

Review

***Stachybotrys chartarum*: cause of human disease or media darling?**

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This is a review of the literature of associations of the saprotrophic fungus *Stachybotrys chartarum sensu lato* with human and animal illnesses. This fungus grows on very wet cellulose-based building materials. *S. chartarum* has been the subject of considerable media attention because of temporal associations of exposure with unexpected and dramatic outcomes such as infant pulmonary hemosiderosis and neurocognitive damage. It is generally accepted that living or working in mouldy environments is associated with building related asthma, exacerbating asthma in mould-sensitive asthmatics and increased rates of upper respiratory disease. However, such relationships are with building-associated moulds, comprising many species that colonize wet or damp building materials, and are not specific to *S. chartarum*. There is limited evidence that severe lung damage can occur from building exposure to *S. chartarum* but possibly only under conditions of exposure that approach those associated with handling contaminated straw. There is no positive evidence in the literature to account for putative neurological damage resulting from exposure to this mould.

Keywords *Stachybotrys chartarum*, toxins, allergens, disease, public health

Introduction

At the beginning of the 21st century, there is a certain irony that one of the few anamorphic Ascomycetes known to the general public in North America is *Stachybotrys chartarum*. The fungus found today on water-saturated wallboard was described as *S. atra* in 1837 from wallpaper collected in a home in Prague. In 2001, *Stachybotrys* figured in the plot of the syndicated US cartoon, 'Rex Morgan MD', which is surely unprecedented attention given to a mould in popular culture. There can be no doubt that several other moulds have a vastly greater impact on human health

and welfare. The mycotoxin-producing fungi, *Fusarium graminearum* (deoxynivalenol, zearalenone), *Aspergillus flavus* (aflatoxin) and *F. verticillioides* (fumonisin) together result in massive economic losses and increased mortality and morbidity [1,2].

In situations where hay or straw has become saturated with water under aerobic conditions, *S. chartarum* thrives. This is no longer common in agriculture and essentially has never been an issue in North America because of an exceptionally favourable climate for farming. In eastern Europe, inhalation exposure to *S. chartarum*-contaminated material in agricultural environments has been demonstrated to harm the health of adult humans and animals. The resulting disease in farm animals and farm workers is mainly due to the potent mycotoxins produced by this fungus. *S. chartarum* and the related species *Memnoniella echinata* out-compete other fungi on water-saturated paper, including the paper covering of wall-

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board. Water leaks that dampen such substrates, and the presence of surfaces on which condensation occurs, have become fairly common occurrences in housing in the USA and Canada. Interior surfaces that are primarily wood and plaster are resistant to mould growth; those where surfaces include paper are correspondingly susceptible. Damage from floods and storms also can result in large amounts of water-saturated paper in homes and buildings.

This change in building technology, perhaps along with other changes making housing less resistant to water incursions, and with lower rates of natural ventilation, have made mould damage in housing, the non-industrial workplace and schools more common than 40 years ago. Since the mid-1980s, this has given rise to new questions on the population health impact of mould growth on building materials, particularly in housing.

Damp housing contains a fairly narrow spectrum of fungi that are capable of colonizing building materials and surfaces. These comprise xerophiles such as *Wallemia sebi*, hydrophilic species including *S. chartarum* and *Chaetomium globosum*, as well as species that thrive at intermediate water activities (e.g. *Aspergillus versicolor*). If paper is dried to a water activity (a_w) below 0.97 at 25°C within 24 h, *S. chartarum* cannot compete with other saprotrophic species; it grows best on substrates close to water saturation. Conditions where *S. chartarum* dominates indicate serious and usually catastrophic water infiltrations, such as from storms, pipe leaks and envelope and roof failures. In general, these are uncommon when the entire housing stock of around 110 million homes in North America is considered.

Varying by genotype, isolates of this species produce metabolites that are the most potent low-molecular-weight inhibitors of protein synthesis known. Other metabolites are produced, including a number of other immunomodulatory metabolites and compounds of unknown toxicity. The trichothecenes produced are in a chemical class that is subject to international regulation in food, mainly because of neurotoxicity.

Short-term studies of *S. chartarum* spores instilled into the lungs of rodents disclose various effects on lung biology. The no-adverse-effect level for installation exposures is not known. Neonate animals are more sensitive. The effects observed are related to several components of the spores, including toxins, beta-glucan and proteases, as well as to the physical damage resulting from inhalation of organic materials. Studies of chronic exposure or nasal-only exposure to spores and mycelial fragments have not been reported. Although it is known that particulate exposure results

in the release from lung tissues of mediators such as endothelins or macrophage-derived neurotransmitters, nothing is known about these secondary effects.

Agricultural exposure and exposure from handling *Stachybotrys*-contaminated building materials or contents are known to result in severe effects on human and animal health. A series of case reports of severe lung damage in sick infants and children has appeared in the literature. In some cases, *S. chartarum* spores or DNA were isolated from lung washings but the role of the toxins in disease has not been unambiguously shown. This is primarily because biomarkers of exposure to the chemicals responsible for the damage in animal models do not yet exist. Antibodies to *Stachybotrys* proteins have been detected in human sera but have not been associated with allergic disease.

From a public health perspective, *S. chartarum* spores in settled dusts or as visible mould on surfaces is an indicator of serious water damage. This translates to unusual indoor exposure to spores and mycelial fragments of this and other building-associated fungi. Mould exposure in buildings is associated with exacerbation of asthma in mould-asthmatic patients, with increasing upper respiratory disease and with building-associated allergic disease [3,4]. The purpose of this review is to summarize the literature on *S. chartarum* and to describe the limits of the disease associations that have been made from building exposure.

Biology of *S. chartarum*

Taxonomy

The taxonomy of the species of *Stachybotrys* and the allied genus *Memnoniella* has been the subject of literature reviews by Jong and Davis [5] and more recently by Koster *et al.* [6].

S. chartarum grows well on common mycological media such as malt extract, potato sucrose agar or V-8 agars. Two studies suggested that cornmeal agar results in the highest recovery from environmental samples [7,8]. Most taxonomic studies employed cornmeal agar as the identification medium (cornmeal agar is commonly used for the culture of lignicolous fungi). In culture or on natural substrates, the fungus sporulates profusely, forming dark masses of conidia from characteristic phialides. The phialides are 9–14 µm in length, in whorls, olivaceous and often with conspicuous collarettes. The phialides produce conidia successively into a slime droplet. Conidia are ellipsoidal, unicellular, 7–12 × 4–6 µm, and dark brown to black with a ridged topography when mature. *S. atra* was described as having smooth spores by Corda [9]. *S.*

chartarum (Ehrenb. ex. Link) Hughes was described as having both smooth and rough conidia [10]. Various mycologists who have examined a large number of strains have noted variation in colony morphology, as well as differences in growth rate, suggesting the presence of two species.

As in *Fusarium graminearum*, chemotypes are known. There are two types of strains: those that produce macrocyclic trichothecenes (satratoxins, roridin) and those that produce atranones plus trichoderms. Both groups produce spirodrimanones. Unlike *F. graminearum*, in which the chemotypes are geographically separated [11], in North America the chemotypes of *S. chartarum* occur in the same econiche [12,13; Miller, unpublished data]. Similarly, it has been reported that different genotypes of *S. chartarum* occur on the same sample [14]. Koster *et al.* have shown that there are two lines within *S. chartarum sensu lato*, but also that there are important problems in naming these according to the rules of mycological nomenclature [6]. Confusion exists as to exactly what was meant by *S. chartarum* or *S. atra*.

The genus *Memmoniella* is similar to *Stachybotrys*, and *S. chartarum sensu lato* may be found together with *M. echinata* (Riv.) Galloway, although the latter is more common in warmer areas. *M. echinata* phialides are similar to those of *S. chartarum*, and bear rough conidia 3–6 µm in diameter. Zuck observed that *M. echinata* developed both *Memmoniella*- and *Stachybotrys*-type conidia [15]. Separation of the two genera has been a matter of controversy for the past 40 years. Smith considered that possession of dry conidia in chains, as in *Memmoniella*, or slimy aggregated conidia, as in *Stachybotrys*, was not sufficient to separate strains into two genera, and reduced *Memmoniella* to synonymy with *Stachybotrys* [16], a position endorsed by Kendrick and Carmichael [17], Barron [18] and Carmichael *et al.* [19]. Zuck observed that *M. echinata* developed conidia in both dry chains and slimy aggregates [15], something also observed by Li *et al.*, who additionally reported that closely related species can also form both types of conidia [20]. Comparative sequence analysis of species of *Stachybotrys* and *Memmoniella* convinced Haugland *et al.* that *M. echinata* should revert to *S. echinata* (Rivolta) Smith [21]. Based on morphological characteristics and comparative sequence analysis of the nuclear ribosomal RNA operon, they also concluded that *Memmoniella* should be relegated to synonymy with *Stachybotrys*. This view was supported by data from Peltola *et al.* [22]. Limited studies of North American strains showed that *M. echinata* produces trichodermol and the

Penicillium metabolite griseofulvin [23]. Whether there are chemotypes in this taxon remains unknown.

