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Review

Mycotoxin production by indoor molds

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Abstract

Fungal growth in buildings starts at a water activity (a_w) near 0.8, but significant quantities of mycotoxins are not produced unless a_w reaches 0.95. *Stachybotrys* generates particularly high quantities of many chemically distinct metabolites in water-damaged buildings. These metabolites are carried by spores, and can be detected in air samples at high spore concentrations. Very little attention has been paid to major metabolites of *Stachybotrys* called spirocyclic drimanes, and the precise structures of the most abundant of these compounds are unknown. Species of *Aspergillus* and *Penicillium* prevalent in the indoor environment produce relatively low concentrations of mycotoxins, with the exception of sterigmatocystins that can represent up to 1% of the biomass of *A. versicolor* at a_w's close to 1. The worst-case scenario for homeowners is produced by consecutive episodes of water damage that promote fungal growth and mycotoxin synthesis, followed by drier conditions that facilitate the liberation of spores and hyphal fragments.

Keywords: *Aspergillus versicolor*; Penicillium; Spirocyclic drimanes; *Stachybotrys chartarum*; Trichothecenes

1. Introduction

During the last 10–15 years case studies have shown that people living and working in damp or moldy buildings have an increased risk of adverse health effects including airway infections, impaired immune function, bronchitis, asthma, recurrent airway infections, and extreme fatigue (Gordon et al., 1999; Gravesen et al., 1994; Hodgson et al., 1998; Johanning et al., 1996; Koskinen et al., 1999; Purokivi et al., 2001; Rylander et al., 1998). Numerous studies point toward the adverse effects of mold exposure in these environments (Brunekreef et al., 1989, Brunekreef, 1992; Dales and Miller, 2002; Dales et al., 1991, 1998; Dekker et al., 1991; Koskinen et al., 1999; Meklin et al., 2002; Platt et al., 1989; Verhoeff and Burge, 1997), but this remains a subject of active debate (Bornehag et al., 2001). Biblical reference to indoor mold growth shows that this has been recognized as a problem for at least 5000 years (Leviticus, ch. 14, v. 33-53). More recently, the presence of *Stachybotrys chartarum* has been associated with the development of idiopathic pulmonary hemosiderosis (IPH) in infants, although the active toxins and mechanisms of exposure remain unclear (Dearborn et al., 1997, 1999; Vesper and Vesper, 2002).

The proportion of buildings with mold growth in Northern Europe and North America is perhaps as high as 20–40%. Data from the UK suggest that 30–45% of buildings are moldy (Hunter et al., 1988; Platt et al., 1989), while 20–25% of buildings may be affected in The Netherlands (Adan, 1994), 20–30% in Finland (Koskinen et al., 1999; Nevalainen et al., 1998), 40% in the USA (Brunekreef et al., 1989), and 30% in Canada (Dales et al., 1991). In Denmark, up to 50% of schools and daycare centers have mold growth (Gravesen et al., 1999). However it should be recognized that this mold growth ranges from small areas covering a few cm^2 to widespread fungal proliferation in heavily contaminated buildings. To address current and future health issues associated with indoor molds, it is essential to obtain clinically-valid analyses of patients' complaints, to identify toxic metabolites present in buildings, and to determine hazardous exposure levels.

Suggested mechanisms of mold-induced illness include Type I allergy (Gravesen, 1994), non-IgE mediated specific histamine release (Larsen et al., 1996a,b)

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and inflammation (Hirvonen et al., 1999; Purokivi et al., 2001), changes in lymphocyte composition (Dales et al., 1998; Johanning et al., 1996), generalized immunosuppression (Miller, 1992), and toxic reactions (Croft et al., 1986; Hodgson et al., 1998; Jarvis et al., 1998; Johanning et al., 1996; Miller, 1992). The following types of agents from molds can be implicated in these diverse health effects:

- Proteins causing Type I allergy and, in rare cases, Type III allergy (Gravesen et al., 1994).
- Structural elements including β-1,3-glucans that may trigger inflammatory reactions related to symptoms observed after exposure to endotoxin (Chew et al., 2001; Rylander et al., 1998), and melanins that can activate the complement system (Rosas et al., 2002).
- Microbial volatile organic compounds (MVOC) produced during growth (though indoor concentrations of MVOCs are significantly lower than those inducing irritation and other adverse effects on human health; Miller et al., 1988; Ström et al., 1994; Wilkins et al., 1997; Pasanen et al., 1998).
- Mycotoxins and other secondary metabolites released from fungal spores and colony fragments after inhalation (Croft et al., 1986; Jarvis et al., 1998; Sorenson et al., 1987). Note that the potential toxicity of colony fragments may explain how a "problem building" can have a low spore count relative to the outdoor air (Garrett et al., 1998; Hyvärinen et al., 2001; Miller et al., 2000).

2. The building-associated mycobiota

Buildings can be considered "new" manmade ecosystems, where—as in other environments—a limited number of fungal species will dominate, depending on humidity and nutrient availability (Filtenborg et al., 1996). Molds growing on building materials can be divided into three groups after Grant et al. (1989) based on their water activity, a_w, requirements on laboratory substrates, and responses to changes in a_w (Nielsen, 2002):

- Primary colonizers or storage molds, capable of growing at $a_w < 0.8$ (many with optimal growth rates at a_w approaching 1): *Penicillium chrysogenum* and *Aspergillus versicolor* are the most common species, followed by others including *A. fumigatus*, *A. niger*, *A. sydowii*, *A. ustus*, several *Eurotium* species, *P. brevicompactum*, *P. commune*, *P. corylophilum*, *P. palitans*, *Paecilomyces variotii*, and *Wallemia sebi*.
- Secondary colonizers or phylloplane fungi, requiring a minimal a_w between 0.8 and 0.9. This group comprises species of *Alternaria*, *Cladosporium*, *Phoma*, and *Ulocladium*. These are able to thrive under conditions where marked changes in humidity occur during the day.
- Tertiary colonizers or water-damage molds, needing $a_w > 0.9$, include many of the most toxic species such as *Chaetomium globosum*, *Memnoniella echinata*, *Stachybotrys chartarum*, and species of *Trichoderma*. Species of *Trichoderma* isolated from moldy buildings include *T. atroviride*, *T. citrinoviride*, *T. harzianum*, and *T. longibrachiatum* (Lübeck et al., 2000). Several of these are considered tropical fungi, which seems consistent with their propensity for growth in humid buildings.

