the bronchial-associated/perivascular lymphoid tissue (1 of 4) were seen. At autopsy, white plaques were found on the peritoneal surface of the abdominal wall and on the liver of immunized mice; the liver had calcification, necrosis, and a large number of macrophages. Sc-immunized animals also had significantly increased production of serum Sc-specific IgG1, IgG$_{2a}$, IgA, and total IgE. Sc aerosol exposure of both Sc-immunized and nonimmunized mice significantly increased the total IgE level but had no effect on the production of increased levels of Sc-specific antibodies.	Korpi et al. (2002)

Abbreviations: ATCC = American Type Culture Collection; BALF = bronchoalveolar lavage fluid; bw = body weight; F = female(s); Ig = immunoglobulin; i.t. = intracheal(ly); M= male(s); n.p. = not provided; PBS = phosphate buffered saline; Sc= *S. chartarum*; VOC = volatile organic compound; wk = week(s)

Intratracheal Instillation

The lungs of juvenile mice exposed to spores of *S. chartarum* strain 58-17 ($1.4x10^6$ conidia/mL or 70,000 spores per animal) via a single i.t. inoculation showed signs of typical granulomatous inflammation reactions. Alveolar type II cells had marked ultrastructural changes (Rand et al., 2002, 2003). In a separate study, *S. chartarum* spores were found capable of significantly altering convertase activity in both the heavy aggregate form of alveolar surfactant and lamellar bodies in juvenile mice (Mason et al., 2001). Experiments with rats (up to $3x10^4$ toxic spores/g) also resulted in a strong inflammatory response and pulmonary injury in the animals (Rao et al., 2000a,b; cited by Yike et al., 2002).

Intratracheal exposure of mice to *S. chartarum* strains JS58-17 (trichothecene-producing) and JS58-06 (atranone-producing) spores (30, 300, or 3000 spores/g bw) produced changes in BALF total protein, albumin, pro-inflammatory cytokine, and lactate dehydrogenase (LDH) concentrations that were significantly strain-, dose-, and/or time-dependent. At the low dose, BALF composition was similar for both strains (Flemming et al., 2004).

Inhalation

[Intranasal/intratracheal administration of *S. chartarum* spores in mouse models were noted to be "fruitless in reflecting the respiratory effects during normal breathing," since the spores were not expected to reach the respiratory organs due to their size. For evaluations of inhalation exposure with rodents, a size of 8-10 x 4.5-5.5 µm *S. chartarum* spores would result in negligible alveolar exposure. Therefore, aerosols generated from extract that is free from large particles (including whole spores) were suggested for natural inhalation experiments (Korpi et al., 2002).]

Mice exposed to airborne emissions (i.e., volatile chemicals, spores, and/or mycelial fragments) from *S. chartarum* cultured in exposure chambers (loading factor, 5.12 m^2/m^3, corresponding to 2.8x the loading in a normal room with all surfaces covered by mold) exhibited weak sensory and pulmonary irritation effects (Wilkins et al., 1998). In naive and *S. chartarum*-immunized mice exposed to aerosolized extract from nontoxic *S. chartarum* strain ATCC 208877 (single exposure of 125 mg/m^3 or 44-83 mg/m^3 twice a week for three weeks), a sensory irritation response in the airways was also observed. Additionally, the extract activated the murine immune system in both groups; serum total immunoglobulin E (IgE) levels were increased (Korpi et al., 2002).

9.1.4 Synergistic/Antagonistic Effects

No data were available.

9.1.5 Cytotoxicity

The details of the following studies, except where noted, are presented in **Table 3**.

In a study in mouse RAW264.7 macrophages (study details not provided), several strains of *Stachybotrys* species stimulated an immediate increase in the production of reactive oxygen species (ROS) and caused the release of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) from the cells (Ruotsalainen et al., 1998). Treatment of the mouse macrophage cell line with spores/microbes from *S. chartarum* (10^4-10^6 spores/10^6 cells or 10^5-10^7 microbes/mL) induced an increase in TNF-α production and dose-dependent cytotoxicity. The spores and microbes, however, failed to induce IL-6 or nitric oxide (NO) production (Huttunen et al., 2003;