When *S. chartarum sensu lato* is actively growing on natural substrates or in culture, the characteristic phialides and conidia are easy to recognize [24]. Polymerase chain reaction (PCR) primers specific for *S. chartarum* are sometimes used in commercial laboratories to identify this fungus. A PCR product analysis using a fluorogenic probe has also been developed to quantify conidia of *S. chartarum* and can be used in the analysis of samples from mould-contaminated indoor environments [25,26]. The half-life of the conidia of *S. chartarum* and *M. echinata* is short compared with that of other moulds (i.e. months versus years). Microscopic examination [27] or even molecular analysis of environmental samples is sometimes required to find this organism [28,29].

Ecology

Stachybotrys chartarum is not pathogenic [30]. The preferred natural substrates of *S. chartarum* are stems of woody plants, including balsa and pine, with soil as a reservoir [5,24,31]. It is strongly hydrophilic, with a minimum a_w for growth of 0.94 and an optimal temperature of 23°C both for UK and for North American strains [32,33]. The growth rate of a North American strain at this temperature was 1.5 mm/day at a_w 0.99, 1.0 mm/day at a_w 0.97 and 0.5 mm/day at a_w 0.95. Conidial germination followed the same pattern. Depending on a_w , good growth rates from 15 to 30°C are possible [32; data reproduced in 34]. It is not competitive in nature outside these ranges.

From the time of the original isolation of *S. chartarum* from wallpaper, it was the fact that it is strongly cellulolytic and will grow under conditions of low nitrogen that attracted attention. The US Army Quartermaster Corps became interested in cellulolytic fungi during WWII because in Southeast Asia and the Pacific the useful life of untreated tarpaulins, tents, ropes and sandbags was very short in areas of high temperature and rainfall. Army tents had to be replaced after 6–8 months. The US Army Quartermaster Laboratories created a massive culture collection of fungi from deteriorated materials from military bases in the South Pacific, as well as others collected in Panama, Florida and elsewhere, as documented by Siu [35]. Among 4500 isolates of fungi from exposed cotton textiles, the most abundant unambiguously cellulolytic species were *Trichoderma viride*, *Chaetomium globosum* and *M. echinata* [34]. Studies of mouldy wallboard in North America reveal that *M. echinata*, as might be expected from the military studies, is more common in

Pacific coastal areas and is replaced by *S. chartarum* in cooler mid-Atlantic areas and in eastern Canada [13,34]. Both *S. chartarum* and *M. echinata* cause soft rot of wood, so that the growth of either results in the degradation of cellulose and hence of the material itself.

Chaetomium globosum and *C. sphaerospermum* are also common on wet wallboard collected across North America. Compared with the latter, the former less commonly occurs with *S. chartarum* [34]. This may mean that one or the other species produces antifungal compounds. As noted by Grant *et al.*, where there are hydrophilic fungi, a prevalence of species with lower optimal a_w can be anticipated in samples because some areas of the substrate are inevitably of lower a_w [33].

Allergens and toxins from *S. chartarum*

The average aerodynamic size of spores in a series of North American strains of *S. chartarum* was noted by Sorenson *et al.* to be 5.6 μm (Table 1) [36]. This average conceals a considerable variation in size. Approximately one-third of the spores were within the respirable range [36]. Furthermore, the degree of hydration of spores, a consequence of the prevailing relative humidity, affects those ranges [37]. Particles at the lower end of that size range can reach the alveoli; others may be swallowed. There is some variation with age: lower airway deposition of 5- μm particles is sixfold higher in newborns than in adults [38]. Fungal fragments $>1\ \mu\text{m}$ in aerodynamic diameter have been reported to occur at between 5 and 300% of the numbers of fungal spores observed in indoor air samples [39–41]. Allergens are also present in these mycelial fragments and

their small size makes their presence relevant for human health [39].

Studies of allergens of *S. chartarum* have been reported in the US. Barnes *et al.* found that 49% of the serum samples collected from a general population in Kansas had IgG, and 9% had IgE, immunoglobulins to *S. chartarum* proteins [42]. These proteins were reported as having molecular weights in the range of 34 and 52 kDa and were present in the spores and mycelia. Similar antigens were reported by Halsey *et al.*, who found that IgE and IgA responses to *S. chartarum* antigens in healthy patients were rare, but occurred in *S. chartarum*-exposed patients [43]. The antigens were identified using sera collected from patients in different locations in the USA. The 34-kDa protein is one of several serine proteases (determined by a functional assay) found in the spores and mycelia of *S. chartarum* from both chemotypes (Miller *et al.*, unpublished data). Antibodies to the 34-kDa serine protease do not materially cross-react with such proteins in some other building-associated fungi (Jensen *et al.*, unpublished data). Proteins with notionally similar molecular weights have been found using sera collected in Finland [44], although at the time of writing it is not clear whether the proteins are in fact the same. These data demonstrate the presence of antibodies to *S. chartarum* antigens in human sera.

Stachybotrys chartarum produces three major classes of secondary metabolite, each with varying biological activities. The predominant class, the one always produced, consists of spirocyclic drimanes and closely related triphenyl phenolics (e.g. stachybotrylactones and stachybotrylactams) (Fig. 1). The spirocyclic drimanes were first described by Japanese workers

Table 1 Percentage of different spore sizes and mean aerodynamic diameters of air dried spores from different strains of *Stachybotrys chartarum*

Strain	Diameter (μm)				Average aerodynamic diameter (μm)
	2.2–3.3	3.3–4.7	4.7–7.0	>7	
SA1	1	12	48	38	6.2 \pm 0.56
SA2	4	26	57	13	5.3 \pm 0.32
SA3	1	22	51	27	5.2 \pm 0.26
SA4	3	37	45	16	6.1 \pm 0.57
SA5	1	21	59	18	5.6 \pm 1.06
SA6	2	20	51	27	5.1 \pm 0.33
SA7	2	28	58	12	5.5 \pm 0.27
SA8	4	32	48	16	5.2 \pm 0.43
SA9	2	23	46	29	5.7 \pm 0.61
SA10	1	23	58	18	5.7 \pm 0.42
SA11	<1	26	64	9	5.7 \pm 0.26
SA12	<1	13	63	24	5.8 \pm 0.27

