Rapport SBUF-projekt: Mykotoxiner i inomhusmiljöer. Förekomst, bestämningsmetoder, immunmodulerande egenskaper.

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Inledning

Första fasen av projektet påbörjades den 1.10 2004 med avslutning den 30.6 2007. För denna fas beviljade SBUF 885 000:-. Erica Bloom knöts till projektet som doktorand och genomförde sin "halvtidskontroll" (motsvarande licentiateexamen) den 25.1 2007. Ett referensgruppsmöte genomfördes i Lund den 18.1 2007 med representanter från såväl akademi som industri där projektets status samt fortsatta inriktning diskuterades. För projektets andra och avslutande fas beviljade SBUF 800 000:- . Projektet i sin helhet avslutas den 31.12 2008. Erica Bloom förväntas lägga fram sin doktorsavhandling i december 2008. Projektet samfinansieras med FORMAS. Mindre bidrag har även erhållits från Astma- och Allergifonden samt Drottning Silvias Jubileumsfond.

Projektet behandlar mögelproblematiken i byggnader. Mögel misstänks kunna orsaka sjukdom genom inhalation av kontaminerat husdamm. Det finns rapporter bla från USA (de s.k. Clevelandfallen) där fall av konstaterad lungblödning – i vissa fall med dödlig utgång - bland barn associerats med massiv vattenskada i hemmet samt starkt ökade mängder av mögel. Ett annat viktigt incitament för igångsättningen av vårt projekt var upptäckten häromåret från tyska forskare att mögelgifter (mykotoxiner) inte bara är toxiska utan även kan påverka immunsystemet på ett sätt som innebär en ökad risk för allergi.

I projektet studerar vi mykotoxiner i husdamm och i byggnadsmaterial med kemisk-analytisk metodik baserad på masspektrometri. Denna metodik har fördelen framför odlingsmetoder att även avdödat mögel kan upptäckas. Metoden har dessutom en fördel över DNA-metoder (som också kan användas för att påvisa mögel): Med DNA kan man påvisa om mögel har en genetisk potentiell förmåga att bilda mykotoxiner men inte huruvida möglet verkligen gör det. I detta projekt använder vi masspektrometri för avgöra huruvida möglet *de facto* bildar mykotoxiner under de aktuella betingelserna.

Projektet represenerar den första kartläggningen över mykotoxinförekomsten i svenska inomhusmiljöer. Projektet kan komma att resultera i ett mer sunt byggande, tex i form av rekommendationer om lämpliga material för fuktiga miljöer samt klargörande om mögelsanering krävs för fuktskadat material vilket innebär direkta ekonomiska besparingar för samhället. Detta kan uppfattas som ett första steg i bildandet av en resurs i Sverige för bestämning av mykotoxiner i såväl luftburet som sedimenterat husdamm i fuktskadade eller på annat sätt mögelangripna byggnader.

Hittills vunna resultat

1. Vi har utvecklat metodik för detektion av mykotoxiner producerade av *Stachybotrys chartarum*, den mögelsvamp som orsakade Cleveland-utbrotten (se ovan). Vi har analyserat byggnadsmaterial (trälister, socklar, gipsskivor etc) samt husdamm från vattenskadade hus med avseende på verrukarol (VER) och trikodermol (TRID), hydrolysprodukter av trikodermin samt samtliga makrocykliska *Stachybotrys*-producerade trikotecener. Proverna extraheras med metanol, hydrolyseras, renas och genomgår kemisk derivatisering innan analys. Genom att använda halogenerade derivat och tandem MS (GC-MSMS) kan vi detektera VER och TRID i mögelskadade materialprov. Dessutom har TRID påvisats, som är ett inflammatoriskt mykotoxin, i sedimenterat damm och detta är första gången som mykotoxin har kunnat detekteras med masspektrometri i damm sedimenterat i andningszonen i en icke-industriell inomhusmiljö. Dessa resultat har redovisats i en publicerad artikel (Bloom et al. 2007a). De presenterades även på Healthy Buildings-konferensen, Lissabon juni 2006 (Bloom et al 2006).