Table 3. Cytotoxicity Studies with *S. chartarum*

Test System	Strain Identification	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
mouse macrophage cell line RAW264.7	*S. chartarum* strain HT580 (spores collected from cores and liners of plasterboard)	incubation with 10^4, 10^5, or 10^6 spores/10^6 cells for 24 h	TNF-α production was induced. A dose-dependent cytotoxicity was observed; a statistically significant level was reached at the highest dose. Both measurements were higher for spores collected from cores than liners. An increase in NO or IL-6 production was not observed.	Murtoniemi et al. (2002)
mouse macrophage cell line RAW264.7	*S. chartarum* (strain n.p.)	incubation with 10^5, 10^6, or 10^7 microbes/mL for 24 h	TNF-α production was induced in a dose-dependent manner. A dose-dependent cytotoxicity was also observed. Strain did not cause IL-6 production.	Huttunen et al. (2003)
alveolar macrophages from male viral-antibody free Sprague-Dawley rats	*S. chartarum* Budapest 1	incubation with 0.052, 0.156, 0.52, 1.56, or 5.2 µg spore extracts/mL	A dose-dependent inhibition of protein synthesis was observed within 1 h. At the lowest dose, inhibition was 104.4% of control, and at the high dose, it was 5.2% of control. (Note: The ED_{50} could not be reported due to values >100% of control.)	Sorenson et al. (1987)*
alveolar macrophages from male viral-antibody free Sprague-Dawley rats	*S. chartarum* Debrecen 1132	incubation with 0.0014, 0.005, 0.014, 0.046, 0.138, 0.46, or 1.38 µg spore extracts/mL	A dose-dependent inhibition of protein synthesis was observed within 1 h. After 2 h, the ED_{50} was 0.05 µg/mL (95% CI, 0.04-0.07). At the low dose, inhibition was 92.7% of control, and at the high dose, it was 5.9% of control.	Sorenson et al. (1987)
thymocytes from female CD-1 mice, 6- to 8-wk old	*S. chartarum* Budapest 1	incubation with 0.020, 0.041, 0.081, 0.163, 0.325, or 0.65 µg spore extracts/mL	The SI was decreased in a dose-dependent manner. With increasing dose, the SI values were as follows: 33.7, 31.2, 26.2, 24.1, 6.5, and 2.3, respectively (SI for untreated control = 38.6). ED_{50} = 0.14 µg/mL (95% CI, 0.12-0.17)	Sorenson et al. (1987)
thymocytes from female CD-1 mice, 6- to 8-wk old	*S. chartarum* Debrecen 1132	incubation with 0.018, 0.036, 0.073, 0.145, 0.29, or 0.58 µg spore extracts/mL	The SI was decreased in a dose-dependent manner. With increasing dose, the SI values were as follows: 41.3, 33.3, 18.9, 6.2, 1.3, and 1.5, respectively (SI for untreated control = 38.6). ED_{50} = 0.06 µg/mL (95% CI, 0.05-0.17)	Sorenson et al. (1987)
human macrophage cell line28SC	*S. chartarum* (strain n.p.)	incubation with 10^5, 10^6, or 10^7 microbes/mL for 24 h	Slight production of IL-6 was induced. NO production was also induced. A dose-dependent cytotoxicity was observed; at the MD, a statistically significant difference was seen when compared to control. No effects on IL-1β and TNF-α production were seen.	Huttunen et al. (2003)

Table 3. Cytotoxicity Studies with *S. chartarum* (Continued)

Test System	Strain Identification	Route, Dose, Duration, and Observation Period	Results/Comments	Reference
human lung epithelial cell line A549	*S. chartarum* (strain n.p.)	incubation with 10^5, 10^6, or 10^7 microbes/mL for 24 h	Slight production of IL-6 was induced. NO production was also induced; statistical significance was seen at the HD compared to controls. A dose-dependent cytotoxicity was observed; at the HD, a statistically significant difference was seen when compared to control. No effects on IL-1β and TNF-α production were seen.	Huttunen et al. (2003)

*Authors also tested purified satratoxin H. In the rat alveolar macrophages, dose-dependent inhibition of protein synthesis was observed. In mice thymocytes, the SI was decreased in a dose-dependent manner.

Abbreviations: BALF = bronchoalveolar lavage fluid; bw = body weight; CI = confidence interval; ED_{50} = median 50% effective dose; h = hour(s); HD = high dose; IL = interleukin; i.t. = intracheal(ly); LD = low dose; LDH = lactate dehydrogenase; MD = mid dose; NO = nitric oxide; NOAEL = no observable adverse effect level; n.p. = not provided; SI = stimulation index; TNF-α = tumor necrosis factor alpha; wk = week(s)

Murtoniemi et al., 2002). In murine alveolar macrophages, however, *S. chartarum* spores (study details not provided) significantly induced NO release, with the amount of NO increasing in a time-dependent manner (Wang and Yadav, 2003 abstr.).

In rat alveolar macrophages, *S. chartarum* strains Budapest 1 (0.052-5.2 µg/mL) and Debrecen 1132 (0.0014-1.38 µg/mL) strongly inhibited protein synthesis. The spore extracts also inhibited mouse thymocytes proliferation (at concentrations of 0.020-0.65 and 0.018-0.58 µg/mL, respectively) (Sorenson et al., 1987).

In human macrophage cell line 28SC and human lung epithelial cell line A549, *S. chartarum* (10^5-10^7 microbes/mL) induced slight IL-6 production, as well as NO production; both, however, failed to produce significant amounts of IL-1β. Cytotoxicity was dose-dependently induced (Huttunen et al., 2003).

9.2 Reproductive and Teratological Effects

White CFW mice were orally administered low doses (3-4000 tissue culture units [TCU]) of *Stachybotrys* toxin from toxicogenic strains 71, 76, and 82 and nontoxicogenic strains 83 and 86 as infected grain, liquid growth medium, or partly purified toxin preparation. Administration was a single dose on day 3 or 5 of pregnancy or during a five-day period in the feed. The percentage of pregnancy for all treated animals was statistically lower than that for controls (70.7% versus 90.5%, respectively). The differences in frequency of nonpregnant mice between the control and the treated groups were also significant. At 100-4000 TCU, a statistically significant increase in the frequency of dead, resorbed, or stunted fetuses and a decrease in the average litter size of live fetuses were seen in treated animals compared to controls. Additionally, uteroplacental hemorrhages occurred in the test animals (Korpinen, 1974).

9.3 Carcinogenicity

No data were available.

9.4 Initiation/Promotion Studies

No data were available.

9.5 Anticarcinogenicity

No data were available.

9.6 Genotoxicity

No data were available.

9.7 Cogenotoxicity

No data were available.

9.8 Antigenotoxicity

No data were available.

9.9 Immunotoxicity

Female BALB/c mice were sensitized by four involuntary aspirations of five isolates of *S. chartarum*, combined and extracted to form a crude extract (SCE-1), over a four-week period. Increases in BALF total protein and LDH were observed. BALF total cells numbers were also elevated, and differential counts of BALF cells showed increased numbers of neutrophilia, eosinophilia, and lymphocytes. Serum and BALF total IgE levels and BALF IL-5 levels were significantly increased. Mice exposed to a single dose of SCE-1 showed inflammatory responses but not allergic responses (Viana et al., 2002).