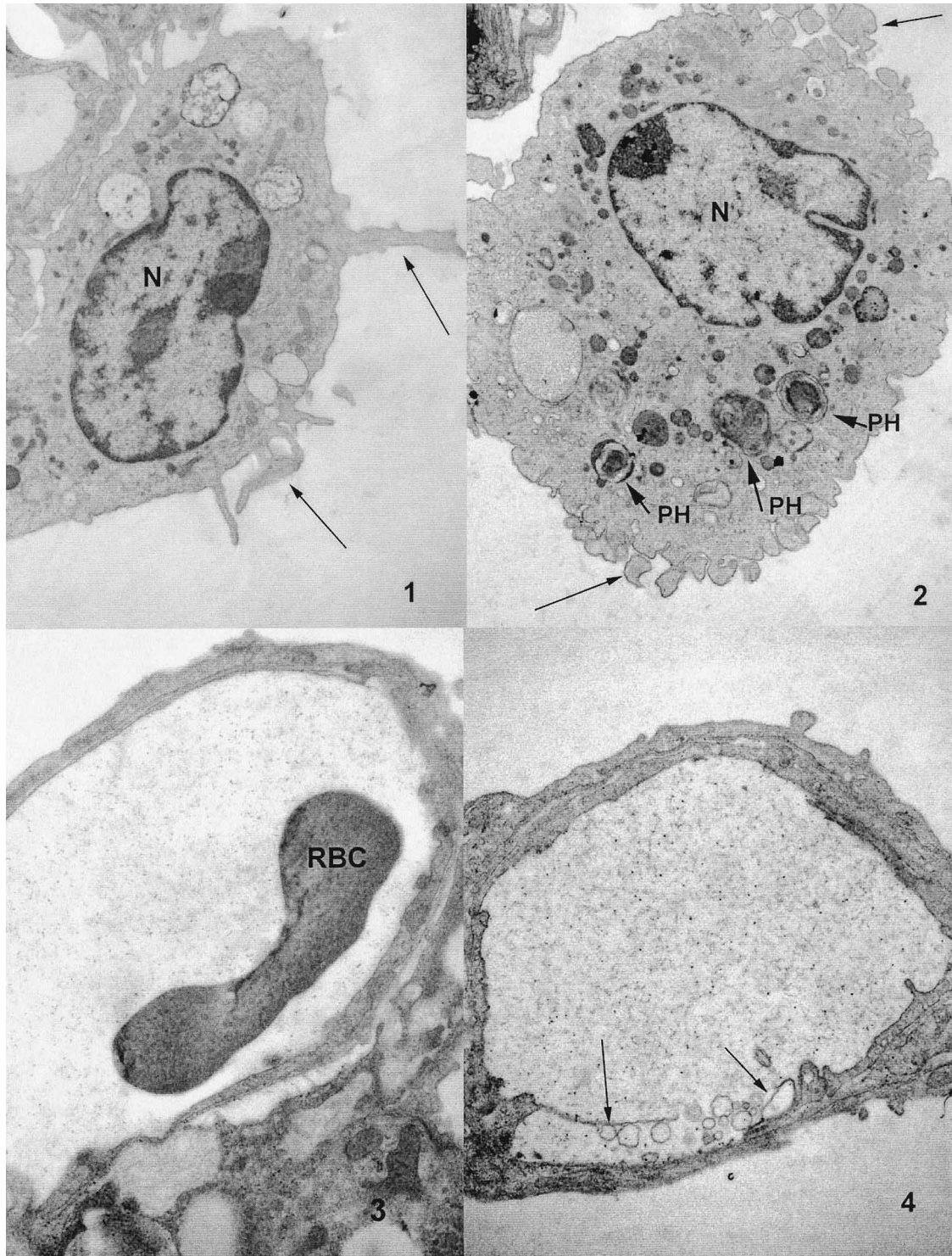


Fig. 1 (a) Alveolar macrophage in saline-treated mouse lung. Note filipodia (arrows) and cytoplasm lacking debris-filled phagolysosomes. N, nucleus. ($\times 12\,500$ magnification). (b) Activated alveolar macrophage in *Stachybotrys chartarum*-spore-impacted mouse lung. Note lobopodia (arrows) and cytoplasm with numerous debris-filled phagolysosomes (PH). N, nucleus. ($\times 12\,500$ magnification). (c) Alveolar capillary with single erythrocyte (RBC) in saline-treated mouse lung. Note that the endothelial surface lining the capillary lumen lacks evidence of vesicularization. ($\times 12\,500$ magnification). (d) Alveolar capillary in *Stachybotrys chartarum*-spore-impacted mouse lung. Note the marked vesicularization of the endothelial surface lining the capillary lumen (arrows). ($\times 12\,500$ magnification).

[45] who attempted to develop them as immunosuppressants (US Patent 4 229 466; 21 October 1980). In addition to their immunosuppressant properties, spirocyclic drimanes from different organisms have been reported as being endothelin receptor antagonists [46], protease inhibitors [47], cholesterol esterase inhibitors [48], inositol monophosphatase inhibitors [49], antiviral components [50] and cytotoxins [51]. The spirocyclic drimanes always appear in cultures of *S. chartarum* grown on building materials [12] as well as in *S. chartarum*-contaminated building materials from mouldy homes and buildings.

The concentrations of spirocyclic drimanes present in spores are not known. Spore concentrations of some of the macrocyclic trichothecenes that may be present have been the subject of very limited study [52,53]. In culture and on building materials, spirocyclics are present at levels typically an order of magnitude higher than the macrocyclic trichothecenes [54].

The class of *S. chartarum* toxins studied most extensively is the trichothecenes. *S. chartarum* produces a variety of macrocyclic trichothecenes in addition to several less toxic simple trichothecenes and trichoveroids (Table 2). Satratoxin H and related macrocyclic trichothecenes (Fig. 2) are held as responsible for the acute toxicity associated with stachybotryotoxicosis in animals.

Chemical analyses of a large number of *S. chartarum* strains isolated from building materials and suspect animal feed from around the world have made clear that: (1) the pattern of mycotoxin production by *S. chartarum* is independent of region, and (2) about one-third of these isolates produce macrocyclic trichothecenes [12,13,54,55]. *S. chartarum* isolates that do not produce macrocyclic trichothecenes typically produce the simple trichothecenes trichodermol and trichodermin.

Studies of metabolite production by the less cytotoxic strains have shown that they consistently produce diterpenes called atranones [51,56]. The atranones are related in structure to the dolabellanes, secondary metabolites produced by marine animals [57]. To date,

eleven atranones (A–K) and two dolabellanes [56] have been described for *S. chartarum* (Fig. 3). Dolabellanes exhibit modest cytotoxicity [57,58]. Atranones are not cytotoxic and do not induce inflammatory mediators in cultured macrophages [59]. At the time of writing, nothing is known about their toxicity nor their presence or absence in *S. chartarum* spores.

A number of additional metabolites occur in *S. chartarum* spores. As with other fungi, the spores contain β -1,3-D-glucan [60; Miller *et al.*, unpublished data]. Beta-1,3-glucan is a potent inflammatory agent [61] that produces a variety of symptoms from inhalation exposure in human volunteers including headache [62,63]. Indirect evidence has been reported indicating that *S. chartarum* produces hydroxylamine-type siderophores [64], uncharacterized proteins with hemolytic activity [65] and proteinase [66]. The potentially immunosuppressive cyclic peptide, cyclosporine, has been reported from a Japanese strain of *S. chartarum* [67]. Limited evidence exists for the production of this compound by North American strains (D. Dearborn, personal communication).

Since early reports of the growth of *S. chartarum* on building materials [68] to the most recent [69], it is its trichothecenes that have been found in such materials. At the time of writing, no investigations have systematically investigated the production of the spirocyclic compounds or the atranones produced on *S. chartarum*-damaged building materials. In addition to the normal problems associated with measuring low levels of chemicals, the matrix in which these compounds are found is often very difficult to extract efficiently. For example, when the mycotoxin standards of the simple trichothecenes, T-2 toxin and trichodermin, roridin A (a macrocyclic trichothecene) and sterigmatocystins are added in dichloromethane solution to building materials, they cannot be detected in organic solvent extracts of that material [70]. Although there are no data comparing distribution of mycotoxins produced in indoor environments in spores (and other fungal particles, such as mycelial fragments) with building material-related particles, it is very likely that substantial amounts of the mycotoxins can find their way from spores to building materials. *S. chartarum* exports trichothecenes to the spore surface where they are readily solubilized in water [71], and presumably can be washed onto building material. In this same regard, toxicological studies in which *S. chartarum* spores, prior to being administered to animals, had been washed with aqueous solutions [52,72] are somewhat flawed, in that > 50% of the trichothecene toxins had been extracted before animal exposure [71].

Table 2 Trichothecene mycotoxins produced by *Stachybotrys chartarum*

Simple Trichothecenes	Trichoveroids	Macrocyclic Trichothecenes
Verrucarol	Trichoverrins A & B	Roridins E, isoE, L-2
Trichodermol	Trichoverrols A & B	Verrucarins B & D
Trichodermin		Satratoxins F, isoF, G, isoG, H and isoH