2. Vi har också utvecklat HPLC-MSMS metoder för sterigmatocystin (produceras främst av *Aspergillus versicolor*) samt satratoxin G och satratoxin H (*Stachybotrys chartarum*). Byggmaterial- och dammprover extraherades med metanol och upprenades innan analys. Av totalt 62 analyserade byggmaterialprov med mögelpåväxt kunde mykotoxiner påvisas i 45 prov (sterigmatocystin i 26, satratoxin G i fem, satratoxin H i fyra, VER i 29, samt TRID i 35). Ofta påvisades flera mykotoxiner i samma prov. Intressant nog kunde sterigmatocystin påvisas i ett dammprov som sedimenterat i andningszonen; detta prov var det samma där också TRID påvisats med GC-MSMS (se ovan). <u>Våra resultat visar således att mykotoxiner regelmässigt bildas av mögel som etablerat sig i inomhusmiljön och att dessa mykotoxiner kan frigöras från mögelkontaminerade material och därmed bli luftburna (och inhalerbara).</u> Dessa data bidrar signifikant till den vetenskapliga litteraturen eftersom det finns mycket få publicerade data om direktdetektion (utan föregående odling) av mykotoxiner i inomhusmiljöer med MS. Dessa resultat har nyligen publicerats (Bloom et al. 2007b).

3. I samarbete med amerikanska forskare har vi nyligen genomfört en studie av miljöer där prov tagits från hem som drabbats av vattenskada i New Orleans i samband med Katrina-orkanen häromåret. Hälsokonsekvenserna av denna naturkatastrof har redan tidigare beskrivits av flera forskare. Bl a har mögelproblematiken uppmärksammats. Vår studie är emellertid den första där mykotoxiner påvisats i dammprov från hus som skadats i denna katastrof. Ett manuskript har nyligen skickats in för publicering.

Publikationer

Originalartiklar:

1. Bloom E, Bal K, Nyman E, Larsson L. Optimizing a GC-MS method for screening of *Stachybotrys* mycotoxins in indoor environments. J Environ Monit. 2007 Feb;9(2):151-6 **(attachas).**

2. Bloom E, Bal K, Nyman E, Must A, Larsson L. Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by *Stachybotrys* and *Aspergillus* spp. in indoor environments. Appl Environ Microbiol. 2007 Jul;73(13):4211-7 **(attachas).**

3. Bloom E, Pehrson C, Grimsley, L. F., Larsson, L. Mold identification and determination of mycotoxins in dust collected in water-damaged homes in New Orleans after Hurricane Katrina. Inskickad i dec 2007 för publicering.

Populärvetenskap:

1. Aime Must och Erica Bloom: Mögelgifter i inomhusmiljön. Bygg & Teknik 5-2007/s45/ **(attachas)**

2. Lennart Larsson, Erica Bloom, Aime Must, Eva Nyman, Christina Pehrson. Ny metod ger alarmerande bild av gifter i husen. Miljöforskning (FORMAS) 5-6 Dec-2007/s30/ **(attachas)**

Presentationer på konferenser:

1. Bloom E, Bal K, Nyman E, Larsson L. Determination of *Stachybotrys* mycotoxins in building material and house dust by GC-MS. ´Healthy Buildings´, June 2006, Lisbon.

2. Bloom, E., Bal, K., Nyman, E., Must, A., and L. Larsson. Mycotoxins produced by molds in water-damaged indoor environments. 'Indoor Climate of Buildings' 2007, Štrbské Pleso, Slovakien.

3. Bloom, E., Nyman, E., Must, A., and L. Larsson. Use of mass spectrometry for determining mycotoxins produced by molds in water-damaged indoor environments. ´American Academy of Allergy, Asthma and Immunology Annual Symposium´, 2008, Philadelphia, USA.

Projektets fortsättning

Som redogjorts för ovan har vi optimerat analysmetodik för bestämning av ett antal relevanta mykotoxiner i byggnadsmaterial samt husdamm. Vi har nyligen utvidgat våra metoder till att omfatta även aflatoxiner (som produceras av *Aspergillus*) samt gliotoxin och ochratoxin A (*Aspergillus* and *Penicillium*). I samarbete med två konsultbolag i branschen - Aimex i Stockholm (kontakperson Aime Must) samt Tekomo i Vellinge (kontaktperson Eva Nyman) – avser vi att analysera ett stort antal (100-200) prov som samlas in konsekutivt i dessa bolags reguljära verksamhet: Närhelst prov (byggnadsmaterial, damm) tas för mögelanalys i deras "ordinarie" verksamheter tas extra prov till oss för analys avseende mykotoxiner. Flertalet av dessa prov har redan analyserats. Vi avser även att utröna huruvida olika metoder (behandling med ånga, boracol, UV-ljus mm) för avlägsnande av mögel på skadade ytor lyckas deaktivera mykotoxiner.

Efter att vår artikel i Miljöforskning publicerats (december 2007) har vår forskning uppmärksammats flitigt i media med stort uppslagna artiklar i flertalet större svenska dagstidningar samt i TV:s och Sveriges Radios nyhetsprogram vilket illustrerar projektets stora samhällsrelevans.