9.10 Other Data

Five strains of *S. chartarum* isolated from PH case homes in Cleveland (51-08, 51-11, 58-02, 58-06, and 63-07) and the strain isolated from the lungs of a child with PH living in Houston produced significantly more of a hydroxamate-type siderophore (i.e., a natural iron binding compound that chelates ferric ions and then is taken up with the metal ion) than those from five control houses (strains 58-07, 58-16, 58-17, 58-18, and 63-01). This suggested that the strains may be more adept at using iron to help them survive in a host. Additionally, all case strains produced significantly more hemolytic activity than four of the five controls (activity of 58-18 was comparable to that of the case strains). When tested on sheep red blood cells (RBC), consistent production of the hemolysin was observed in the conidia from the Houston strain (Vesper et al., 2000b). The hemolysin produced from *S. chartarum* is stachylysin. Sheep erythrocytes began lysing six hours after treatment with stachylysin and completed the process by 48 hours (Vesper et al., 2001).

10.0 Structure-Activity Relationships

No data were available.

11.0 Online Databases and Secondary References

11.1 Online Databases

DIALOG Files
CEH (Chemical Economics Handbook)
DIOGENES

National Library of Medicine Databases
EMIC and EMICBACK (Environmental Mutagen Information Center)

STN International Files

AGRICOLA	CANCERLIT	MEDLINE	Registry
BIOSIS	CAPLUS	NIOSHTIC	RTECS
CA	EMBASE	NTIS	TOXCENTER
CABA	LIFESCI	PROMT	

TOXLINE includes the following subfiles:

Toxicity Bibliography	TOXBIB
International Labor Office	CIS
Hazardous Materials Technical Center	HMTC
Environmental Mutagen Information Center File	EMIC

Environmental Teratology Information Center File (continued after 1989 by DART)	ETIC
Toxicology Document and Data Depository	NTIS
Toxicological Research Projects	CRISP
NIOSHTIC®	NIOSH
Pesticides Abstracts	PESTAB
Poisonous Plants Bibliography	PPBIB
Aneuploidy	ANEUPL
Epidemiology Information System	EPIDEM
Toxic Substances Control Act Test Submissions	TSCATS
Toxicological Aspects of Environmental Health	BIOSIS
International Pharmaceutical Abstracts	IPA
Federal Research in Progress	FEDRIP
Developmental and Reproductive Toxicology	DART

Databases Available on the Internet
Code of Federal Regulations (CFR), National Archives and Records Administration

In-House Databases
Current Contents on Diskette®
The Merck Index, 1996, on CD-ROM

11.2 Secondary References
No secondary sources were cited.

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Appendix A: Units and Abbreviations

°C = degrees Celsius
µg/L = microgram(s) per liter
µg/m^3 = microgram(s) per cubic meter
µg/mL = microgram(s) per milliliter
µM = micromolar
AIHA = American Industrial Hygiene Association
ATCC = American Type Culture Collection
BALF = bronchoalveolar lavage fluid
bw = body weight
CI = confidence interval
ED_{50} = median 50% effective dose
F = female(s)
g = gram(s)
g/mL = gram(s) per milliliter
GC = gas chromatography
GLC = gas-liquid chromatography
h = hour(s)
HPLC = high-performance liquid chromatography
i.c. = intracerebral(ly)
Ig = immunoglobulin
IL = interleukin
i.p. = intraperitoneal(ly)
IPH = idiopathic pulmonary hemorrhage
i.t. = intratracheal(ly)
i.v. = intravenous(ly)
kg = kilogram(s)
L = liter(s)
LC = liquid chromatography
LD_{50} = lethal dose for 50% of test animals
M = male(s)
mg/kg = milligram(s) per kilogram
mg/m^3 = milligram(s) per cubic meter
mg/mL = milligram(s) per milliliter
min = minute(s)
mL/kg = milliliter(s) per kilogram
mm = millimeter(s)
mM = millimolar
mmol = millimole(s)
mmol/kg = millimoles per kilogram
mo = month(s)
mol = mole(s)
mol. wt. = molecular weight
NIOSH = National Institute for Occupational Safety and Health
nm = nanometer(s)
NO = nitric oxide

n.p. = not provided
PH = pulmonary hemorrhage
s = second(s)
s.c. = subcutaneous(ly)
TCU = tissue culture units
UV = ultraviolet
VOC = volatile organic compound
wk = week(s)
yr = year(s)

Appendix B: Summary Information on Major *S. chartarum* Mycotoxins

S. chartarum produces the following mycotoxins: satratoxins, roridins, verrucarins, and stachybocins (Albright, 2001). The majority of available toxicological studies focus on the macrocyclic trichothecenes (satratoxins G and H and roridins A and E). Nonmacrocyclic trichothecenes include trichodermol (roridin C), trichodermin, trichoverrins, and roridin L-2. The structures and characteristics (when available) for those compounds for which toxicity data were available for this report are found below. The fungus also produces phenylspirodrimanes (spirolactones and spirolactams), cyclosporin, atranones A-G, dolabellane diterpenes, and stachylysin.