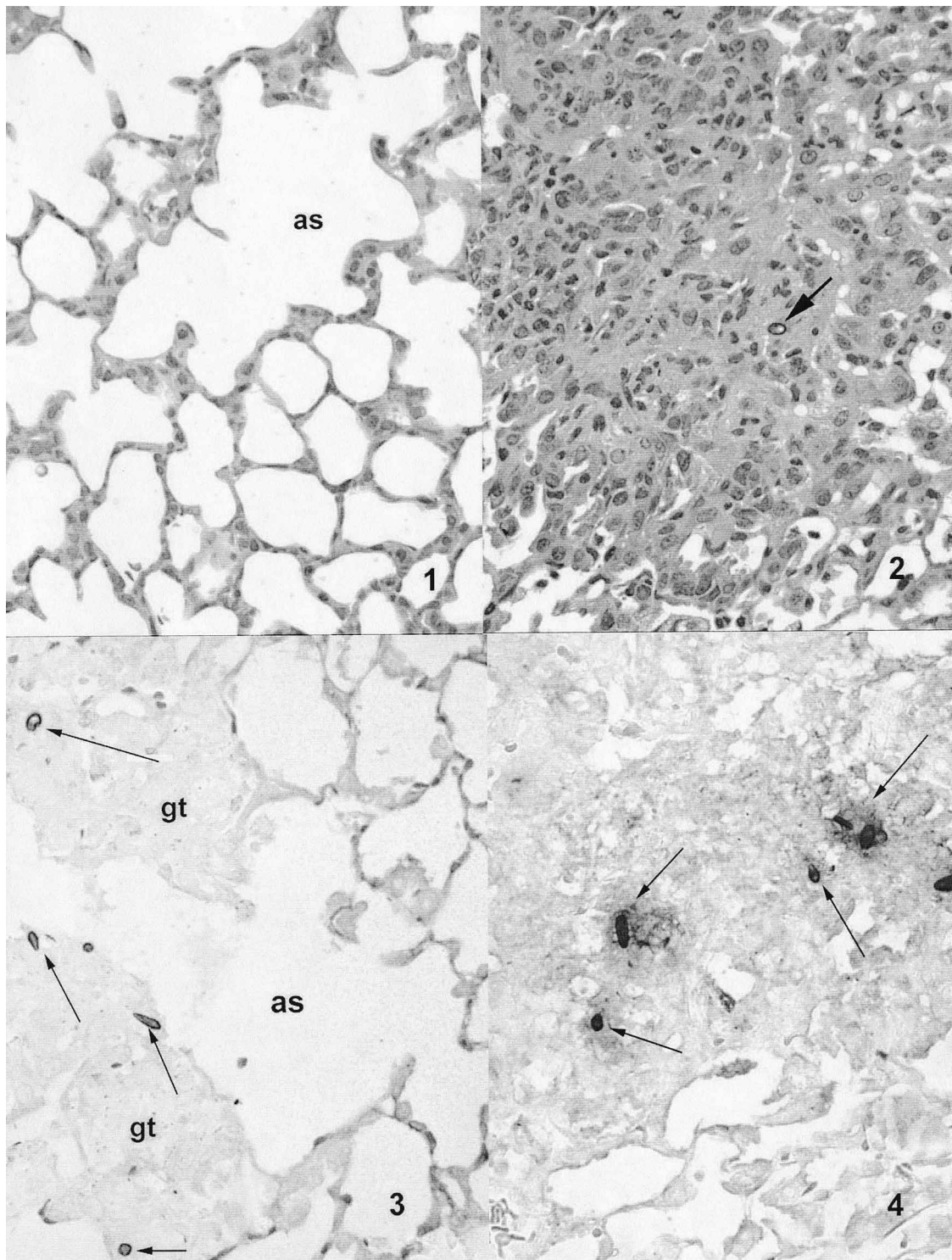


Fig. 2 (a) Distal lung area in saline-treated mouse lung inflated at 18 cm H₂O pressure. AS, alveolar space. ($\times 400$ magnification). (b) Distal lung area of *Stachybotrys chartarum*-spore-impacted mouse lung inflated at 18 cm H₂O pressure. Note the lack of alveolar space surrounding *S. chartarum* spore (arrow) in granuloma tissue. ($\times 400$ magnification). (c) Collagen-IV-labelled mouse lung granuloma tissue (GT) from a *Stachybotrys chartarum*-spore-exposed animal (48 h post exposure). Note the apparent lack of labelling in granuloma tissue surrounding spores (arrows) with labeled alveolar walls. AS, alveolar space. ($\times 400$ magnification). (d) Distribution of stachylysin surrounding *Stachybotrys chartarum* spores (arrows) in mouse lung granuloma tissue. ($\times 400$ magnification).

Human and animal diseases linked to *S. chartarum*

Occupational environments

In Russia and Hungary during the late 1920s through the 1940s, there were outbreaks of a disease in livestock, especially horses. Symptoms reported included irritation of the mouth, throat and nose leading to hemorrhage, shock, decrease in resistance, septicemia and death [73; Jarmay 1929, cited in 74]. In 1938, Russian scientists determined that the disease was associated with *S. chartarum* growing on the straw and grain fed to the animals. Intensive studies then resulted in the first demonstrated toxicity of *S. chartarum* to animals. When horses were fed cultures of the fungus, material from one Petri dish resulted in sickness and from 30 others resulted in death of individual horses. By 1945, extracts of cultures were shown to contain a toxic principle. That trichothecenes were responsible was not discovered until 1973. Eppley and Bailey reported that a strain from the US Army Quartermaster Laboratories collection, originally isolated from cardboard in Finland, produced satratoxin H [75]. Unsurprisingly, outbreaks were associated with hay or feed stored under wet conditions. The syndrome stachybotryotoxicosis was subsequently reported in a deer in France [76], horses in North Africa [77], sheep in Hungary [74] and South Africa [78], cows in Bosnia Herzegovina [79] and pigs in Hungary [74].

It is often said that the cases of animal toxicosis offer no information on building-related exposures to *S. chartarum* because the animals consumed the hay. It is obvious that the animals had their noses directly on and above the mouldy hay, so that part of their exposure must have been by inhalation. Oral exposure to toxins is different from inhalation exposure. The bioavailability of fungal toxins is usually greater by inhalation than by oral ingestion. This is the case for trichothecenes [80,81]. In ruminants such as cows and sheep, it is known that trichothecenes are modified by rumen bacteria and, in comparison to swine, both species are resistant to the agriculturally important trichothecenes [82,83]. This is reflected in guidelines on the amount of the trichothecene deoxynivalenol permitted in feed in the US and Canada. Lungs do not have the barriers to toxins offered by the gut microflora and intestinal cells. Regardless, because the toxicological descriptions of bovine or ovine stachybotryotoxicosis have been weak, it is impossible to comment on the relative impact of oral ingestion versus inhalation exposures in the historical case. Fortunately, there is a modern case report from South Africa [78,84].

The South African report [78,84] is a detailed toxicological picture of ovine stachybotryotoxicosis by the pathologist, Kriek, and the mycologist/mycotoxicologist, Marasas. The disease was characterized by hemorrhagic septicemia, anemia and leukopenia. Observations during the first phase of the disease included elevated body temperature, listlessness and intermittent hemorrhagic diarrhea, followed by a progressively worsening anemia and leukocytopenia, followed by death. Opportunistic bacterial infections were noted. On autopsy, the primary findings included purpuric hemorrhage on serosal and mucosal surfaces and in most of the organs, enterorrhagia, severe pulmonary congestion and edema. On histological examination, atrophy and necrosis of the lymphoid tissue, aplastic anemia and a 'markedly' impaired inflammatory response. The authors determined that the cultures of *S. chartarum* isolated contained trichothecenes (see figures in original report).

A feature of this detailed pathology report was severe pulmonary congestion and edema. The oral toxicology of trichothecenes is very well known [80,85]. Edema is a feature of oral and inhalation exposure at lethal doses of trichothecenes, because at high doses heart damage occurs. In experimental oral exposure to lethal doses of trichothecenes, severe pulmonary congestion has not been reported [85], even in studies with swine [80], which offer a good model for the toxic effects of trichothecenes in humans. This indicates that part of the pathology observed in animal stachybotryotoxicosis is due to inhalation exposure to fungal particles and dusts containing trichothecenes.

For the Russian cases from the 1930s and 1940s, there are contemporary reports of illnesses in people who handled hay or feed grain infested with *S. chartarum* or who slept on straw-filled mattresses. Claims appear in these reports that the straw was black from fungal growth. Symptoms included skin and mucous membrane irritation, dermatitis, pain and inflammation of the mucous membranes of the mouth and throat, conjunctivitis, a burning sensation of the eyes and nasal passages, tightness of the chest, cough, bloody rhinitis, fever, headache and fatigue. Symptoms developed within days of exposure. The mortality rate was reported to be high. When Russian scientists investigating this disease rubbed their skin with the fungus, this induced local and systemic symptoms similar to those observed on farms [73,86].

There are modern case reports of human stachybotryotoxicosis that has arisen from handling straw by farm workers from Hungary [74,87] and Bosnia Herzegovina [Ozegovica *et al.* 1970, cited in 74], and from France among stable workers [88,89] who handled

'highly' contaminated straw. Symptoms were reported to be similar to those described in Russia. In the more recent Hungarian cases, *S. chartarum* was cultured from scrapings of symptomatic areas of the skin and from samples taken from the nose and throat. Most workers recuperated when they stopped handling the infested straw. In the French cases, precipitins were not found in the serum of the workers [89].

The French researchers handled a portion of highly contaminated straw from a 300-kg round bale for 5 s in an experimental chamber. Air samples were taken after 3, 9, 13, 27 and 81 min with an Andersen cascade sampler. At 3 min, spore clouds in excess of 10^7 spores per m^3 air were measured, decaying to around half that value by 15 min, with some spores still in the air at 81 min. (Recco *et al.* [89] only assessed intact spores and did not include substrate dusts, which would have contained fungal toxins, endotoxin and allergens.) From the results, they estimated that a stable worker would inhale 3–5 mg of spores alone. They judged that when the strains were good producers of cytotoxins, this would pose a direct toxic hazard, but it is to be remembered that when Le Bars and Le Bars wrote their paper, the existence of the atranone chemotype was not known [88].

Workers at a horticultural facility in Germany developed painful inflamed lesions on their fingertips, followed by peeling skin, after handling pots made from recycled paper colonized by *S. chartarum* [90].

In both the earlier and the newer reports, symptoms in humans and animals were recorded as appearing after some delay and involved a mixture of inflammatory and toxic responses. Farm workers exposed to organic dust can experience Organic Toxic Dust Syndrome (ODTS), a non-allergic disease [91]. The acute inflammatory response is produced on exposure to endotoxin, fungal spores and components of fungal spores including β -1,3-D-glucan (and other materials). These particles mainly affect macrophages, which then secrete inflammatory mediators, particularly polyclonal cell activators. This results in the release of plasma from the lung capillaries into the alveolar and lung tissue, and also in the invasion of these cells by neutrophils. Blood–air diffusion capacity may be reduced and there may be an increase in the number of white blood cells. Symptoms such as fever, general malaise, headache, chills, body aches, cough and shortness of breath are seen. Antibodies to the agents concerned are usually absent. ODTS is not an allergic disease, hence is usually characterized by a higher attack rate than hypersensitivity pneumonitis, and is not considered fatal [91–93].

In the human cases, farm exposure produced ODTS-like symptoms along with toxic symptoms consonant with exposure to trichothecenes, particularly immunological and skin/mucosal irritation and hemorrhage [85,94,95]. There is, then, unambiguous evidence that occupational exposure in the order of 10^3 – 10^7 spores/ m^3 air results in symptoms of OTDS and trichothecene toxicosis.