Optimizing a GC-MS method for screening of *Stachybotrys* mycotoxins in indoor environments

Erica Bloom,[a] Karol Bal,[a] Eva Nyman[b] and Lennart Larsson*[a]

Presence of *Stachybotrys chartarum* in indoor environments has been linked to building-associated disease, however, the causative agents are unknown. Verrucarol (VER) and trichodermol (TRID) are hydrolysis products of some major *S. chartarum* mycotoxins, *i.e.* macrocyclic trichothecenes and trichodermin. We optimized gas chromatography–mass spectrometry (GC-MS) methods for detecting VER and TRID in *S. chartarum*-contaminated indoor environmental samples. Heptafluorobutyryl derivatives of both VER and TRID exhibited little MS fragmentation and gave much higher detection sensitivity (sub-picogram injected onto the GC column), both in GC-MS and GC-MSMS, than trimethylsilyl derivatives. Optimal detection sensitivity and specificity was achieved by combining chemical ionization and negative ion (NICI) detection with MSMS. With this method, VER and TRID were detected in building materials colonized by *S. chartarum* and TRID was demonstrated in dust settled in the breathing zone in a house where an inner wall was colonized. In summary, we have shown that NICI-GC-MSMS can be used to demonstrate mycotoxins in house dust in *S. chartarum*-contaminated dwellings.

Introduction

At high humidity moulds tend to grow well on different building materials including gypsum board, wood, and paper insulation, ceiling tiles, chipboard, wall paper *etc.*[1,2] Dampness inside buildings or in building constructions may therefore result in growth *e.g.* of *Stachybotrys spp.*, *Aspergillus spp.*, *Penicillium spp.*, *Trichoderma spp.*, and *Cladosporium spp.* There are clear associations between moulds in indoor environments and the development of adverse health effects.[3–6] A prominent mycotoxin producer, *S. chartarum*[7,8] has been linked repeatedly to indoor environment-associated disease outbreaks including the so-called Cleveland cases in the 1990's.[9,10] *S. chartarum* can be divided into two chemotypes, A and S, depending upon the mycotoxins they may produce. Chemotype A strains produce atranones, dolabellanes, simple trichotecenes *etc*, whereas chemotype S strains produce macrocyclic trichothecenes (MTRs),[11] such as, for example, satratoxins, roridins and verrucarins.[12,13] MTRs are cytotoxic, *i.e.* they inhibit peptidyl transferase in the protein synthesis process[14] and suppress the immune system by means of apoptosis.[15] Trichodermol and trichodermin may be produced by various *Stachybotrys* species.[16] In damp indoor environments, different *Stachybotrys* species often grow together with other moulds.
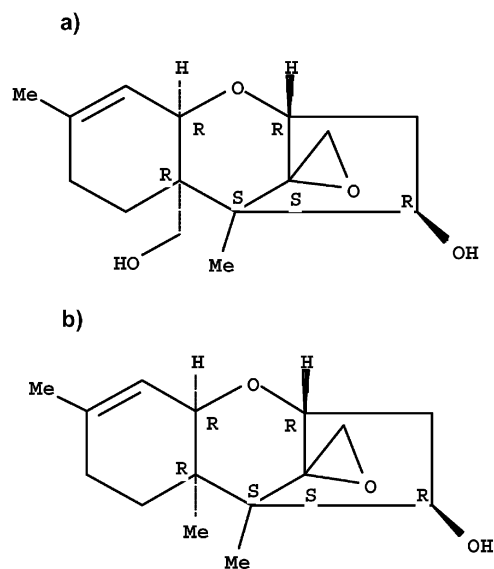
The direct detection of *Stachybotrys* mycotoxins in indoor environmental samples has been demonstrated repeatedly.

Verrucarin B and J, trichoverrin A and B, and satratoxin H were detected in a ceiling fiber board contaminated with *S. chartarum* by using thin-layer chromatography and high performance liquid chromatography (HPLC); the results were confirmed with NMR.[17] Similarly, Satratoxin G and H were demonstrated in ceiling tiles.[18] Flappan *et al.* in 1999[19] identified Roridin L-2, Roridin E, and Satratoxin H at 0.5, 0.7 and 3.2 ng cm^{-2}, respectively, on a *Stachybotrys*-infested closet ceiling by HPLC. HPLC was also used to identify satratoxin H in paper material from a water-damaged subbasement office area[20] and in gypsum board liner from a water-damaged children's day care center,[21] and to demonstrate the presence of dolabellanes, atranone B and C, and spirocyclic and spirocyclic-like drimanes in airborne particles collected in a home where an infant had developed pulmonary hemorrage.[9] Tuomi *et al.*[22,23] used HPLC with mass spectrometry (HPLC-MS) to demonstrate satratoxins in *Stachybotrys*-affected interior materials collected from buildings with a history of water damage. Finally, Brasel *et al.*[24] used an ELISA-based method (antibodies raised against Satratoxin G) to identify *Stachybotrys* MTRs in airborne dust samples in mould-affected buildings.