Chemical Identification

Atranone B

Atranone B ([$C_{25}H_{34}O_7$]; mol. wt. = 446.59) is also called:

2*H*-1,4,8-Trioxabenzo[7,8]cycloundeca[1,2,3-*cd*]pentalene-3,9-dione,2*a*,6,7,7*a*,11*a*,12,14*a*,14*b*-octahydro-2-hydroxy-10-methoxy,2,5,7*a*,14-tetramethyl-11-(1-methylethyl)-, (2*R*,2*aS*,4*aE*,7*aS*,11*aR*,13*Z*,14*aR*,14*bR*)-rel- (9CI)

Isororidin A

Isororidin A ([$C_{29}H_{40}O_9$]; mol. wt. = 532.69) is also called:

Verrucarin A, 7′-deoxo-7′-[(1*S*)-1-hydroxyethyl]-, (7′*R*)- (9CI)
Spiro[16,18-methano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]benzopyran-17(18*H*),2′-oxirane], verrucarin A derivative
Verrucarin A, 7′-deoxo-7′-(1-hydroxyethyl)-, [7′*R*(*S*)]-

Roridin A

Roridin A ([$C_{29}H_{40}O_9$]; mol. wt. = 532.69) is also called:

Roridine A
Spiro[16,18-methano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]benzopyran-17(18*H*),2′-oxirane], verrucarin A derivative
Verrucarin A, 7′-deoxo-7′-[(1*R*)-1-hydroxyethyl]-, (7′*R*)- (9CI)
Verrucarin A, 7′-deoxo-7′-(1-hydroxyethyl)-, [7′*R*(*R*)]-

Roridin E

Roridin E ([$C_{29}H_{38}O_8$]; mol. wt. = 514.67) is also called:

Roridine E
Spiro[16,18-methano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]benzopyran-17(18*H*),2′-oxirane], verrucarin A derivative
Verrucarin A, 2′,3′-didehydro-7′-deoxo-2′-deoxy-7′-[(1*R*)-1-hydroxyethyl]-, (2′*E*,7′*R*)- (9CI)
Verrucarin A, 2′,3′-didehydro-7′-deoxo-2′-deoxy-7′-(1-hydroxyethyl)-, [2′*E*,7′*R*(*R*)]-

Satratoxin G

Satratoxin G ([$C_{29}H_{36}O_{10}$]; mol. wt. = 544.65) is also called:

Satratoxin H, 2′,3′-epoxy-2′,3′-dihydro-
Spiro[10,12:19,22*a*-dimethano-4*H*,5*H*,22*aH*-oxireno[8,9][1,6,12]trioxacyclooctadecino[3,4-*d*]-[1]benzopyran-11(10*H*),2′-oxirane]-2,14(1*aH*,19*H*)-dione,6,8*a*,11*a*,12,21,22-hexahydro-23-hydroxy-19-(1-hydroxyethyl)-7,11*a*-dimethyl- (9CI)

Spiro[10,12:19,22*a*-dimethano-4*H*,5*H*,22*aH*-oxireno[8,9][1,6,12]trioxacyclooctadecino[3,4-d]-[1]benzopyran-11(10*H*),2′-oxirane], satratoxin H derivative

Satratoxin H

Satratoxin H ([$C_{29}H_{36}O_9$]; mol. wt. = 528.65) is also called:

Spiro[5,9:16,18-dimethano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]-benzopyran-17(18*H*),2′-oxirane]-3,14(9*H*)-dione,6,7,16,16*a*,19*a*,22-hexahydro-25-hydroxy-9-[(1*S*)-1-hydroxyethyl]-16*a*,21-dimethyl-, (2′*R*,4*E*,9*R*,10*E*,12*Z*,16*R*,16*aS*,18*R*,19*aR*,23*aR*,25*R*)- (9CI)

Stereoisomer of 6,7,16,16*a*,19*a*,22-hexahydro-25-hydroxy-9-(1-hydroxyethyl)-16*a*,21-dimethylspiro[5,9:16,18-dimethano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*]-[1]benzopyran-17(18*H*),2′-oxirane]-3,14(9*H*)-dione

Verrucarin A (7CI, 8CI, and 9CI)

Verrucarin A ([$C_{27}H_{34}O_9$]; mol. wt. = 502.61) is also called:

Muconomycin

Muconomycin A

[4*S*-(4*R**,5*S**,10*E*,12*Z*,16*S**,16*aR**,17*S**,18*S**,19*aS**,23aS*)]-4,5,6,7,16,16*a*,19*a*,22-Octahydro-4-hydroxy-5,16*a*,21-trimethylspiro[16,18-methano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]benzopyran-17(18*H*),2′-oxirane]-3,9,14-trione

Spiro[16,18-methano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]benzopyran-17(18*H*),2′-oxirane]-3,9,14-trione,4,5,6,7,16,16*a*,19*a*,22-octahydro-4-hydroxy-5,16*a*,21-trimethyl-, [4*S*-(4*R**,5*S**,10*E*,12*Z*,16*S**,16*aR**,17*S**,18*S**,19*aS**,23*aS**)]-

Spiro[16,18-methano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]benzopyran-17(18*H*),2′-oxirane], verrucarin A derivative

Verrucarine A

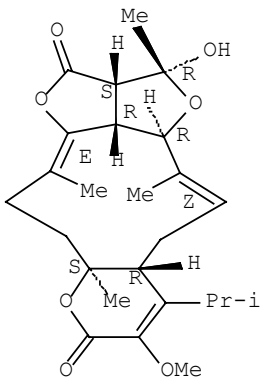
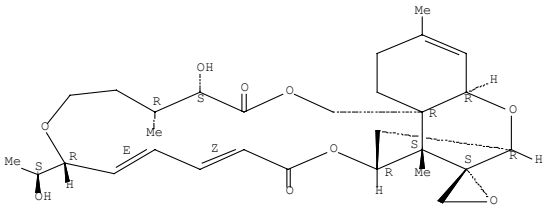
Verrucarin J (7CI, 8CI)

Verrucarin J ([$C_{27}H_{32}O_8$]; mol. wt. = 484.59) is also called:

Muconomycin B

Verrucarin A, 2′,3′-didehydro-2′-deoxy-, (2′*E*)- (9CI)

Spiro[16,18-methano-1*H*,3*H*,23*H*-[1,6,12]trioxacyclooctadecino[3,4-*d*][1]benzopyran-17(18*H*),2′-oxirane], verrucarin A derivative

Atranone B [227597-62-8]	Isororidin A [84773-08-0]
	