Built environment

Perhaps the first indication that mould damage in US buildings where *S. chartarum* was amongst the fungi isolated, can be found in Kozak *et al.* [96]. The focus of that study was on the allergy-related symptoms that are acknowledged today. Exposure to *S. chartarum* toxins as a potential cause of human disease indoors was first noted from a case in a house in Chicago [68]. What is known is that *S. chartarum* was growing on debris in the heating/air conditioning system of the house and on a bedroom ceiling. Trichothecenes were measured in the colonized debris. No other environmental information was reported. No clear case report of the symptoms of the family exists and none of authors of the published report was a clinician. Symptoms were said to have disappeared after the house was cleaned but this was not documented. Symptoms reported in the Croft *et al.* paper included headaches, sore throat, general malaise, diarrhoea and fatigue, which have been characterized as 'consistent with chronic trichothecene exposure' [97]. Symptoms of human exposure to trichothecenes include vomiting (because of neurotoxicity), hemorrhage and skin lesions. In contrast, the reported symptoms are more consistent with the symptoms from living or working in a mouldy environment [98]. This widely cited case provides no clear information as to whether *S. chartarum* toxins contributed to the symptoms reported.

The so-called New York *S. chartarum* cluster [99–101] could more fairly be described as two clusters. The group initially presenting to the New York Mount Sinai Clinic involved three workers who, without personal protection, had been cleaning and removing mouldy cardboard boxes for 2 or 3 days [100] in a sub-basement that had had recurrent flooding [99]. They suffered an acute response and sought medical attention. The attending physician noted throat irritation, fatigue, fever, muscle aches, stomach aches and, importantly, skin rash on the hands (E. Johanning, personal communication). Bulk sampling of wallboard and books indicated massive contamination of these surfaces with *S. chartarum*. In addition, one of its trichothecene toxins was found in bulk samples of

contaminated material [100]. Area measurements of the affected location resulted in estimated airborne concentrations of the order of 10^5 viable spores per m^3 [99,101], which could possibly translate into much greater total airborne *S. chartarum* spore loads. Measurements by others of airborne levels of *S. chartarum* spores arising from handling contaminated materials have been reported to be in the range of 7×10^5 per m^3 [102]. In addition, preliminary data suggest that the concentrations in the breathing zone of workers carrying such materials are higher than area measurements (unpublished report; D Hamlin, Center for Toxic Environmental Health, University of Texas). Several of these workers established claims with the New York State workers' compensation system. The workers were diagnosed with chronic laryngitis, sinusitis, bronchitis, asthma, allergy and toxic encephalopathy [103]. It is reasonable to assume that these workers were exposed to spore concentrations associated with agricultural stachybotryotoxicosis. The time of onset, the fever and the skin symptoms are consistent with ODS plus exposure to the potent dermal toxins of *S. chartarum* trichothecenes.

After unspecified remediation steps had been taken [100], a case-control study was conducted on some of the original cases and an additional group of approximately 50 workers from the same workplace, together with 21 matched controls without contact with the problem site [101]. This group was exposed to damp-building fungi, including *S. chartarum*, perhaps in quantity at the time of handling the uncontained contaminated materials. In this study, immune modulation and respiratory and non-respiratory symptoms of the type associated with living or working in mouldy environments were reported [98].

This incident, which can be more fairly characterized as two separate situations, provides limited evidence of a toxic effect consequent on handling *S. chartarum*-damaged materials in buildings. The consequences for the remaining building inhabitants, certainly by the time the larger formal study was conducted, are hard to distinguish from those reported in studies of damp buildings.

Finally, *S. chartarum* has been associated in six locations with infant pulmonary hemosiderosis. Most cases on the public record have been reported in Cleveland, but other cases have been reported in Texas [104], Kansas City [105], Belgium [N. Nolard, cited in 59] and in Quebec (P. Auger, personal communication).

Physicians at the Rainbow Babies and Childrens' Hospital in Cleveland recognized that there was an unusually high number of cases of idiopathic pulmonary hemosiderosis between January 1993 and Decem-

ber 1994. Ten were diagnosed during this time-period, compared to only three in the previous 10 years. They were clustered within a zone of 9.5-km radius in an area prone to flooding because of drainage from other parts of the city. A case-control study was conducted with the 10 diagnosed during their first 6 months of life and 30 age-matched controls living within 9.5 km of these cases, who were mostly black people, in low-income families, living in poorly maintained older homes with water damage [106–108]. Odds ratios were 9 for male sex, 16 for water damage and, 10, it was subsequently reported, for high levels of *Stachybotrys* in the home. Parental smoking was also a potentially important contributing factor.

The hypothesis that exposure to high levels of *S. chartarum* contributed to the development of pulmonary hemosiderosis in the Cleveland infants was challenged because of the discovery of serious problems with the exposure assessments [109]. The scientific reports noted water damage, but this aspect was not adequately characterized. The authors of the present review have each examined one or more of these houses. The basements had often suffered repeated flooding and severe mould growth on beams, and sometimes on debris on dirt floors, was seen. Mould growth on wood is uncommon in housing because mould growth requires a wood moisture content 15% upwards by weight. An idiosyncratic configuration of the forced-air heating system is also common among houses in these Cleveland neighborhoods: the return spills directly onto the basement floor. This means that contamination of whatever kind can be returned to the house during the heating season.

The clinical data on the Cleveland infants were evaluated and the disease remained idiopathic in the sense that all known risk factors for infant hemosiderosis in these children were examined and none was found. Most presented with severe pulmonary symptoms requiring intensive support, but a few cases had less severe haemorrhage. Three-quarters of the patients required ventilator support and blood transfusions. Removal from the damp home reduced lower respiratory rebleeding in the surviving infants. Several patients continued low-grade haemorrhage for months, even after removal from their original home [110].

The CDC review of the original epidemiology [109] noted that the hypothesis that *S. chartarum* toxins contributed to the disease observed could not be excluded. This assessment was based on evidence of nasal and tracheal bleeding in highly exposed adult humans and animals and the pathophysiology of the toxins. Direct evidence of personal exposure to *S. chartarum* has not been obtained in the reported

Cleveland cases; the evidence that the toxins from this fungus or the fungus itself were involved remains inferential. In a recent unreported case, *S. chartarum* DNA from lavage was detected by the EPA laboratory (D. Dearborn, R. Haugland, S. Vesper *et al.*, personal communication). CDC created three working groups to develop better protocols for investigation of future clusters, and has begun a surveillance program in conjunction with the USA [111]. In 2002, the CDC published a case definition of idiopathic pulmonary hemosiderosis which will be used here as part of the analysis of the published cases. The CDC working group noted that cases of acute idiopathic pulmonary hemorrhage in infants (AIPHI) are characterized by the sudden onset of pulmonary hemorrhage in a previously healthy infant. Evidence of pulmonary hemorrhage includes hemoptysis, and finding blood in the nose or airway with no evidence of upper respiratory or gastrointestinal bleeding. Patients present with acute, severe respiratory distress or failure requiring mechanical ventilation and often demonstrate bilateral infiltrates on chest radiograph. A clinically confirmed case is illness in a previously healthy infant aged <1 year with a gestational age of >32 weeks and no history of neonatal medical problems that could cause an episode of pulmonary hemorrhage meeting all of the following three criteria: (1) abrupt or sudden onset of overt bleeding or patent evidence of blood in the airway; (2) a severe condition progressing to acute respiratory distress or failure that results in hospitalization with intubation and mechanical ventilation in a pediatric intensive care unit; and (3) diffuse, bilateral pulmonary infiltrates on chest radiograph or computerized tomography of the chest [112]. Only the few premature babies considered by Dearborn *et al.* do not meet this new case definition [110].

At the time of writing, there have been two published reports of additional geographic locations where idiopathic pulmonary hemorrhage in young children has occurred in environments where *S. chartarum* was found. Water damage leading to high airborne *Aspergillus* and *Penicillium* counts, as well as *S. chartarum* spores, was found in the room of an infant case in Kansas. The baby was admitted with pulmonary hemorrhage and the clinical work-up [105] was similar to that in Cleveland (D. Dearborn, personal communication) and meets the new CDC case definition. A third case occurred in Delaware, where a 2-week-old infant was admitted to intensive care with pulmonary hemorrhage [29]. Again the clinical work-up was similar to that in Cleveland and meets the CDC case definition. The environmental assessment report in this case is weak, but the building was reported as having

unremediated storm damage, something that can result in serious mould growth. *S. chartarum* was not recorded for the limited number of samples tested, but in subsequent undescribed samples examined by the method of Haugland *et al.* [25] *S. chartarum* DNA was detected.

A case of pulmonary hemorrhage also associated with *Stachybotrys* was reported in a 7-year-old child in Texas. The child underwent a bronchial lavage within hours of hospitalization and viable *S. chartarum* spores were recovered from the lavage [104]. The identification of the fungus was confirmed by D. Malloch. For this case, both the circumstantial and personal exposure evidence is sufficient enough to be able to say that exposure to *S. chartarum* contributed to the disease observed.