3. Factors affecting growth on building materials

Water activity, also referred to as equilibrated relative humidity, is undoubtedly the most important factor in determining whether or not mold growth is initiated on building materials (Adan, 1994; Ayerst, 1966; Galloway, 1935; Hukka and Viitanen, 1999; Rowan et al., 1999; Scott, 1957). It is essential to understand that local differences in ventilation and surface temperature can generate micro-climates with very high a_w in a room with an otherwise low relative humidity (RH).[1] For this reason, a measurement of indoor RH is a very poor predictor of mold problems (Adan, 1994; Becker, 1984; Grant et al., 1989; Gravesen et al., 1999; Hukka and Viitanen, 1999).

On construction materials, xerophilic molds, such as the penicillia and aspergilli will begin growth at a_w between 0.78 and 0.90, depending on the composition of the substrate (Adan, 1994; Chang et al., 1995; Grant et al., 1989; Nielsen, 2002; Pasanen et al., 1992; Rowan et al., 1999). Wood, wood composites (plywood, chipboard, OSB board, etc.), and materials with a high starch content are capable of supporting mold growth at the lowest values of a_w (Hukka and Viitanen, 1999; Nielsen et al., 2000; Nielsen, 2002; Viitanen and Bjurman, 1995). Plasterboard reinforced with cardboard and paper fibers, or inorganic materials coated with paint or treated with additives that offer an easily-degradable carbon source, are excellent substrates for molds, but will not support growth unless a_w reaches 0.85–0.9 (Adan, 1994; Chang et al., 1995, 1996; Nielsen, 2002). Other inorganic materials with traces of organic materials seem to support growth at higher water activities (a_w 0.9–0.95). A few authors report extraordinarily low a_w limits for growth on building materials (Chung et al., 1999; Ezeonu et al., 1994; Nikulin et al., 1994; Pasanen et al., 1994), but this may be due to the provision of water from the inoculum, or poor experimental control of water activity (Adan, 1994). An excellent review of

[1] *Abbreviations used*: a_w, water activity; DAD, diode array detection (UV light); GC, gas chromatography; LC liquid chromatography, including high performance liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; RH, relative humidity.

fungal growth in buildings can be found in Flannigan and Miller (2002).

4. Significance of transient humidity conditions

Variations in indoor humidity and temperature exert a profound influence upon mold growth (Adan, 1994; Viitanen and Bjurman, 1995), where the period of time when water availability exceeds the threshold a_w for mold growth, and the number of dry periods during the day are key parameters (Adan, 1994). A bathroom is a good example of an indoor environment with transient high humidity, and has a specific mycobiota dominated by phylloplane fungi including species of *Alternaria*, *Aureobasidium*, *Cladosporium*, *Phoma*, and *Ulocladium* (Moriyama et al., 1992; Samson et al., 2002). The appearance of these fungi is due to their capacity to resume growth from dry hyphal tips within 1 h after rewetting—storage and water-damage molds do not begin growing again for 1–2 days (Park, 1982). Recently, it has been shown that *C. sphaerospermum* can out-compete *P. chrysogenum* on various plaster materials, paints and plasterboards under conditions of variable a_w (during the day), whereas *P. chrysogenum* out-compete *C. sphaerospermum* at constant a_w (Adan, O.C.G., van der Wel, G., Nielsen, K.F., unpublished data; Nielsen, 2002).

5. Factors affecting mycotoxin production by indoor molds

Microfungi produce many secondary metabolites which are thought to play a crucial role in their natural habitats (Frisvad et al., 1998; Williams et al., 1989). Most of these compounds function as toxins against plants, bacteria, and other fungi (Gloer, 1995). Fungal metabolites that cause a toxic response at low doses in vertebrates are referred to as mycotoxins. Most are non-volatile with a molecular weight below 1500 Da. Numerous reviews on mycotoxigenic fungi in the indoor environment have been written, but none of them adequately address the mycotoxins and other metabolites that molds generate in buildings. Many issues must be considered when assessing mycotoxin production in buildings: (i) secondary metabolites and mycotoxins are species-specific, making identification of isolates to the species level extremely important (Frisvad et al., 1998; see next section); (ii) the literature is scattered with false reports of mycotoxins produced by different mold species (Frisvad, 1989); (iii) mixtures of metabolites can produce synergistic effects (Miller, 1992; Sorenson et al., 1984; Stoev et al., 2002); (iv) certain mycotoxins can be masked as derivatives (Gareis et al., 1990; Jarvis, 1992); (v) the biological effects of many fungal secondary metabolites are poorly documented, and very few of them have been evaluated in animal studies (especially the effects of inhalation); (vi) in vitro experiments show that metabolite production is influenced by medium composition, temperature, and water activity, indicating that molds are likely to generate different metabolites when they grow on building materials (Frisvad and Gravesen, 1994; Nielsen, 2002; Ren et al., 1999), and (vii) toxin-production is affected by the fact that molds grow in mixed cultures with other fungi and bacteria in buildings (Anderson et al., 1997; Peltola et al., 2001; Räty et al., 1994).