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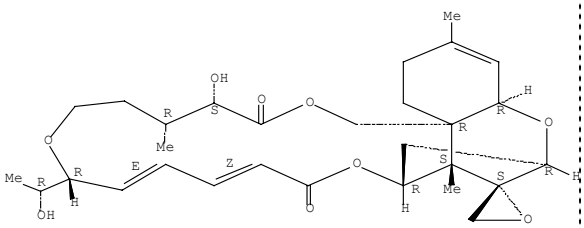

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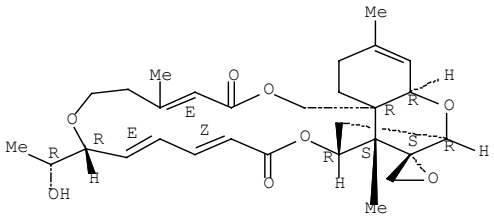

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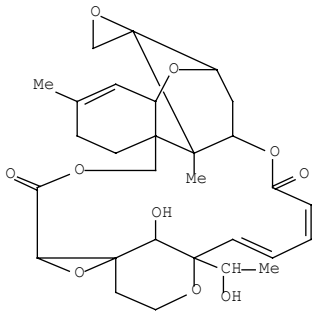

Satratoxin H
[53126-64-0 or 54385-59-0]

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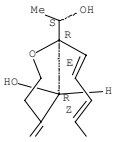

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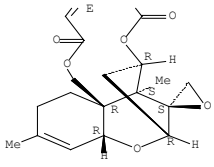
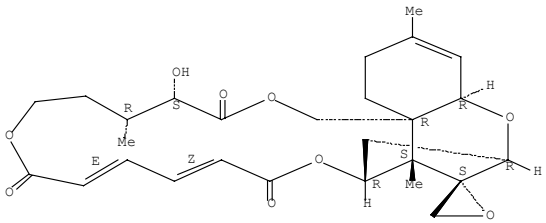
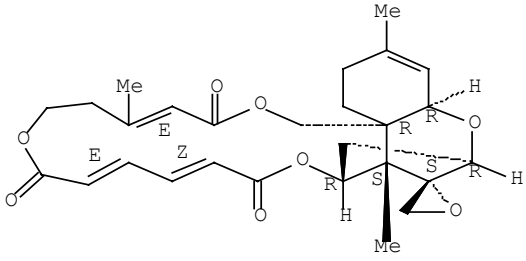


Verrucarin A
[3148-09-2]



Verrucarin J
[4643-58-7]



Source: Registry (2002)

Mycotoxins Associated with *S. chartarum*

Mycotoxin	CASRN	OTHER FUNGAL SOURCES	CHEM CLASS trichothecene	CAPLUS	TOXLINE	MEDLINE	AGRICOLA	NIOSHTIC	EMBASE	CABA	BIOSIS	ESBIOBASE	IPA	NAPRALERT	LIFESCI	TOXCENTER	Total
Atranone A	227597-61-7			3													3
Atranone B	227597-62-8			3													4
Atranone C	227597-63-9			3													3
Atranone D	227597-64-0			3													3
Atranone E	227597-65-1			3													3
Dechlorogriseofulvin	3680-32-8	√		17		2	1		2	1	4		1	3	1	2	17
Epidechlorogriseofulvin	177697-34-6	√		2		1										1	2
Epiroridin E	249744-08-9	√		3		1										2	3
Isororidin A	84773-08-0	√		4							1			2		3	6
Isororidin E	64726-84-7	√	√	12		1	2			1	1			7		7	19
Isosatratoxin F	220983-34-6			5		4										1	5
Isosatratoxin G	163648-23-5			2			1									1	2
S-Isosatratoxin H	163648-24-6			1			1										1
Phenylspirodimane(s)		√			2											1	3
Roridin	11113-83-0																
Roridin A; Roridine A [Only in combination with *Stachybotrys* except CAPLUS]	14729-29-4	√	√	156													?
Roridin C; Roridine C; Trichodermol	2198-93-8			47													31*
Roridin E; Roridine E	16891-85-3	√	√	68		16	9	1	8	20	9			28		27	118
Roridinic acid	14682-20-3	√		1		1										1	2
Roridin L 2; Roridin L-2; (+)-Roridin L-2	85124-22-7	√	√	4			1		1	1	1			6		2	12
Satratoxin OR Satratoxins	113689-89-7				38	29	5	2	11	38	6	3		9	2	26	131
Satratoxin F	73513-01-6 replaced: 53126-10-6	√	√	6		2	1		1		2			4		4	14
Satratoxin G	53126-63-9		√	38		15	2	2	2	4	3			7	1	18	54
Satratoxin H	53126-64-0 replaced: 54385-59-0			46		21	1	3	5	9	5			8	1	24	77
Solaniol; Neosolaniol [Only in combination with *Stachybotrys*]	36519-25-2			219													[21]
Stachybocin A	158827-60-2			4													
Stachybocin B	158827-61-3			4													
Stachybocin C	158827-62-4			4													
Stachybocin D	174423-35-9			1													
Stachybotramide; Stachybotrin	149598-71-0			5		3			4	2						3	12
Stachybotrydial; Mer-NF 5003F; NF 5003F; F 1839M	149598-70-9			9		3			1							4	8
Stachybotrylactam	163391-76-2			1				1								1	2
Stachybotrylactone; Stachybotrolide	149691-31-6			5				1								4	5
Stachybotryotoxin; Stachybotriotoxin	12698-94-1	√		15		5	3		12	18	3					11	52
Stachybotrys atra; *Stachybotrys alternans*; *Stachybotrys chartarum*	67892-26-6				166												
Trichoverrin A	76739-70-3	√	√	5		1								4		4	9
Trichoverrin B	76685-82-0	√	√	8		1				1	2			6		6	16
Trichoverrol A	76739-71-4		√	10		1					3			4		8	16
Trichoverrol B	76685-83-1		√	9							2			3		7	12
Verrucarin; Verrucarine [Only with *Stachybotrys*]	54018-05-2			17		7	2		2	7	3			2	2	4	29
Verrucarin A	3148-09-2	√	√														
Verrucarin B	2290-11-1																
Verrucarin C	4643-58-7																
Total after automated duplicate detection						62	22	3	34	78	29	1	1	45	4	72	351
Total after automated plus manual duplicate detection						62	14	3	31	71	26	1	1	21	1	68	302
Total after matched with duplicate TOXLINE (12) and CA records (31) already printed						60	13	2		70	26	1	1	21	1	64	250