Exposure to *S. chartarum* via the lung in laboratory animals

Preliminary reports

A crude allergen extract of *S. chartarum* induced allergic asthma-like responses in the BALB/c murine model [113]. It was only relatively recently that it was recognized that spores of toxigenic fungi contain high concentrations (1–650 µg/g) of one or more species-specific toxins, including in some cases, spore-specific toxins. Moulds have been shown to contain mixtures of the toxins associated with the species. These include: *Fusarium graminearum* (DON), *F. sporotrichioides* (T-2), *F. verticillioides* (fumonisin), *S. chartarum* (satratoxins), *Penicillium expansum* (citrinin), *P. chrysogenum* (roquefortine C), *P. brevicompactum* (mycophenolic acid), *Aspergillus versicolor* (sterigmatocystin), *A. flavus/parasiticus* (aflatoxins), *A. fumigatus* (fumitremorgen B, verruculogen) [53,114–118]. It is often overlooked that the concentrations in spores range up to the mm levels.

Experimental inhalation exposure to the pure trichothecene T-2 toxin have been studied in mice, rats, guinea-pigs and swine. The LD₅₀ is typically one order of magnitude less by inhalation than by systemic administration [80,81]. The macrocyclic trichothecenes are highly toxic compounds, being among the most potent inhibitors of protein synthesis known [94,119]. Trichothecenes cause membrane damage in proportion to the diphosphatidylcholine concentration of the membrane type [120,121]. Yang *et al.* reported that satratoxin G was the most cytotoxic of eight trichothecenes tested on mammalian cells [122]. Other researchers have also reported the high toxicity of satratoxins

compared with that of other trichothecenes. The LD₅₀ in mice for satratoxins is < 1 mg/kg [84,123].

Towards the no adverse effect level (NOAEL)

In rodents, intratracheal or intranasal installation of *Stachybotrys* spores can result in massive lung damage and acute lethality, both in mice [52,124,125] and in rats [30]. The experiments reported are not particularly informative because they were conducted at rates above the maximum tolerated dose, generally using doses in the range 3000–30 000 spores/g bdy wt. However, they have shown that such exposures can result in a variety of biochemical, anatomical and gross pathological lung changes, and in dose-, time- and spore-strain-dependent ways. These experiments have also provided evidence that exposure to high *S. chartarum* spore loads can cause disease signs in rodents that accord with those reported with agricultural exposure of domestic animals, farm workers and with high exposure from the built environment.

At the biochemical level, alveolar type II cells and alveolar macrophages (AM) appear to be particularly sensitive. Mason *et al.* [126,127], Macrae *et al.* [128] and Sumarah *et al.* [129] have shown that *S. chartarum* spore and isosatratoxin F exposure induces significant changes in the phospholipid composition of pulmonary surfactant in bronchoalveolar lavage fluid (BALF). *S. chartarum* spores and isosatratoxin F induce changes in regulation of both the secretion and synthesis of pulmonary surfactant, and in the pattern of phospholipid targeting the pulmonary surfactant pools in mice [126]. These agents alter the activity of convertase responsible for the conversion of the surface-active to the metabolically used surfactant fraction [127]. The surfactant changes in mice may be due to an increase in pulmonary surfactant phospholipids associated with alveolar type II cell damage [128]. *S. chartarum* spore or toxin exposure induces other changes in lung surfactant phospholipid composition, including depressed disaturated phosphatidylcholine (DSPC) [129], which is the major phospholipid responsible for maintaining surface-tension properties of lung surfactant. Hastings *et al.* have recently shown that depressed DSPC synthesis in *S. chartarum* spore exposed mice is probably related to modulation of a key enzyme, CTP:cholinephosphate cytidyltransferase (CPCT), in the phosphatidylcholine synthesis pathway [130].

These experiments have also shown that intratracheal exposure of rats and mice to *S. chartarum* spores causes a significantly elevated protein content, particularly albumin, in the BALF. This accords with the acute inflammation response seen in several reports

[72,127,131,132]. These differential effects of *Stachybotrys* spores were almost eliminated in the case of methanol-extracted spores [132]. In mice, exposure to single doses of spores from two different *S. chartarum* isolates stimulated pro-inflammatory cytokine (IL-1, IL-6, TNF α) and other responses (total protein, albumin, LDH). These responses are significantly elevated even at doses as low as 30 spores/g bdy wt compared to saline and *Cladosporium cladosporioides*-spore-treated animals. (*C. cladosporioides* is the most common fungal spore in outdoor air.) The pattern of responses in mice was dependent on dose, time and strain differences in the toxicity of the spores [131]. These authors estimated the NOAEL for pro-inflammatory cytokine production to be in the range of 10–15 spores/g bdy wt. Rosenblum *et al.* also revealed that mice exhibited dose-dependent pulmonary responses to *S. chartarum* spore loads [133]. Again, the degree of response to exposure was also dependent on mouse strain used: strain Balb/C mice exhibit the most, while C57bl/6J show the least inflammation and injury.

There is clear *in vivo* evidence that intratracheal exposure of mice and rats to *S. chartarum* spores results in a variety of micro-anatomical (Fig. 4a–d), histopathological and gross pathological changes. Transmission electron microscopy (TEM) combined with stereology has shown that exposure to *S. chartarum* spores and isosatratoxin F in mice results in significant alveolar type II cell changes *in vivo* different to those associated with exposure to the same concentrations of *C. cladosporioides* [125]. These changes included condensed mitochondria with separated cristae, scattered chromatin and poorly defined nucleolus, cytoplasmic rarefaction, and distended lamellar bodies with irregularly arranged lamellae. These cytological changes are consistent with apoptosis and parallel results of some of the *in vitro* studies. For example, Pertola *et al.* reported mitochondrial swelling as a cytological lesion in boar spermatozoa exposed to *S. chartarum* spores and T-2 toxin [134]. Okumura *et al.* found that exposure of a mouse cell line to T-2 toxin caused mitochondrial condensation [135]. However, the latter study suggested that the cellular changes observed in mouse cell lines exposed to T-2 toxin may be linked to apoptosis. Yang *et al.* also provided molecular evidence *in vitro* that other trichothecenes derived from *S. chartarum* isolates induce apoptosis [122].

At the histopathological level, *Stachybotrys* spores instilled in the lungs of neonate Sprague–Dawley rats resulted in extensive hemorrhage with an LD₅₀ of 10⁵/g bdy wt and growth of surviving animals was impaired in a dose-dependent fashion. Histology of the lungs revealed fresh hemorrhage, hemosiderin-laden macro-

phages and evidence of inflammation, including thickened alveolar septa infiltrated by lymphocytes and mononuclear cells and intra-alveolar macrophages. None of these effects were observed in animals instilled with spores treated with ethanol to remove the toxins [118]. Similar pathological changes have been reported in mice [52,124,125]. Amongst the most consistent histopathological features described in both rats and in mice are granuloma formation (Fig. 5a, 5b), hemorrhage and hemosiderin deposition.

Nikulin *et al.* showed that intranasal instillation of spores of a relatively non-toxic and a toxic *S. chartarum* strain into NMRI mice resulted in a variety of histopathological features that were dependent on spore dose and the relative toxicity of spores [52]. They also revealed that despite the often massive lung changes in mice, spleen, thymus and intestinal tissues showed little apparent damage. This suggested that inhalant response to *S. chartarum* spores and their toxins is different from that associated with inhalant exposure to pure *S. chartarum* toxins, something observed in the South African case of ovine stachybotryotoxicosis [78]. Rand *et al.* showed that intratracheal exposure to *C. cladosporioides* and *S. chartarum* conidia stimulated granuloma formation in the lungs of Carworth Farms White (CFW) mice [125]. Hemorrhage, erythrocyte accumulation in the alveolar air space, dilated capillaries engorged with erythrocytes and hemosiderin accumulation at spore impaction sites were features associated with *S. chartarum* spore exposure but not *C. cladosporioides* spore exposure. Quantitative light microscopy showed that granulomatous lesions were more severe and persistent in *S. chartarum*- than in *C. cladosporioides*-spore-treated animals. Erythrocyte abundance in *S. chartarum*- but not in *C. cladosporioides*-spore-treated animals also exhibited significant time-dependent accumulation in the alveolar space. None of these effects were observed in the lungs of untreated, saline- or isosatratoxin-F-treated animals, suggesting it is the spore exposure, not exposure to pure toxin, that stimulates inflammatory tissue reactions.

Marked differences in inflammatory response in lungs of *S. chartarum*-spore- and isosatratoxin-F-impacted animals are also manifest as BALF biochemical changes and in gross pathology of lungs. For example, total protein content in the BALF from spore-impacted lungs is significantly higher than in that of the toxin-impacted lungs, which is not different from concentrations recovered from control animals (Rand, unpublished data). Additionally, lungs of spore-exposed animals often appear abnormally pale and mottled with hemorrhagic foci at sites of spore impac-

tion, while toxin-exposed lungs appear uniformly pink and similar in appearance to those of the control animals (Rand, unpublished observations). These observations indicate that the magnitude of tissue reaction and seriousness of disease outcome in *S. chartarum*-spore-impacted lungs is far greater than it is in lungs exposed to pure toxin.