6. Identification

Identification of mold species is vital for studies on mycotoxin production by indoor molds. Chemotaxonomy based on the species-specific production of metabolites is an important tool that can help exclude problems caused by incorrect identification based on microscopy, contamination of cultures, and erroneous claims of mycotoxin production (Frisvad, 1989; Mantle, 1987). Chemical analysis has proven very effective in studying *Aspergillus* and *Penicillium*, where there are clear relationships between metabolite production and certain species, and where even experts find it very difficult, or impossible, to discriminate between species by classical microscopic examination (Andersen and Frisvad, 2002; Frisvad et al., 1998). In most cases, the identification of aspergilli and penicillia can be greatly enhanced by examining colony morphology (diameter, color, and surface texture) on several media including Czapek yeast autolysate (CYA), yeast extract sucrose (YES) and creatine sucrose agar (CREA; Frisvad, 1981; Samson et al., 2002). Analysis of culture morphology, growth rate (colony diameter), and color, on one or more types of medium using image analysis software can help automate the identification of species (Dörge et al., 2000) and clones (Hansen et al., 2003). Molecular biological methods are becoming increasingly important, but their usefulness is limited by the availability of data on reference strains that have been correctly identified (De Hogg et al., 2002; Geiser et al., 1998; Skouboe et al., 2000; Tuthill et al., 2001).

7. Analytical methods

The detection of mycotoxins is a difficult task requiring careful work by analytical chemists with knowledge of fungal metabolites plus access to state-of-the-art instrumentation. Building materials represent new ''matrices'' with an infinite number of combinations of materials (e.g., wallpapers, paints, dust, etc.) that may interfere with the analytical methods. The identification of specific mycotoxins is further complicated by the fact that fungi generate so many different metabolites.

Many studies concerned with indoor health problems and mycotoxins focus on trichothecenes, although this group of metabolites is interesting only when growth of *Stachybotrys*, and perhaps *Memnoniella*, has occurred. For instance, the identification of trichothecenes in dust from a ventilation system by Smoragiewicz et al. (1993) was based on false positive results originating from the use of non-specific thin layer chromatographic (TLC) and liquid chromatographic (LC) methods. Specificity is extremely important, and ideally, a combination of LC (or gas chromatography, GC) and mass spectrometry (MS) should be used. If possible, partial purification of samples with an immunoaffinity column (Richard et al., 1999), or some other method employing a different phase than the one used in the final analytical column, is desirable. LC with diode array detection (DAD) should be used with caution and only for analysis of compounds with highly-characteristic UV-spectra, not for components with end absorption or a single UV max.

However, even when analyses are performed by LC with MS or tandem MS (MS/MS), trichothecene analysis can be difficult due to the lability of these metabolites and their tendency to form adducts (Razzazi-Fazeli et al., 2002). For instance, the report of deoxynivalenol, verrucarol, 3-acetyl-deoxynivalenol, diacetoxyscirpenol, and T-2 tetraol in moldy materials by Tuomi et al. (2000) may have resulted from false positives, because no trichothecene-producing fungi were isolated, no T-2 toxin was detected in samples containing T-2 tetraol, and no deoxynivalenol was found in samples containing 3-acetyl-deoxynivalenol. Such odd results, in which no apparent producer is present and in which there is an inconsistency in the list of detected compounds, must be verified using different analytical methods.

Besides immunoassays, a variety of biological methods have been used for mycotoxin analysis, including cytotoxicity or inflammation assays (Johanning et al., 1999; Ruotsalainen et al., 1998; Smith et al., 1992). Trichothecene concentrations can also be estimated via their inhibitory effect on protein translation (Yike et al., 1999).

8. Sample collection

Sample collection is a key step in the analysis of mycotoxins in buildings. The collection of pieces of building material can be problematic because: (i) biomass can be lost during handling of the material or can be transferred to the inner surface of the sampling container (and lost in subsequent analyses); (ii) large quantities of components that interfere with the chemical analyses are introduced which necessitates several clean-up steps (Nielsen et al., 1998b, 1999); and (iii) from the point of view of human exposure, only the mycotoxin-containing particles that can become airborne are interesting. Therefore, surface sampling using ultra-clean swabs (Nielsen, 2002), or by vacuuming (e.g., onto 0.1 μm pore size Teflon filters), is recommended. This approach can be extended to quantitative assessment of exposure based on particle release using a Particle—Field and Laboratory Emission Cell (P-FLEC; Jensen and Kildesø, 2001; Kildesø et al., 2000).

9. Mycotoxins produced by *Stachybotrys*

Since the association between growth of *Stachybotrys chartarum* in buildings and idiopathic pulmonary hemosiderosis (IPH) was published (Dearborn et al., 1997; Jarvis et al., 1996; Montana et al., 1995) this mold has become the subject of intensive investigation. The apparent toxicity of this mold makes sense from a chemical point of view because the biomass released from areas infested by *Stachybotrys* contain higher quantities of secondary metabolites (based on LC–DAD and LC–MS results) than other indoor molds (Nielsen, 2002).