Acute Toxicity

Route	Species (sex and strain)	LD_{50} (mg/kg)	Reference(s)	Acute Exposure Studies
Roridin A				
i.v.	mouse (sex and strain n.p.)	1.0 (1.9 µmol/kg)	Wannemacher and Wiener (1997)	In male rats, a sublethal dose of roridin E (2 mg/kg body weight [bw]) significantly increased glucose-6-phosphatase in the liver. A combination of the compound and linoleic acid (300 µmol/kg bw) significantly decreased blood glucose and glutathione levels and kidney superoxide dismutase and glucose-6-phosphatase levels and markedly increased lipid peroxidation in the liver (Omar et al., 1997).
s.c.	rat (sex and strain n.p.)	0.9±0.14 (1.7 µmol/kg)	Bergmann et al. (1992; cited by Bergmann and Yagen, 1993)	
Satratoxin G				
i.p.	mouse (ddY, M)	1.23±0.08 (2.26 µmol/kg)	Yoshizawa et al. (1986)	In four-week-old ddY male mice, a single intraperitoneal (i.p.) injection of satratoxin G or H (dose not provided) produced extensive ulceration of the small intestine and mild damage of the lymphoid and hematopoetic tissues. Additionally, satratoxin G caused vascular and fatty degeneration of the hepatic cells (Yoshizawa et al., 1986). Five-week old adult mice intranasally instilled with 10^6 *S. chartarum* spores containing satratoxins G and H exhibited severe alveolar, bronchiolar, and interstitial inflammation (i.e., neutrophils, macrophages, lymphocytes) with luminal hemorrhagic exudates; development of immunoglobulin G (IgG) antibodies to the fungus did not occur (Nikulin et al., 1996; cited by Dearborn et al., 1999).
Satratoxin H				
i.p.	mouse (sex and strain n.p.)	1.0 (1.9 µmol/kg)	Wannemacher and Wiener (1997)	
	mouse (sex and strain n.p.)	1.4 (2.6 µmol/kg)	Bata et al. (1986b)	
	mouse (ddY, M)	5.69±0.43 (10.8 µmol/kg)	Yoshizawa et al. (1986)	

Acute Toxicity

Route	Species (sex and strain)	LD_{50} (mg/kg)	Reference(s)	Acute Exposure Studies
Stachybotriotoxin A*				
i.p.	mouse (sex and strain n.p.)	51.6 mg/kg	Palyusik (1970)	Mice administered stachybotriotoxin (dose not provided) not only showed circulatory alterations and dystrophies similar to those noted in carbon tetrachloride poisoning but also had two distinct functional zones in the liver lobules (Mitroiu et al., 1973). A separate study found peritonitis in mice, rats, and guinea pigs. Rats also had enteritis and some liver hematomas (Palyusik, 1970). Intradermal injection of the material suspended in propylene glycol into rabbits caused local inflammation, and at high concentration, necrosis (Palyusik, 1970). Stachybotriotoxin was most toxic on the skin when in cultivation for 12 days (Azimov and Ibragimov, 1981). Additionally, 1 mg/mL caused inflammation of the rabbit ear skin (Seredyuk et al., 1972).
	rat (sex and strain n.p.)	44.5 mg/kg		
	guinea pig (sex and strain n.p.)	62.4 mg/kg		
	1-day-old chick (sex and strain n.p.)	92.0 mg/kg		
	6-day-old embryo (sex and strain n.p.)	0.359 mg/kg		
	10-day-old embryo (sex and strain n.p.)	1.7575 mg/kg		
Verrucarin A				
i.v.	mouse (sex and strain n.p.)	1.5 (3.0 µmol/kg)	Wannemacher and Wiener (1997)	
	rat (sex and strain n.p.)	0.8 (2 µmol/kg)		
	rabbit (sex and strain n.p.)	0.54 (1.1 µmol/kg)		
s.c.	rat (sex and strain n.p.)	1.7±0.3 (3.4 µmol/kg)	Bergmann et al. (1992; cited by Bergmann and Yagen, 1993)	

*Substance was identical to stachybotriotoxin A; molecular formula was not available in the Registry file.

Abbreviations: bw = body weight; i.p. = intraperitoneal(ly); i.v. = intravenous(ly); LD_{50} = lethal dose for 50% of test animals; M = male; n.p. = not provided; s.c. = subcutaneous(ly)

Cytotoxicity (ED_{50}) Values for Several *S. chartarum* Mycotoxins

Compound	Dose; ED_{50} (µg/mL)	Pathological System	Results
satratoxin G	6.3	CA-SK-HEP-1[a]	weakly active
satratoxin H	1.6	CA-SK-HEP-1	active
satratoxin J	0.2	CA-SK-HEP-1	active
roridin A	>0.1	CA-SK-HEP-1	active
roridin J	0.4	CA-SK-HEP-1	active
verrucarin A	0.01	CA-SK-HEP-1	active
	>50.0	CELLS-MDCK[b]	inactive
	12.5	HELA CELLS[c]	weakly active
verrucarol	>50.0	CA-SK-HEP-1	inactive
neosolaniol	12.5	CA-SK-HEP-1	weakly active