Gas chromatography–mass spectrometry (GC-MS) analysis of verrucarol (VER) and trichodermol (TRID) (Fig. 1), hydrolysis products of, respectively, MTRs and trichodermin[1,16,17,25,26] has been suggested as a convenient method for screening of building material samples suspected of being contaminated by mycotoxin-producing strains of *Stachybotrys*.[2,27] Previous experiments have included use of trimethylsilyl (TMS), pentafluorobutyryl, and heptafluorobutyryl (HFB) derivatives and analysis in both electron ionization (EI) and chemical ionization (CI) modes by MS and tandem MS (MSMS). The aim of the present study was to compare different GC-MS methods for determining VER and TRID in indoor environmental samples for achieving the highest

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Fig. 1 (a) Verrucarol (VER) and (b) trichodermol (TRID), hydrolysis products of, respectively MTRs and trichodermin.

possible detection sensitivity and specificity. We demonstrate that by applying GC-MSMS of HFB derivatives, particularly when combined with negative ion CI (NICI) detection, *S. chartarum* mycotoxins may be detected not only in building materials but also in house dust settled in the breathing zone in mould-affected dwellings.

Material and methods

Chemicals and standards

Solvents and reagents were of analytical or HPLC grade and used without any further purification. Methanol, dichloromethane and sodium hydroxide were purchased from Fischer Chemicals (Leicester, UK) and acetonitrile, toluene and acetone from Lab Scan (Dublin, Ireland). *N*-Heptafluorobutyrylimidazole (HFBI) and verrucarol (VER) were purchased from Sigma (Schnelldorf, Tyskland). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), *N*-trimethylsilylimidazole (TSIM), and trimethylchlorosilane (TCMS) were obtained from Macherey-Nagel (Düren, Germany) and pyridine from MP Biomedicals, Inc., (Ohio, USA). Trichodermin was a kind gift from Poul Rasmussen (Leo-pharma, Denmark). Trichodermol (TRID) was derived by hydrolysis of trichodermin.

Samples

Samples of building materials (Table 1) and settled house dust (Table 2) were collected from dwellings with a history of water damage. Mould growth in all buildings (Table 1) was found inside the construction and the damage was caused by water diffusion from the outside. The fungi were identified by direct microscopy of the affected surface, and by cultivation on malt extract agar. Colony forming units were detected in all materials except in the pipe isolation sample. The dust samples (Table 2) were collected on filters (which, according to the manufacturer, retain 74% of particles 0.3–0.5 μm in size, 81%

Table 1 Building materials from water-damaged dwellings

Name	Source	Mold content	cfu per cm^2
T1	Wooden base strip	*Stachybotrys sp.*, *Aspergillus versicolor*, *Penicillium sp.* and *Alternaria sp.*	9000
T20	Wooden block	*Stachybotrys sp.*, *Aspergillus sp.*, *Aspergillus versicolor* and *Penicillium sp.*	11 000
BS1	Gypsum board	*Stachybotrys chartarum*, *Cladosporium sp.*, *Trichoderma sp.* and *Alternaria sp.*	> 20 000
Pipeisol	Paper pipe-isolation	*Stachybotrys sp.*	No growth

of particles 0.5–1.0 μm , and 95% of particles 1–10 μm) by using a vacuum cleaner. The water damage in Object 1 was caused by flooding in the apartment above; the situation was further worsened by leaky waste-pipes. Mould growth (approx. 2–3 m^2) was found on indoor wall surfaces. In Object 2, moisture load from an outer wall caused water damage inside the construction; previously, *S. chartarum* colony forming units had been detected by cultivation of dust taken from the air duct (unpublished data). All samples were kept at 4 °C before chemical analysis.