A number of other *in vivo* studies have reported a lack of an inflammatory lung response in animals exposed to pure mycotoxins [80,81,136–139]. Pang *et al.* [138] have suggested that lack of an apparent tissue response to T-2 toxin exposure may be a consequence of its lipophilic nature, making it rapidly absorbed from lung tissue, whereupon it is metabolized and excreted. However, results of immunohistology and immunocytochemistry studies do not support the rapid clearance of *S. chartarum* metabolites, including trichothecenes, from spore-impacted mouse and rat lungs. To the contrary, they indicate that spores act as time-release capsules – liberating their metabolites into the extracellular space relatively slowly.

Using immunocytochemistry, Gregory *et al.* evaluated the distribution of the trichothecene, satratoxin G, in spores and mycelia of *S. chartarum* and in lung tissues of intratracheally exposed mice [140]. Heavy satratoxin G labelling was observed predominantly in AM, particularly in the lysosomes, heterochromatin and rough endoplasmic reticulum, even 48 h post-exposure. Alveolar type II cell heterochromatin and endoplasmic reticulum showed only modest presence of the toxin. These observations indicated that spore-derived satratoxin G was relatively long lived in tissues, and that it displayed a high degree of cellular specificity with respect to its uptake in mouse lung tissues, and that AM appear to play an important role in providing cellular defence against trichothecene toxins. In a separate study, Gregory *et al.* [141] described the distribution of stachylysin, which causes lysis of red blood cells *in vitro* [64,65,142]. Granulomatous lesions enclosing spores in rat and mouse lungs, labeled lightly for stachylysin at 24 h and more heavily for stachylysin after 72 h exposure. This indicated that stachylysin production/release is a relatively slow process. They also reported localization of stachylysin in AM phagolysosomes and suggested that AM may be involved with its inactivation and clearance from the lung environment. Localization of the satratoxin G in lysosomes in the AM, and stachylysin in the phagolysosomes (Fig. 5d) suggests that there are probably different strategies for the sequestration, detoxification and clearance of these substances from the lung.

Animal experiments have indicated that the slow disappearance of *S. chartarum* spores from lung tissue

has other consequences. Using antibodies to collagen IV, lung tissue in untreated mice, and saline-, isosatratoxin-F- and *C. cladosporioides*-spore-treated animals labels heavily for collagen IV. Collagen IV is the important basement membrane material that lends structural and functional support to the basal surface of all epithelial lining cells. It also labels heavily in alveoli not directly impacted by *S. chartarum* spores (Fig. 5c). However, in granulomas surrounding *S.*

chartarum spores in mice exposed for 24–96 h post-inoculation, there is reduced labeling. Kordula *et al.* [66] and Yike *et al.* [118] showed that *S. chartarum* spores produce extracellular proteases that hydrolyse collagens (I, IV, X). Changes in the expression and distribution of extracellular ground substances such as collagens I, II and IV in lung tissue, due to exposure to proteases sequestered in *S. chartarum* spores in the lungs, may have several effects. The integrity of lung

Table 3 Summary of demonstrated or suspected* effects of *Stachybotrys chartarum* spore components on rodent lung biology†

Component	Effect
Spore particle, 3000 spores/g bdy wt	Gross pulmonary injury (hemorrhage) Granuloma formation Reduced pulmonary function Hemodynamic adjustments (vasodilation/constriction) BALF eosinophilia Alveolar macrophage activation/ phagocytosis Hemodynamic adjustments (transpulmonary pressure changes)*
30 spores/g bdy wt	BALF IL-1, IL-6 cytokine production
Multiple exposures <i>S.c.</i> allergen extract	Increased BALF cell abundance BALF Neutrophilia BALF Eosinophilia BALF IgE production BALF Total protein production BALF LDH accumulation BALF Lymphophilia TNF α production Immunomodulation*
β 1,3 glucan, 2.1 ng/g bdy wt	TNF α release
β 1,3 glucan, 0.02 ng/g bdy wt	Tissue damage
Serine proteinases in 3000 spores/g bdy wt	Depressed collagen IV expression Pulmonary "Convertase" perturbation* Alveolar capillary vascular permeability changes (increased BALF total protein/albumin content)* Alveolar type II cell necrosis* Alveolar capillary wall vesicularization* Increased BALF LDH concentration* Hemorrhage*
Serine proteinases, 30 spores/g bdy wt	Alveolar capillary vascular permeability changes (increased BALF total protein/albumin content)* Increased BALF LDH production
Hemolysin in 3000 spores/g bdy wt	Incorporation into phagolysosomes Erythrocyte plasmalemma binding Hemosiderosis*
Satratoxin, 2.1 ng/g bdy wt	Alveolar type II cell damage Pulmonary surfactant homeostasis perturbation Depressed CTP:cholinephosphate cytidylyltransferase Lamellar body perturbation Mitochondrial damage Plasma membrane vesicularization Alveolar macrophage: Incorporation into lysosomes Incorporation into heterochromatin Ribosomal binding

†See text for further detail.

granuloma tissue surrounding *S. chartarum* spores could be affected by depressing collagen IV synthesis in the alveolar capillary endothelium basement membrane. This in turn could weaken alveolar capillary lining cells making them susceptible to rupture during cough spasm.

None of these experiments has produced a biomarker that might document an effect of toxin exposure in human tissues. They have, however, produced an emerging picture of the complexity of potential damage resulting from inhalation of *S. chartarum* spores, mycelial fragments and substrate dusts. Table 3 summarizes the effects noted in the above experiments, making an effort to account for the known or suspected cause of the damage observed. Although it has been noted for years that any effects of inhalation exposure to moulds would represent the combined effects of the chemicals present in the spores, most authors have chosen to focus only on the trichothecene toxins.

Observations and conclusions

Living or working in a mouldy building can cause building-associated asthma, is associated with exacerbation of asthma and increased rates of upper respiratory diseases (e.g. [3,4]). In damp or water-damaged buildings, *S. chartarum* is often associated with other hydrophilic species and a variety of other species that grow on progressively drier materials [34]. The evidence reviewed in this paper is that *S. chartarum* contributes both to the allergic and to the non-allergic components of the human diseases seen in damp buildings. As is the case for essentially all building-associated fungi, it is currently impossible to assess the individual contribution of any building-associated fungus or indeed all such fungi on a population health basis [4].

Nevertheless, every cognizant authority review since the first New York Guidelines [143] has explicitly acknowledged that exposure to some species of building-associated fungi, including *S. chartarum*, represents

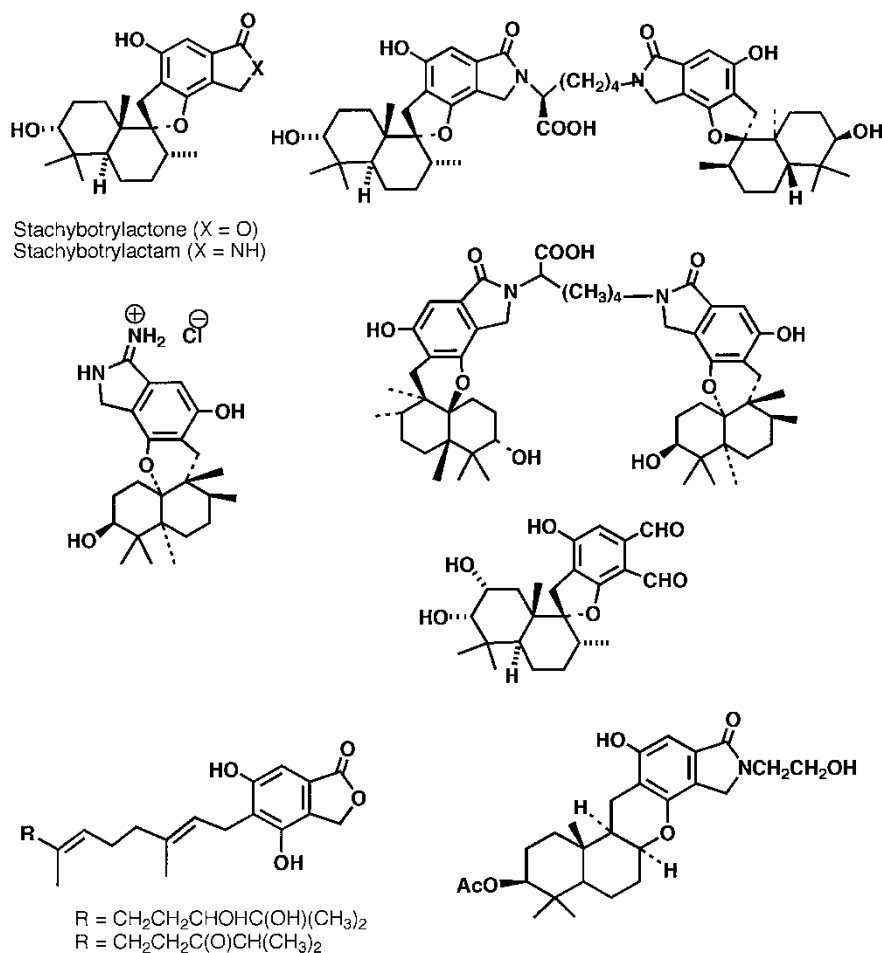


Fig. 3 Structures of spirocyclic drimanes and related compounds.