[a]liver cancer cell line; [b]Madin-Darby Canine Kidney, kidney epithelial cell line; [c]cancer cell line
Source: Hanelt et al. (1994)

- Lymphocyte cultures isolated from the spleens of male CD-1 mice and incubated with up to 10^{-4} M of roridins A, D, and E and verrucarins A and J, LC_{50}s for cytotoxicity went up to $20.1x10^{-6}$ M (Hughes et al., 1990).
- Roridin A inhibited blastogenic responses to concanavalin A, *Escherichia coli* lipopolysaccharide, phytohemagglutinin P, and pokeweed mitogen at concentrations 2-5 orders of magnitude lower than its LC_{50} (Hughes et al., 1990).
- Roridin A and satratoxin G were cytotoxic to Hep-2 cells (Okazaki and Yoshizawa, 1997).
- Stachybotriotoxin had no effect on the mitotic activity of cultures of Vero cells and Hep-2 cells (Nadgornaya et al., 1981).
- Atranone B was moderately phytotoxic and weakly cytotoxic with mammalian cell lines (Abbas et al., 2002).
- In two myeloid models, RAW 264.7 murine macrophage and U937 human leukemic cells, cytotoxicity of trichothecenes was evident according to the following order: satratoxin G, roridin A, and verrucarin A > T-2 toxin, satratoxin F, and satratoxin H > nivalenol and vomitoxin (Yang et al., 2000).

Genotoxicity

- Satratoxin G and roridin A did not induce unscheduled DNA synthesis in HeLa S-3 cells (Okazaki and Yoshizawa, 1997).
- Stachybotriotoxin was not mutagenic in *Drosophila melanogaster* larvae (somatic mutagenesis) (0.4, 4, or 11 mM) or human embryonic liver or adult rat liver cell cultures (DNA repair synthesis) (0.006, 0.01, 0.05, 0.3 mM) (Belitsky et al., 1985).

Immunotoxicity

- In EL-4 thymoma cells, incubation with low doses of satratoxin H, isosatratoxin F, roridin A, and verrucarin A significantly increased IL-2 concentrations at 24 and 72 hours. At higher concentrations, they significantly decreased the levels. All compounds decreased cell viability in a concentration-dependent manner (Lee et al., 1999).
- Oral, intraperitoneal (i.p.), and i.t. administration of trichothecenes from cultures of an *S. chartarum* strain to BCBA-F1 mice (1.25-20 mg/kg bw) and WAGb1 rats (0.16-2 mg/kg bw) caused temporary increases in lymphocyte and granulocyte counts. Pathological examination showed that both species had severe thymus damage during the first days after dosing (Bergers et al., 1987).
- In mice, a single i.p. injection (dose=1/2 LD_{50}) of a toxin from *S. chartarum* caused a reduction in the blood lymphocyte count 24 hours after administration without a reduction in spleen and thymus weights (Fromentin et al., 1982).
- In mice with experimental stachybotryotoxicosis, stachybotriotoxin caused mild functional injury of the mononuclear phagocyte system, delimitation of adaptability, and severe functional and morphological damage to the lymphoid system (Bojan et al., 1976).
- Rabbits immunized with a roridin A-hemisuccinate derivative coupled to human serum albumin produced antibodies in the sera (Martlbauer et al., 1988).

Structure-Activity Relationships

The following are mycotoxins potentially found in indoor environments, as indicated by the American Conference of Governmental Industrial Hygienists (ACGIH):

Mycotoxin	Produced By	Health Effects
Aflatoxins	*Aspergillus flavus* and other *Aspergillus* spp.	forms DNA adducts, hepatotoxic, carcinogenic, immunotoxic
Alternariol	*Alternaria* spp.	cytotoxic, teratogenic
Citrinin	*Penicillium expansum*	carcinogenic
Chaetoglobosins	*Chaetomium globosin*	inhibits cell division
Cytochalasins	*Aspergillus clavatus*	inhibits cells division
Epicladosporic acid	*Cladosporium* spp.	immunosuppressive
Fumonisins	*Fusarium* spp.	inhibits sphingolipid biosynthesis, neurotoxic, hepatotoxic, nephrotoxic, carcinogenic
Fumitremorgens	*Aspergillus fumigatus*	tremorgenic
Gliotoxin	*Aspergillus fumigatus*	blocks membrane thiol groups, immunosuppressive, cytotoxic
Griseofulvins	*Memnoniella*, *P. griseo-fulvum*, and *P. viridicatum*	hepatotoxic, tumorigenic, teratogenic
Mycophenolic acid	*Penicillium brevicompactum*	blocks inosine monophosphate dehydrogenase, immunosuppressive
Ochratoxins	*Aspergillus ochraceus* and *Penicillium viridicatum*	forms DNA adducts, inhibits protein synthesis (phenylalanyl-t-RNA synthetase), nephrotoxic, carcinogenic
Patulin	*Paecilomyces variatii* and *P. expansum*	inhibits potassium uptake, possible carcinogen

Mycotoxin	Produced By	Health Effects
Sporidesmin	*Pithomyces chartarum*	hepatotoxic
Sterigmatocystin	*Aspergillus versicolor*	hepatotoxic, carcinogenic
Tenuazoic acid	*Alternaria alternata* and *Phoma soghina*	nephrotoxic, hepatotoxic, hemorrhagic
Trichothecenes	*Fusarium* spp.	inhibits protein and nucleic acid synthesis, immunosuppressive, hemotoxic, hemorrhagic
Verrucosidin	*Penicillium polonicum*	neurotoxic