Sample preparation, extraction and purification

Sample preparation before analysis was performed largely according to Andersen *et al.*[16] Dust (0.4–0.6 g) and building materials (0.5–1 g) were placed in 10 ml glass test tubes with teflon-lined screw caps. Samples were covered with methanol (3 ml) and stored in the dark, overnight, at room temperature. Then the tubes were sonicated for 1 h to improve extraction. Small portions of ice were occasionally added in the water-bath during sonication to avoid excessive heating. After, extraction samples were centrifuged at 3200 rpm for 5 min and the supernatants were decanted into new tubes. Extracts were evaporated under a gentle stream of nitrogen and re-dissolved in 1 ml of dichloromethane. Samples were then purified using polyethyleneimine (PEI) bonded silica gel columns (JT Baker, Phillipsburg, NJ, USA) that had been pre-conditioned with 4 ml methanol and 4 ml dichloromethane. Samples were eluted with 6 ml dichloromethane, again evaporated under nitrogen, re-dissolved in 1 ml methanol, filtered through 0.45 μm Millex syringe filters (PTFE, Millipore, Bedford, MA, USA) into new teflon-capped analysis vials, and kept at −20 °C until further preparation.

Table 2 Dust samples (< 1 g) from water-damaged dwellings

Name	Building	Source
1	Object 1	Top of a doorframe (approx. 150 cm^2)
2	Object 1	Floor (approx. 900 cm^2)
3	Object 2	Floor
4	Object 2	Movable surfaces
5	Object 2	Air duct leading from inside to outside of house
6	Object 2	Air duct leading to inside from outside of house

Hydrolysis and derivatization

The hydrolysis and derivatization steps were performed mainly according to Nielsen and Thrane.[26] The sample extracts were evaporated under a gentle stream of nitrogen and hydrolyzed in 200 μl of 0.2 M methanolic NaOH, at room temperature, overnight. Samples were evaporated, 1.5 ml of distilled water was added, and tubes were vigorously vortexed. Approximately 1 ml of dichloromethane was added and tubes were vortexed again. Tubes were then centrifuged at 3200 rpm for 2 min and the dichloromethane phase was taken to new tubes and evaporated under nitrogen. The dried extracts were then subjected to either TMS or HFB derivatization. TMS derivatization was performed by adding 50 μl of derivatization mixture (MSTFA : TSIM : TCMS, 3 : 3 : 2, v : v : v) and 5 μl pyridine and heating the tubes at 60 °C for 30 min. Then, 45 μl of dichloromethane was added and samples were transferred to autosampler vials. HFB-derivatization was made by adding 200 μl of acetonitrile-toluene (1 : 4, v : v) and 15 μl of HFBI followed by heating at 70 °C for 60 min. Then, samples were washed with 1 ml of sterile distilled water and the upper phase was transferred into autosampler vials. The derivatized samples were all stored at 4 °C pending analysis.

GC-MS

Samples were analyzed on a CP-3800 gas chromatograph equipped with a fused-silica capillary column (FactorFOUR™, VF-5ms, 30 m × 0.25 mm i.d., 0.25 μm film thickness) and connected to a 1200L triple quadropole MSMS detector (Varian Inc., Walnut Creek, CA, USA). Derivatives were analyzed both in EI mode, at an energy of 70 eV and an ion source temperature of 250 °C (TMS derivatives) or 200 °C (HFB derivatives), and in NICI mode with methane as ionization gas at a pressure of 0.8 kPa and a source temperature of 200 °C. Volumes of 1–2 μl were injected in the splitless mode with a helium carrier gas pressure of 69 kPa, using a CombiPAL autosampler (CTC Analytics AG, Zwingen, Switzerland). Column flow was 1.0 ml min^{-1}. The injector syringe was washed 6 times with methanol and toluene, respectively, before and after each sample injection. The temperature of the column was programmed from 90 to 280 °C at 20 °C min^{-1}; the injector temperature was 280 °C and transfer line temperature was 280 °C.

The MSMS conditions were optimized by repeatedly injecting 0.1–1 ng amounts of standards at different collision energy, ion source temperature, and argon pressure in the collision cell. The parameters that gave the largest product ion peak area were selected. Detection sensitivity, defined as amount of standards injected with a signal-to-noise ratio >4 (software calculated peak-to-peak values), was determined by analysing derivatized standard preparations diluted in dichloromethane (TMS-derivatives) or acetonitrile–toluene (1 : 4, v : v) (HFB-derivatives) injecting 0.1, 0.2, 1, 2, 10, 20, 100, and 200 pg in SIM and MSMS modes. Calibration was made by adding VER (0, 0.25, 0.5, 0.75, 1.25, 1.75, and 2.5 ng), TRID (0.0625, 0.125, 0.1875, 0.25, 0.375, 0.5 ng), and 1,12-dodecanediol (internal standard, 1.25 ng in the case of VER and 0.25 ng in the case of TRID) in 0.5 ml-aliquots of methanol. Reproducibility was evaluated by preparing seven samples with 5 ng and seven samples with 0.5 ng of TRID and VER, plus 5 ng of the internal standard, in 0.5 ml-aliquots of methanol. All mixtures went through the sample preparation procedure, and each sample was injected three times.