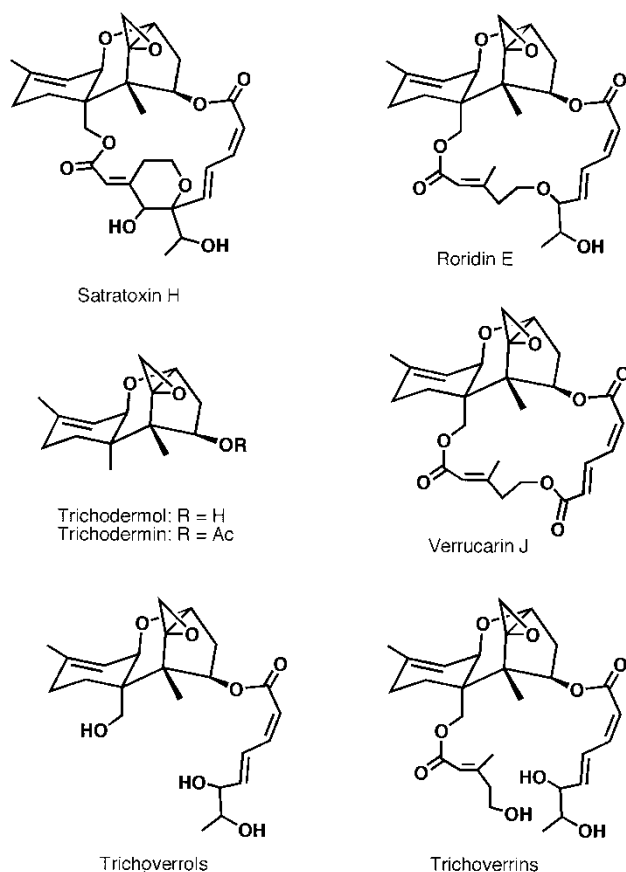


Fig. 4 Structures of trichothecenes.

an increased hazard compared to other fungi and to the high levels of fungi in outdoor air. We found that this position is well supported by the information we found on the properties of *S. chartarum*. The reasons for this can be briefly summarized as follows. Because *S. chartarum* requires near water saturation for growth on cellulose-derived materials in nature, its growth implies that some part of the building has been very wet. Wet buildings can become highly contaminated by fungi.

Handling contaminated materials generates total spore concentrations capable of causing severe disease in humans for several reasons, including the presence of agents such as cytotoxins, organic material, allergens and endotoxin. However, the symptoms documented from handling materials contaminated with *S. chartarum* indicate a greater hazard than that for organic materials in general, including that for materials damaged by other moulds. The weight of evidence from agricultural exposure and the so-called first cluster of the New York cases suggests that the disease observed is partially a consequence of exposure to the

potently cytotoxic macrocyclic trichothecenes present in the spores and substrate dusts associated with *S. chartarum*-contaminated straw and building materials. In the two cases where personal exposure to *S. chartarum* spores was documented (first in a child in Texas, by isolating a spore in lung lavage, and second in an infant in Cleveland, by DNA), it is reasonable to conclude that at least part of the symptoms seen resulted from exposure to macrocyclic trichothecenes, because such symptoms are similar to those seen in experimentally exposed laboratory animals. It must be noted that even in these cases, no evidence of toxin-associated injury was measured biochemically.

In a regulatory sense, all of the conditions necessary to make a public health recommendation have been met in terms of hazard identification. The allergens and toxins of *S. chartarum* can result in disease in humans as well as in domestic and laboratory animals. The mechanisms that result in cell damage in animals apply to humans. Handling materials naturally contaminated by *S. chartarum* is relatively dangerous. Living in a building where it is possible to recover *S. chartarum* spores or even DNA from lung lavage is also apparently dangerous, and is probably more dangerous to infants and children than to adults. For several reasons, we cannot describe what this might mean in terms of the extent of mould damage required to produce those conditions, except to note that, at least twice, they have been met.

Several authors have characterized the risk of toxin-associated disease from exposure to *S. chartarum* in building materials as low. We feel that it is too soon to draw such a conclusion because many data are missing. Perhaps foremost, there is no reliable theory on the nature of exposure, except to say that spores, mycelial fragments and substrate dusts are involved. As noted above, spores of *S. chartarum* are produced in a range of sizes; however, spore and mycelial fragments are known to be an important component of the exposure yet are virtually never measured, and substrate dusts containing fungal toxins and perhaps allergens have never been measured in a building. These shortcomings prevent a reasoned assessment of the potential exposure that at the present time can only be inferred, typically from measurements of the area of substratum bearing intact spores or, less usefully, viable propagules.

Some of the laboratory studies reported above show that fairly modest intratracheal exposure in a number of mouse strains results in clear adverse effects on critical aspects of lung biology and damage. The presence of trichothecenes in the spores used for such experiments explains some of the effects seen, but the potential effects of other concurrent components of the

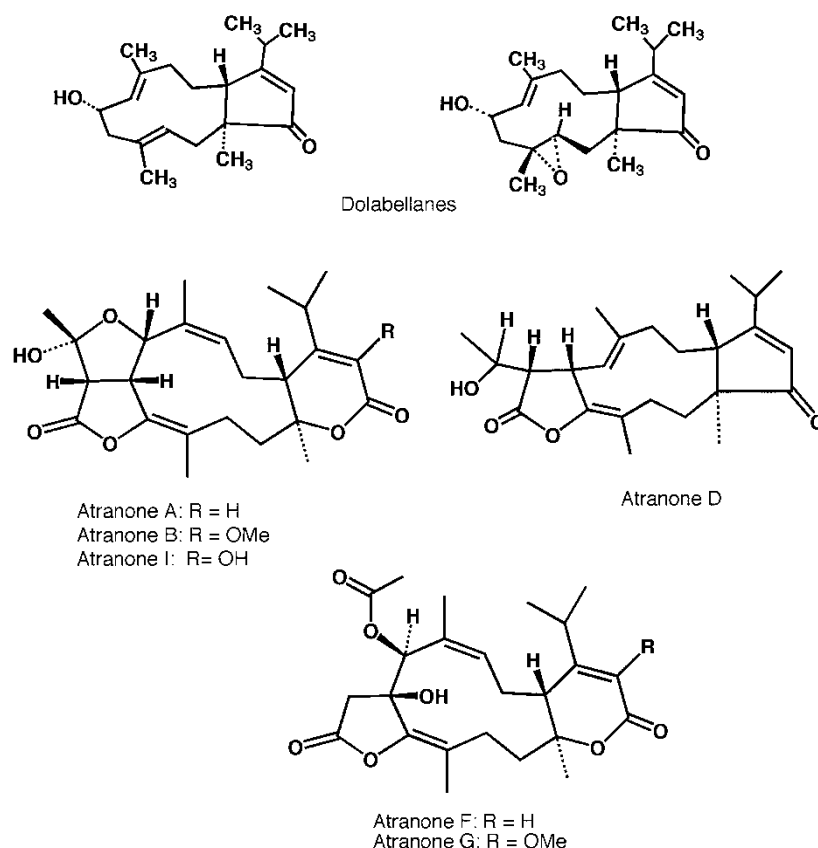


Fig. 5 Structures of atranones and dolabellanes.

exposure measured (including other metabolites such as glucan, allergen, haemolytic factors and proteases) have neither been individually attributed nor, perhaps, measured. Although there is much speculation about the effect of systemic exposure to trichothecene mycotoxins, such an effect is unlikely, despite non-lung consequences of lung damage [116]. The effects of inhalation exposure to *S. chartarum* on lung mediators such as endothelins [144] or alveolar macrophage-derived neurotransmitters [145] have not been assessed. Headaches have been reported in investigations of mouldy buildings with or without reports of *Stachybotrys* [61,98,146]. As noted above, headaches were reported by volunteers exposed to glucan [63]. One abstract suggested that neurocognitive damage resulted from *Stachybotrys* exposure [147] although no mechanism for this was suggested and none are immediately obvious.

The most useful studies on the impact of trichothecene-containing spores of *S. chartarum* are those that have involved a number of different strains of experimental mice. Mice are more resistant to the toxic effects of trichothecenes than are non-human primates [94].

Whether the consequences of *S. chartarum* exposures would be greater when other fungal materials, endotoxin and building dusts are present remains unknown.

Further risk characterization cannot be reliably made without obtaining one or more of the following advancements. (1) Better characterization of the total exposure (spores, spore and mycelial fragments), such that nose-only inhalation experiments can be carried out, is needed. (2) A better understanding of the relative roles of the chemicals present in an exposure arising from *S. chartarum*-contaminated building materials (trichothecenes, atranones, sporocyclics, protease, allergen, cyclosporine) needs to be obtained from several animal models. (3) Biomarkers of the impact of one or more *S. chartarum* need to be developed and applied to human tissue.

It appears that the outcome of very high exposure to *S. chartarum* can differ from that of exposure to other building-associated fungi. It is evident that there is a NOAEL of exposure to *S. chartarum* in the built environment, but this may differ according to age, medical history and concurrent exposures. Much needs to be done to clarify the risk. That said, the public

policy in several countries is that living or working in damp buildings is unacceptable. Perhaps this will in the end render the question of the hazard of *S. chartarum* irrelevant.

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