Interestingly, very little attention has paid to the 10–40 different spirocyclic drimanes (Fig. 1), and their precursors, produced by *Stachybotrys*. Large quantities of these compounds are synthesized, suggesting that they may play an important role in the biology of the mold and its effects on human health (Andersen et al., 2002b; Hinkley and Jarvis, 2000; Nielsen, 2002). This class of metabolites has a broad spectrum of activity, including enzyme inhibition, disruption of the complement system, inhibition of TNF-α liberation, cytotoxicity and neurotoxicity, and stimulation of plasminogen, fibrinolysis, and thrombolysis (the structures and effects of several of these compounds have been patented; Hasumi et al., 1998; Kaneto et al., 1994; Kohyama et al., 1997; Nozawa et al., 1997). A number of precursors, such as SMTP-1 in which the drimane part is not cyclized, have also been described (Hasumi et al., 1998). The spirocyclic drimanes are produced partly by the terpenoid pathway, which generates two lower rings under the spiro bond, and partly by the polyketide pathway, which fabricates the upper part of the molecule.

Stachybotrys chartarum is best known as a producer of the highly cytotoxic macrocyclic trichothecenes that inhibit protein synthesis: satratoxin H, G, F, and iso-F, roridin L-2 (Fig. 1), several roridin E epimers, hydroxyroridin E, and verrucarin J and B (all are products of the sesquiterpenoid biosynthetic pathway). However, when analyzing isolates from contaminated buildings, only 30–40% of the strains (chemotype S) actually produce the macrocyclic trichothecenes (mainly satratoxin H, and roridins E and L-2; Andersen et al., 2002b; Croft et al., 1986; Jarvis et al., 1998; Nielsen et al., 1998a, 2001; Vesper et al., 2000). The remaining isolates produce the diterpenoid atranones and their dolabellane

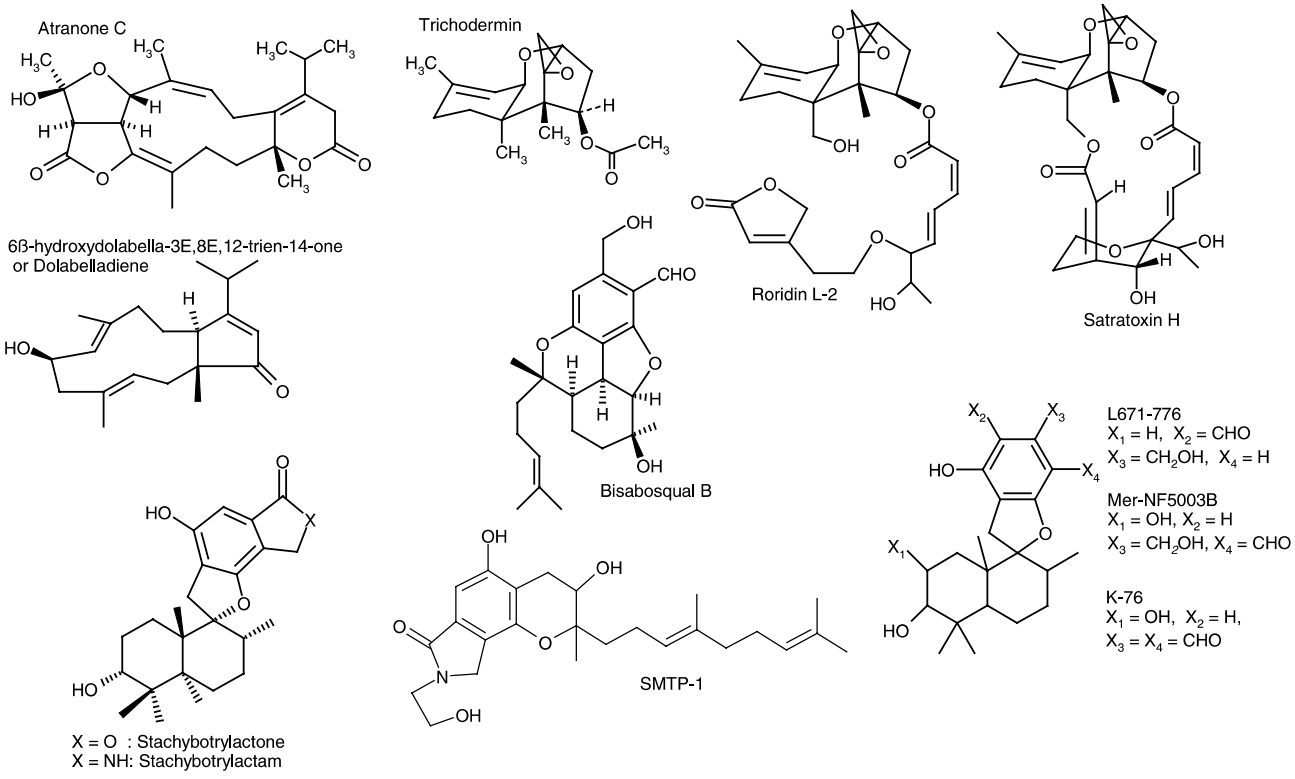

Fig. 1. Variety of metabolites produced by *Stachybotrys* spp.

precursors (Hinkley et al., 2000), and small quantities of simple (non-macrocyclic) trichothecenes (Andersen et al., 2002b).

The majority (70–80%) of the strains that do not produce macrocyclic trichothecenes belong to *S. chartarum* chemotype A, and the remaining strains to a new species of *Stachybotrys* (Andersen et al., 2002b; Cruse et al., 2002; Peltola et al., 2002). *S. chartarum* chemotype A occasionally produces traces of the satratoxin precursors roridins E and L-2. Other interesting metabolites from *S. chartarum*, include a cyclosporin analogue (Sakamoto et al., 1993), and a novel compound called stachylysin (Vesper et al., 1999; Vesper and Vesper, 2002). Stachylysin production may be associated with the IPH cases in Cleveland.