Results

Standards

Mass spectra of TMS- and HFB-derivatized VER and TRID standards are shown in Fig. 2. An overview of the precursor and product ions monitored, detector voltages and additional information, is shown in Table 3. In the following, detection limits have been expressed as injected amounts that gave a signal-to-noise ratio of at least 4 (peak-to-peak values).

TMS derivatives of both VER and TRID showed excessive fragmentation. Ions used in SIM analysis of VER–TMS$_2$ were m/z 320 (probably representing M–TMSOH), m/z 307 (M–CH$_2$OTMS), m/z 277 (M–CH$_2$OTMS–2CH$_3$), and m/z 217 (M–CH$_2$OTMS–TMSOH). The detection limit was difficult to determine since the derivative co-eluted with an unknown compound present in the blank. Fragment ions of m/z 217 gave product ions of m/z 174 used for monitoring in MSMS; detection limit was 20 pg. Notably, other ions (for example m/z 320 and 277) gave excessive fragmentation in MSMS without any prominent product ions being formed. Ions used in SIM analysis of TRID–TMS were m/z 217 (probably M–TMSOH–CH$_3$), m/z 176, and m/z 161. Of these, m/z 176 gave best results in MSMS monitoring product ions of m/z 161. The detection limit was 10 pg both in MSMS and SIM (for all ions monitored).

HFB derivatives analysed in EI mode produced abundant ions in the high mass range. Ions used in SIM analysis of VER–HFB$_2$ were m/z 445 (M–HFBO), m/z 444 (M–HFBOH), and m/z 416 (M–CH$_2$OHFB–CH$_3$). In MSMS, ions of m/z 444 were fragmented, and product ions of m/z 123 were monitored. The detection limit both in MSMS and SIM was 10 pg. TRID–HFB gave abundant ions of m/z 446 (M) and m/z 431 (M–CH$_3$) used in SIM. In MSMS, fragment m/z 446 gave distinct product ions of m/z 431 that were monitored. The detection limit both in SIM and MSMS was 2 pg. HFB derivatives analysed in NICI mode gave very little fragmentation. The VER–HFB$_2$ spectrum showed mainly ions of m/z 638 (M–HF) and m/z 213 (HFBO); the former was selected for MSMS monitoring product ions of m/z 213. In SIM, m/z 638, m/z 302, and m/z 213 were monitored. In case of TRID–HFB, ions of m/z 426 (M–HF) were used both in MSMS monitoring product ions of m/z 159 (C$_3$F$_5$CO) and SIM. The detection limits both of VER and TRID were 0.1 and 0.2 pg in, respectively, MSMS and SIM.

The peak area ratios of the mycotoxin standards/internal standard *vs.* the amounts of the mycotoxin standards in the samples followed the equations $y = 0.5082x$ (VER, $R^2 = 0.998$) and $y = 0.3301x$ (TRID, $R^2 = 0.959$). The coefficient of variation was 5.3% (5 ng VER), 3.2% (5 ng TRID), 18% (0.5 ng VER), and 33% (0.5 ng TRID).