Sources: Burge and Ammann (1999); Jacobsen et al. (1993); both cited by va.gov (Undated) [URL: http://www.va.gov/vasafety/OSH-Issues/OtherOSHIssues/IndoorFungi.htm]

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Appendix C: Designations for *S. chartarum* Strains

ATCC #	Designation	Isolation	Mycotoxins	Source(s)
Cleveland, OH[a]				
201210	JS5802 [58-02]	basement drain pipe in home	roridin L-2 satratoxins H trichoverrol B	Jarvis et al. (1998); Vesper et al. (1999, 2000b); Sorenson (personal communication)
201211	JS5817 [58-17]	dust below bathtub	isosatratoxin G roridin L-2 satratoxins H *low stachylysin producer*	Jarvis et al. (1998); Sorenson (personal communication)
201212	JS5818 [58-18]	wallboard	isosatratoxin F isosatratoxin G roridin E roridin L-2 satratoxin G satratoxin H	Jarvis et al. (1998); Vesper et al. (2000b); Sorenson (personal communication)
201858	JS5105 [51-05]	home of infant with IPH		Vesper et al. (1999)
201859	JS5106 [51-06]	home of infant with IPH		Vesper et al. (1999)
201860	JS5108 [51-08]	home of infant with IPH		Vesper et al. (1999, 2000b)
201861	JS5111 [51-11]	home of infant with IPH		Vesper et al. (1999)
201862	JS5119 [51-19]	home of infant with IPH		Vesper et al. (1999)
201863	JS5806 [58-06]	home of infant with IPH	*high stachylysin producer*	Vesper et al. (1999, 2000b, 2001)
201864	JS5807 [58-07]	house not associated with IPH (control house strain)		Vesper et al. (1999)
201865	JS5808 [58-08]	house not associated with IPH (control house strain)		Vesper et al. (1999)
201866	JS5815 [58-15]	house not associated with IPH (control house strain)		Vesper et al. (1999)
201867	JS5816 [58-16]	house not associated with IPH (control house strain)		Vesper et al. (1999)
201868	JS5819 [58-19]	house not associated with IPH (control house strain)		Vesper et al. (1999)
201869	JS6301 [63-01]	house not associated with IPH (control house strain)		Vesper et al. (1999)
201870	JS6307 [63-07]	home of infant with IPH		Vesper et al. (1999, 2000b)
Finland[a]				
26384	682	barley	mycotoxin, n.o.s.	Korpinen (1973); Korpinen and Ylimaki (1972)
26385	7011a II-12	wheat	mycotoxin, n.o.s.	Korpinen (1973); Korpinen and Ylimaki (1972)
26386	69116 II-14	commercial pig feed	mycotoxin, n.o.s.	Korpinen (1973); Korpinen and Ylimaki (1972)
48610	10T/72 [NC-5]	barley	mycotoxin, n.o.s.	Harrach et al. (1982); Vesper et al. (1999)
Hungary[a]				
34915	Jaszapati	oats	mycotoxin, n.o.s.	Szathmary et al. (1976)
34916	Miskolc	oats	aflatoxin B2	Szathmary et al. (1976)
34917	Petrivente	oats	mycotoxin, n.o.s.	Szathmary et al. (1976)
62752	S-2 [Budapest 1]	straw	trichothecenes	Jarvis et al. (1986)
62753	S-3 [Budapest 2]	straw	trichothecenes	Jarvis et al. (1986)
62754	S-4 [Miskolc 2]	straw	trichothecenes	Jarvis et al. (1986)
62755	S-6 [Miskolc 30	straw	trichothecenes	Jarvis et al. (1986)

ATCC #	Designation	Isolation	Mycotoxins	Source(s)
62756	S-5 [Belvardgyula]	straw	trichothecenes	Jarvis et al. (1986)
62757	S-7 [Karcag 1]	straw	trichothecenes	Jarvis et al. (1986)
62758	S-8 [Karcag 2]	straw	trichothecenes	Jarvis et al. (1986)
62759	S-9 [Debrecen 1132]	straw	trichotheccnes	Jarvis et al. (1986)
62760	S-10 [Debrecen 23]	straw	trichothecenes	Jarvis et al. (1986)
62761	S-12 [My 276]	straw	trichothecenes	Jarvis et al. (1986)
62762	S-13	not provided	trichothecenes	Jarvis et al. (1986)
62763	S-14 [Petrivente]	straw	trichothecenes	Jarvis et al. (1986)
62764	S-15 [Perkata]	not provided	trichothecenes	Jarvis et al. (1986)
62765	S-17 [Nagytarcsa]	straw	S-isostratoxin H epiisororidin E isororidin E isosatratoxin G roridin L-2 trichothecenes	Jarvis et al. (1986, 1995)
Egypt[a]				
46994	61	wheat straw	satratoxin	El-Kady and Moubasher (1982)
46996	128	wheat straw	satratoxin	El-Kady and Moubasher (1982)
MYA-2106	SC-SB1	root of soybean, Glycine max		Li and Hartman (2000); Li [S] et al. (2001)
Miscellaneous[b]				
208877				Korpi et al. (2002)
	IBT 14916	n.p.	satratoxin H	Wilkins et al. (198)
	s.29	rice flour agar	stachybotrylactone stachybotrylactam	Nikulin et al. (1996); Leino et al. (2003)
	s.72 [NRRL 6084]	rice flour agar	satratoxins G and H stachybotrylactone stachybotrylactam	Nikulin et al. (1996); Leino et al. (2003)
	DAOM 225489	Hawaiian hotel		Mason et al. (2001)
	HT580	cores and liners of plasterboard		Murtoniemi et al. (2002)
	71, 76, and 82		"toxicogenic"	Korpinen (1974)
	83 and 86		"nontoxicogenic"	

[a]ATCC (2004)
[b]additional sources cited in this report