Studies on the toxicity of extracts from *Stachybotrys* spores and/or mycelium have revealed significant differences between the two chemotypes (Korpi et al., 2002; Nielsen et al., 2001; Nikulin et al., 1997b,a; Ruotsalainen et al., 1998). Both induce inflammation, but high concentrations of extracts from chemotype S are, in addition, highly cytotoxic. Animal studies indicate that the two chemotypes have very different effects on lung homeostasis and surfactant production (Mason et al., 2001; Rand et al., 2002). Rao et al. (2000) have verified that inflammation is caused by methanol extractable metabolites (that include all of the non-protein metabolites mentioned previously).

Stachybotrys is commonly found on water-saturated gypsum boards, which it can overgrow within 2–3 weeks (Gravesen et al., 1999; Nielsen et al., 1998b). Here, as well as on hay (one of its natural growth substrates), the most common metabolic profile includes a variety of spirocyclic drimanes (Nielsen, 2002), traces of trichodermin, and, depending on the chemotype, atranones (as the two dolabellanes and atranones B and C; Vesper et al., 2000) or macrocyclic trichothecenes (Croft et al., 1986; Hodgson et al., 1998; Jarvis et al., 1998; Knapp et al., 1999). The primary spirocyclic drimanes have UV spectra resembling Mer-NF5003B (Kaneto et al., 1994; Roggo et al., 1996; and see Fig. 1 in Andersen et al., 2002b) and the bisabosquals (Minagawa et al., 2001), but our LC–MS analyses indicates that they have molecular masses 50–200 Da higher than the two known metabolite groups (Nielsen, unpublished). Other important drimanes detected are stachybotryamide, stachybotrylactams, and di-aldehydes. The quantities of metabolites based on LC–UV, LC–MS, and bioassays are significantly higher than those detected in other molds growing on gypsum boards (Anderson et al., 1997; Johanning et al., 1996, 1998; Nielsen et al., 1998b, 1999; Nielsen, 2002).

Recently, satratoxins, stachybotryamide, stachybotrylactams, and the metabolites resembling Mer-NF5003B and/or bisabosquals, were detected by combined LC–DAD and GC–MS/MS in air (sampled

using filters attached to a high volume pump) from a house with massive *Stachybotrys* growth. The satratoxin content of the samples rendered them highly toxic to cell cultures (MTT test; Johanning et al., 2002).

Analysis of material samples from four IPH case homes identified *S. chartarum* chemotype S in one home (Knapp et al., 1999), and chemotype A in three other homes (Nielsen, 2002; Nolard, 2000; Vesper et al., 2000; Johanning, Gareis, Nielsen, unpublished). In other studies chemotype S was not associated with cases of IPH (Andersen et al., 2002b; Jarvis et al., 1998).

A number of active *S. chartarum* metabolites await identification, including a mitochondrial toxin (Peltola et al., 1999, 2002), and a compound, or compounds, with molecular mass exceeding 3000 Da that induces specific and non-specific inflammation in mice as well as an asthma-like response (Viana et al., 2002). The latter effect may be induced by the proteins and glycoproteins described by Raunio et al. (2001).

10. Mycotoxins produced by other indoor molds

10.1. Alternaria

The *A. tenuissima*-species group predominates in buildings (Andersen et al., 2002a; Nielsen et al., 1999) rather than the rare species, *A. alternata*, which is often identified incorrectly when any black-colored *Alternaria* is found (Andersen et al., 2001). On laboratory media *A. tenuissima*-species group produce alternariols, tentoxin, tenuazonic acids, altertoxin I, and a number of unknown metabolites (Andersen et al., 2002a). On building materials, *A. tenuissima* produce alternariol and alternariol monomethyl ether (both very low toxic and not considered true mycotoxins), but none of the other metabolites generated on laboratory media (Nielsen et al., 1999; Ren et al., 1998). However, none of the methods used in these studies offered high sensitivity for tenuazonic acid. Interestingly, we have not been able to obtain samples of materials contaminated with substantial quantities of *Alternaria* biomass; most of the samples suspected of housing *Alternaria*, actually housed *Ulocladium* which does not produce any known mycotoxins (Nielsen et al., 1999).

10.2. Aspergillus flavus

A. flavus is occasionally isolated from building materials, and since this species produces the most potent naturally-occurring carcinogen, aflatoxin B_1 (Davis et al., 1966; Frisvad and Thrane, 2002), mycotoxin synthesis by building isolates of this species has been studied by several groups. In addition to aflatoxin B_1, *A. flavus* can also produce metabolites called kojic acid and aspergillic acid, as well as the mycotoxins 3-nitropropionic acid, and cyclopiazonic acid (Frisvad and Thrane, 2002). When aflatoxinogenic strains were inoculated on various building materials they did not produce aflatoxins (Rao et al., 1997; Ren et al., 1999). Research by the author found that building materials contaminated with *A. flavus* did not contain kojic acid, aspergillic acid, cyclopiazonic acid, nor aflatoxin B_1 (Nielsen, 2002).

10.3. Aspergillus fumigatus

A. fumigatus is frequently isolated in moldy buildings and especially from dust. It has an amazing arsenal of biological active metabolites, including fumigaclavines, fumitoxins, fumitremorgens, gliotoxins, tryptoquivalins, and verruculogen (note that these names are sometimes spelled differently; Debeaupuis and Lafont, 1978; Frisvad and Thrane, 2002; Steyn and Vleggaar, 1985). When cultivated on wood pieces it produces compounds that act as tremorgenic agents in rats (presumably the fumitremorgens; Land et al., 1987). Isolates capable of producing verruculogen and helvolic acid, failed to synthesize these compounds on ceiling tiles and plasterboard (Ren et al., 1999). Recently, it was shown that gliotoxin (up to 40 ng/cm^2) and several gliotoxin analogues are produced by *A. fumigatus* inoculated on wood, plasterboard, and chipboard at high a_w (Nieminen et al., 2002).