Building materials and dust samples

The building material and dust samples from water-damaged dwellings were analyzed for VER and TRID as HFB
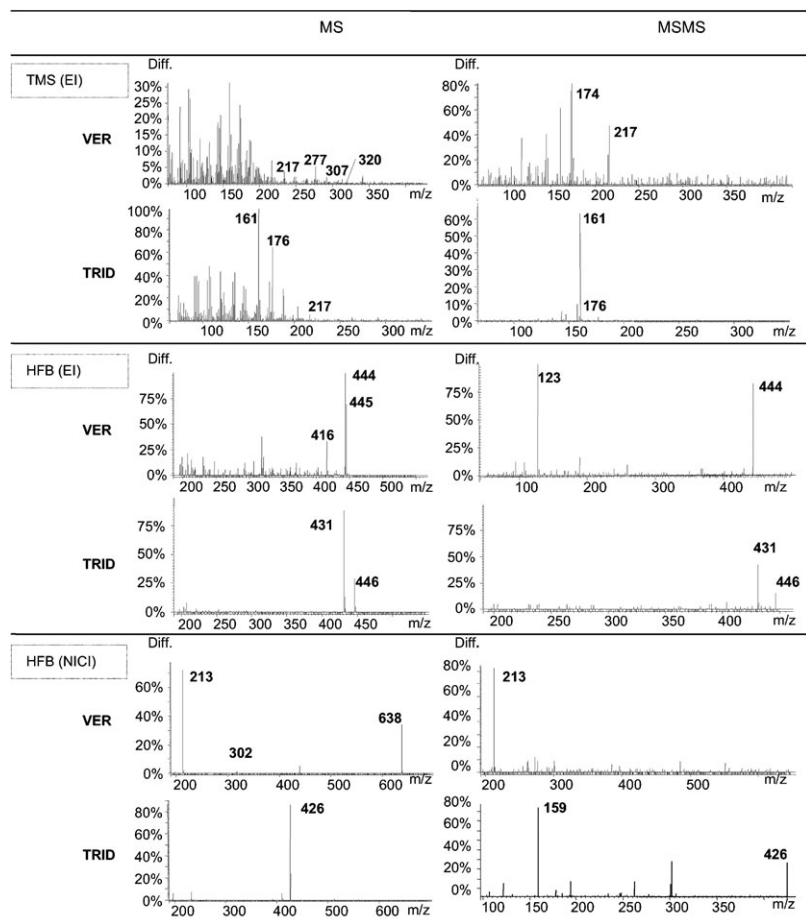
Fig. 2 MS (left) and MSMS (right) spectra of verrucarol (VER) and trichodermol (TRID) analyzed as TMS (upper) and HFB (center) derivatives in electron ionization (EI) mode and as HFB (lower) derivatives in chemical ionization–negative ion (NICI) mode.

derivatives. VER (0.4–3.6 pg mg^{-1} sample) and TRID (0.05–0.2 pg mg^{-1} sample) were detected with both MSMS and SIM (NICI) in all of the four studied building materials.

Representative analysis results are shown in Fig. 3. Clearly, the SIM analyses revealed peaks at the correct retention times but with a high background noise level and, in the case of TRID, a disturbance from a partially co-eluting compound. In comparison, MSMS resulted in much lower background noise and improved detection specificity (Fig. 3). TRID (approximately 1 pg mg^{-1}), but not VER, was detected both with MSMS and SIM in the dust sample that had been collected

from the top of a doorframe in a building infested with *S. chartarum*; this finding was confirmed by additional MS and MSMS analyses in the EI mode (Fig. 4). The other studied dust samples were negative for both VER and TRID.

Discussion

Epidemiological studies have revealed clear associations between damp indoor environments and adverse health effects.[6,20] However, since no causal relationships have been demonstrated between symptoms/diseases and findings of

Table 3 Mass spectral characteristics of VER-TMS$_2$, TRID-TMS, VER-HFB$_2$, and TRID-HFB

Derivative	Ret. time/min	MSMS				SIM	
		Precursor (m/z)	Product (m/z)	Exc. voltage/V	Detect. voltage/V	(m/z)	Detect. voltage/V
TMS (EI)							
Verrucarol	9.40	217	174	−15	1800	320, 307, 277, 217	1800
Trichodermol	8.66	176	161	−15	1800	217, 176, 161	1800
HFB (EI)							
Verrucarol	7.96	444	123	−10	1800	445, 444, 416	1800
Trichodermol	7.91	446	431	−10	1800	446, 431	1800
HFB (NICI)							
Verrucarol	7.96	638	213	15	1900	638, 302, 213	1800
Trichodermol	7.93	426	159	20	1900	426	1800
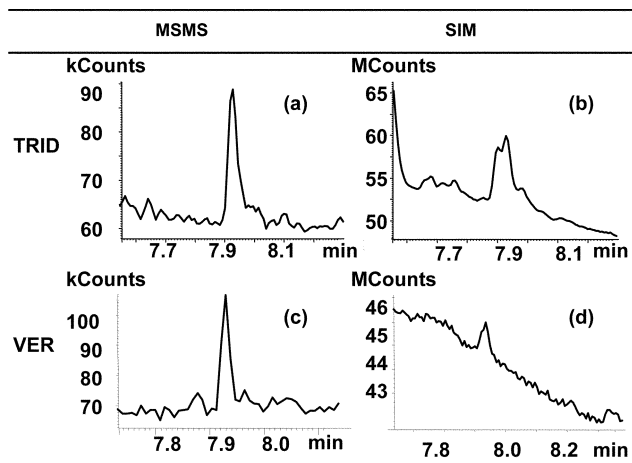
Fig. 3 Identification of TRID (upper) and VER (lower) in a sample of a wooden base strip by using MSMS (left) and SIM (right) NICI analysis.