10.4. Aspergillus niger

It is difficult to differentiate between *A. niger* and other members of *Aspergillus* section *Nigri*, but all of the isolates we have examined from buildings have been *A. niger sensu stricto* (Nielsen et al., 1999). Ochratoxin A is the only mycotoxin reported from *A. niger*, and is produced by 3–10% of the isolates (Schuster et al., 2002). Other metabolites include naphtho-γ-pyrones, tetracyclic compounds, nigragillin, kotanin, orlandin and malformin A, B, and C (Schuster et al., 2002). On wet building materials, nigragillin, orlandin, and more than 20 unknown metabolites including naphtho-γ-pyrones and tetracyclic compounds were detected from two isolates examined (Nielsen et al., 1999). Neither produced ochratoxin A (according to LC with fluorescence detection) when grown on a variety of substrates (Nielsen, 2002). (The analytical methods used in this study would not have detected malformins: these should be analyzed by LC–MS.)

10.5. Aspergillus ochraceus

A. ochraceus is occasional isolated from building materials, and produces a variety of mycotoxins including ochratoxin A, penicillic acid, xanthomegnin, viomellein, and vioxanthin (Frisvad and Thrane, 2002). High levels of ochratoxin A (up to 1500 ppb) have been

detected in dust samples taken from one home by Richard et al. (1999). This finding was confirmed by several highly selective methods, but the investigators did not determine whether the ochratoxin A originated from mold growth on construction materials in the house or on food scraps in the ventilation system.

10.6. *Aspergillus* cf. *ustus*

Isolates of *A. ustus* from cereals produce the highly carcinogenic austocystins (related to aflatoxins; Steyn and Vleggaar, 1974), versicolorin C, austalides, and the highly toxic austamides and austdiols (Steyn and Vleggaar, 1976). Most building-derived isolates have a totally different metabolite profile, and appear to belong to a new species related to *A. ustus* var. *pseudodeflectus*. When five building-derived strains were grown on chipboard and gypsum (with and without wallpaper), the only metabolites that could be unambiguously identified were kotanins, and from the >40 unknown components detected, a number of peaks in the HPLC-DAD chromatograms were probably produced by ophibolins. Austamide, austdiol, and austocystins were not detected, even though reference standards were available (Nielsen et al., 1999).

10.7. *Aspergillus versicolor*

A. versicolor and *P. chrysogenum* are the most common species of indoor mold. *A. versicolor* is able to grow on very nutrient-poor materials such as concrete and plaster. This mold has highly variable culture morphology, but produces a consistent chemical profile on laboratory substrates, usually generating high quantities of the carcinogenic mycotoxin called sterigmatocystin (especially on CYA and 2% malt agar), and related compounds (e.g., versicolorins; Frisvad and Gravesen, 1994; Frisvad and Thrane, 2002). On YES agar, *A. versicolor* produces versicolins but only minute quantities of the sterigmatocystins (Nielsen et al., 1998b). On wallpaper paste, conidia of 50% of the isolates contained sterigmatocystin (Larsen and Frisvad, 1994). Reports of cyclopiazonic acid and ochratoxin A production are probably due to culture contamination and misidentification (Frisvad, 1989; Frisvad and Thrane, 2002).

On water-saturated materials, *A. versicolor* produces 5-methoxy-sterigmatocystin and sterigmatocystin in quantities up to 7 and 20 $\mu g/cm^2$ respectively (up to 1% of biomass; Engelhart et al., 2002; Nielsen et al., 1998b, 1999; Tuomi et al., 2000), whereas they are not produced when $a_w < 0.9$ (Nielsen, 2002). Interestingly, non-sporulating red-colored samples of contaminated materials contained the largest quantities of sterigmatocystins; areas with many conidia contained small quantities (Nielsen et al., 1998b, 1999). Recently, sterigmatocystin was detected in 20% of household dust samples at levels up to 4 ng/g (Engelhart et al., 2002). Sterigmatocystin is not very cytotoxic by itself, but becomes carcinogenic after activation in the liver by the cytochrome P450 mono-oxidase (McConnell and Garner, 1994). In addition to these toxic properties, sterigmatocystin also acts as a strong inhibitor of tracheal ciliary movement (Piecková and Jesenská, 1998).

Rats exposed to spores of *A. versicolor* originating from mold growth on the walls of their cages suffered severe lung damage and developed granulomatous lesions after a month of exposure; these symptoms are thought to have resulted from IL-1 production from activated macrophages (Sumi et al., 1987, 1994). More recently, instillation of single doses of spores has been shown to produce similar effects in mice, with the recruitment of inflammatory cells in the lungs for at least one month (Jussila et al., 2002). The inflammatory properties were also observed from *A. versicolor* spores from wet gypsum boards (Murtoniemi et al., 2001).

10.8. *Chaetomium globosum*

Chaetomium globosum is the most common species of *Chaetomium* found in buildings (Andersen and Nissen, 2000). It is known to produce the highly cytotoxic chaetomins and chaetoglobosins that inhibit cell division and glucose transport (Ueno, 1985). Other *Chaetomium* species produce sterigmatocystins (Sekita et al., 1981), but these have not been found in buildings. On wallpapered plasterboard six building-derived isolates of *C. globosum* produced large quantities of chaetoglobosin A and C (up to 50 and 7 $\mu g/cm^2$), and more than 10 unknown metabolites (Nielsen et al., 1999). Subsequent analyses of naturally contaminated plasterboard, wood, and textiles have shown the same metabolite profiles as well as numerous products of chaetoglobosin hydrolysis (Nielsen, 2002).

10.9. *Memnoniella echinata*

This species is found in the same places as *Stachybotrys* in buildings, and on laboratory media *M. echinata* produces large quantities of griseofulvin, dechlorogriseofulvins, spirocyclic drimanes (different than the ones from *Stachybotrys*), components related to mycophenolic acid, and two simple trichothecenes (trichodermin and trichodermol; Hinkley et al., 1999; Jarvis et al., 1996, 1998; Nielsen, 2002). A sample of contaminated plasterboard studied by the author contained griseofulvin, dechlorogriseofulvin, and epi-dechlorogriseofulvin (Nielsen, 2002).

10.10. *Penicillium brevicompactum*

P. brevicompactum is another common indoor mold (Frisvad and Gravesen, 1994) and is often isolated from

wood (Seifert and Frisvad, 2000). It is capable of producing many metabolites that affect plant growth (Macias et al., 2000). Mycophenolic acid and its analogues are produced most consistently; mycophenolic acid was the first fungal metabolite to be purified and crystallized (as early as 1893; see Bentley, 2000). The most toxic metabolite is the mutagenic compound botryodiploidin (Frisvad and Filtenborg, 1989; Fujimoto et al., 1980). Other metabolites include asperphenamate, brevianamides and Raistrick phenols (Andersen, 1991). When inoculated on building materials, *P. brevicompactum* produced mycophenolic acid, asperphenamate, a tanzawaid acid analogue, and at least two unknown metabolites (Nielsen et al., 1999; Nielsen, 2002).

10.11. Penicillium chrysogenum

Penicillium chrysogenum is the most common indoor mold, and since penicillin was first identified in isolates of this species, its secondary metabolism has been the subject of intensive study for decades. Secalonic acid D is the only toxin generated by this species, and the few strains in which this has been detected were not isolated from the indoor environment. Roquefortine C is consistently produced by *P. chrysogenum* (Frisvad and Filtenborg, 1983, 1989) and seems to be present in most blue mold fermented cheeses (Krusch et al., 1977; Ware et al., 1980). Fortunately, its toxicity is limited! Other metabolites include ω-hydroxyemodine, pyrovoylaminobenzamides, chrysogine, meleagrin, and xanthocillin X (Frisvad and Filtenborg, 1989). On building materials small quantities of chrysogine, 2-pyrovoylaminobenzamide, and meleagrin have been detected (Nielsen et al., 1999; Nielsen, 2002). On isolation media such as V8, DG18, and MEA, *P. chrysogenum* strains can show substantial differences in coloration. For example, cultures of strains that produce xanthocillin X stain agar bright yellow (J.C. Frisvad, personal communication). It is easy to misidentify different species, but sub-cultivation onto YES agar enables the researcher to distinguish *P. chrysogenum* from other penicillia based on colony color, texture, and growth rate (Samson et al., 2002).

10.12. Penicillium expansum

P. expansum is occasionally isolated from wooden substrates in buildings (Frisvad and Gravesen, 1994), and most isolates produce a highly toxic mixture of patulin, citrinin, chaetoglobosins, and communesins, as well as the less toxic roquefortine C (Frisvad and Thrane, 2002). Land and Hult (1987) showed that some *P. expansum* isolates could produce patulin on wood. This is among the most toxic *Penicillium* metabolites. This cytotoxic compound has an inhibitory effect upon the activity of mouse peritoneal macrophages (Bourdiol et al., 1990), and increases animal susceptibility to fungal and bacterial infections (Pestka and Bondy, 1990).

10.13. Penicillium polonicum

P. polonicum is a member of *Penicillium* series *Viridicata* (*P. aurantiogriseum* complex) all of which are associated with cereals. Species belonging to this series are often falsely cited as ochratoxin producers, due to confusion with *P. verrucosum* from *Penicillium* series *Verrucosa* (Frisvad and Thrane, 2002; Lund and Frisvad, 1994). No building derived isolates of *P. verrucosum* have been identified by *Penicillium* experts.

P. polonicum can produce at least three types of highly toxic compounds: (i) the tremorgenic verrucosidins (Steyn and Vleggaar, 1985); (ii) the cytotoxic penicillic acid, which is extremely active against rodent lung macrophages (Sorenson and Simpson, 1986); and (iii) nephrotoxic glycopeptides which may have been involved in the Balkan nephropathy endemic (Frisvad and Thrane, 2002). Other important metabolites include viridicatins, anacine, and cyclopenins. On building materials two *P. polonicum* isolates produced 3-methoxy-viridicatin, verrucosidin, and verrucofortine, although it should be noted that the analytical method (LC–DAD) would not have detected nephrotoxic glycopeptides (Nielsen, 1999).

10.14. Trichoderma

Six species of Trichoderma have been isolated from buildings: *T. longibrachiatum*, *T. harzianum*, *T. citrinoviride*, *T. atroviride*, *T. viride*, and *T. harmatum* (Lübeck et al., 2000). *T. viride* was originally known for producing the trichothecenes trichodermol and its acetyl ester trichodermin (Godtfredsen and Vangedal, 1964) and later the related harzianum A (esters of trichodermol; Corley et al., 1994) have been isolated from *T. harzianum* Cvetnic and Pepelnjak (1997) reported diacetoxyscirpenol synthesis from an indoor isolate of *T. viride* (using TLC), which is unlikely as it would require trichothecene synthesis over iso-trichodermol (Desjardins et al., 1993). Production of gliotoxin and viridin has also been reported from several *Trichoderma* species, but most or all of these were *T. virens* (Frisvad and Thrane, 2002; Thrane et al., 2001). Other bioactive metabolites from *Trichoderma* include: (i) a number of small and often volatile pyrones and lactones (<300 g/mol; Claydon et al., 1987); (ii) membrane active peptides and especially peptaibols (peptaibols belong to a special class of modified peptides; Chugh and Wallace, 2001; Duval et al., 1998); (iii) cytotoxic proteins that inactivate ribosomes (Lin et al., 1991); (iv) iso-nitriles (Faull et al., 1994); and (v) numerous low molecular weight metabolites.

Peltola et al. (2001) showed that a *T. harzianum* strain isolated from a building produced compounds that

damaged the plasma membrane of sperm cells. None of eight strains of *T. atroviride, T. harzianum, T. longibrachiatum* and *T. viride* incubated on different building materials produced detectable quantities of trichodermol (after hydrolysis), or other trichothecenes (Nielsen et al., 1998b)—nor did culture extracts from the 36 indoor isolates studied by Lübeck et al. (2000) or 150 isolates (mostly *T. harzianum*) from various other environments (Nielsen and Thrane, 2001). The only isolate in which trichothecene synthesis has been measured was a strain of *T. harzianum* described by Corley et al., 1994; the original isolate described by Godtfredsen and Vangedal 1964 is no longer available). These data suggest that trichothecene production by *Trichoderma* is very limited.

11. Human exposure

Until recently it was assumed that the inhabitants of mold-contaminated buildings were exposed to mycotoxins via the inhalation of spores and spore-sized fragments of mycelia (Sorenson, 1999). For this reason, spore counts were valued for assessing the potential for mycotoxin exposure. Recently, however, it has been shown that particles far smaller than spores (down to 0.3 μm) are released from colonies growing on building materials (Gorny et al., 2002; Kildesø et al., 2000). Large quantities of these particles can be liberated from colonies creating a 300-fold higher concentration of particles compared with the number of spores (Gorny et al., 2002). There is no correlation between the concentration of particles and spores. Numerous factors that further complicate exposure assessment include (i) the fact that low humidity stimulates spore release (Chang et al., 1995; Kildesø et al., 2000; Pasanen et al., 1991); (ii) the effects of physical activity within a building on spore and particle liberation (windspeeds within the building envelope are very low and physical activity plays a more important role in dispersing spores and colony fragments; Kildesø et al., 2000; Lehtonen et al., 1993; Reponen et al., 1992); (iii) the effects of external wind pressure on airflow patterns within the building envelope and inside the building, and (iv) changes in ventilation and the degree of physical disturbance caused by sampling. For these reasons, particle liberation varies greatly. Combined with lack of techniques for quantifying particles smaller than spores, it is easy to appreciate why it has proven so difficult to correlate data on viable or total airborne fungi with health problems in mold-contaminated buildings.

Airborne mycotoxins have been detected in environments with high concentrations of airborne spores, including farms and compost handling facilities (Fischer et al., 1999; Sorenson, 1999). But mycotoxins have only been detected sporadically in indoor air, where (as mentioned in Section 9) trichothecenes and spirocyclic drimanes have been detected in dust and air samples obtained from homes contaminated with *Stachybotrys* (Johanning et al., 2002; Nielsen, 2002; Vesper et al., 2000; Yike et al., 1999). These studies have shown that the detection of airborne mycotoxins requires the investigator to sample large volumes of air ($\gg 10 m^3$) using filters with very small pore sizes $\leqslant 0.2\,\mu m$; Johanning et al., 2002; Kildesø et al., 2000; Pasanen et al., 1993). Analysis of household dust can also provide useful data on the levels of other mycotoxins (Engelhart et al., 2002; Richard et al., 1999).

Exposure assessment using human biomarkers is very effective for determining exposure to aflatoxin B_1 (Autrup et al., 1991). There has been little work to test the value of this approach for estimating exposure to macrocyclic trichothecenes, though these mycotoxins may be detected in serum as verrucarol and detected after acetylation using antibodies against diacetoxyscirpenol (Dietrich et al., 1999). It would also be interesting to explore the use of sterigmatocystin-guanine or lysine adducts to determine mycotoxin exposure in buildings contaminated with *A. versicolor*. The lack of reference standards for many mycotoxins has complicated exposure assessment, by making it impossible to develop analytical methods to measure traces of these compounds.

12. Conclusions

Mycotoxin production in buildings seems to occur when a_w at the surface of the construction material exceeds 0.9, but significant toxin synthesis does not begin until a_w reaches 0.95. For this reason, the worst-case scenario for the development of an indoor mold problem involves a series of water intrusion events that allow large quantities of biomass and mycotoxins to form, then a period of drying that promotes the dispersion of spores and colony fragments, followed by their deposition throughout the building.

Compared with other molds, *Stachybotrys chartarum* produces a large number of secondary metabolites and substantial quantities of these compounds can accumulate when this mold grows in buildings. Growth of *Chaetomium globosum* should also be treated with caution. When colonies of this mold are dry, fragments containing large quantities of chaetoglobosin mycotoxins may be dispersed. *Penicillium* species produce very small quantities of secondary of metabolites and mycotoxins when they grow on building materials. The same is true for *Aspergillus* species, with the exception of *A. versicolor*, in which sterigmatocystins may represent up to 1% of its biomass. Exposure to sterigmatocystins might occur via micro-fragments derived from colonies, because very few spores are formed by this species.

Research on mycotoxins in buildings is a multidisciplinary task, demanding knowledge of chemotoxonomy, fungal metabolism and biosynthetic pathways, fungal physiology and growth, analytical chemistry, and toxicology. Collaboration with specialists in fungal identification is also essential. Therefore a taskforce of specialists in these disciplines is needed for future advances in this field.

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Further reading

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