moulds that may grow—or volatile chemicals that may be released or produced—in damp conditions, possible health consequences of mould and mycotoxin exposure indoors are still unclear.[28,29] Moulds may produce highly toxic mycotoxins depending upon species, chemotype, and a number of environmental factors such as substrate, water activity, and co-existing microbial flora. Mycotoxin production can even vary within a single isolate over time.[8] Some mycotoxins have recently been shown to possess strong immunomodulatory effects, driving the immune system towards a T-helper 2 type of cytokine pattern even at very low concentrations.[30,31] Moulds may disperse very small air-borne mycotoxin-containing particles, much smaller than conidia, hence the exposure may be much higher than assumed previously.[24,32–34] For example, in one study, the average concentration of released fungal fragments from *S. chartarum* was 380 particles cm^{-3}, about 514 times higher than that of spores.[35] Almost nothing is known about how long-term exposure to air-borne
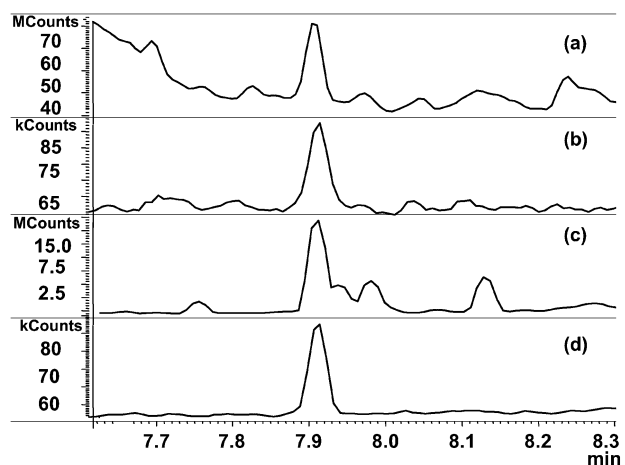
Fig. 4 Detection of TRID in a sample of settled house dust collected from the top of a doorframe in a building infested with *S. chartarum*. The analyses were performed with SIM (a, c) and MSMS (b, d) in both NICI (diluted sample) (a, b) and EI (c, d) modes.

mycotoxins at low concentrations may affect our health and well-being; little is also known about synergistic effects between different mycotoxins, glucans, endotoxins, indoor air chemicals and other molecules in indoor environments. A strong toxicity synergy was found between trichodermin and *Streptomyces californicus*.[36]

Clearly we need to know more about the prevalence of mycotoxins in indoor environments, hence, better methods are required for detecting the mycotoxins. The different analysis methods that have been used for application in indoor environments include immunoaffinity-based methods, which may suffer from cross-reactions, PCR, which may prove the presence of genes but says nothing of gene-expression and actual mycotoxin production, HPLC, and TLC.[37] In comparison, MS-based methods (GC-MS, HPLC-MS) offer superior detection specificity, especially when tandem MS is applied. Tuomi *et al.*[22] used HPLC-MSMS in electrospray mode for demonstrating Satratoxin G and H in methanol extracts of mould-affected building materials. With the quadropole ion-trap instrument used the detection limits were *ca.* 0.2 ng (injected amount).

The present study was prompted by earlier works[38–43] where VER was used as an *S. chartarum* MTR marker molecule after hydrolysis. Later, TRID, a hydrolysis product of trichodermin, was also included in the concept. The combined analysis of VER and TRID of hydrolysed samples provides information of the total content of MTRs and TRID/trichodermin.[1,25,27] This method has previously been successfully applied to screen authentic water-damaged building material samples for *S. chartarum* mycotoxins.[2,27] It should be borne in mind, however, that there may be also other sources of VER and TRID in indoor environments, such as *Stachybotrys* species other than *S. chartarum*,[13,16,44] *Memnoniella echinata*[12,26,45] and *Trichoderma*.[26]

We found that HFB derivatives gave higher detection sensitivity than TMS derivatives, and that SIM analysis, at the same detector voltage, provided similar detection sensitivity as MSMS for standards and extracts of pure cultures (data not shown). However, when applied to the building material and dust samples, the background noise level in SIM was very high. In MSMS, background noise was reduced considerably (so the detector voltage could be increased) resulting in an improved signal-to-noise ratio and a dramatically improved detection specificity. The NICI mode analysis was consistently preferred over EI because of lower detection limit, superior background reduction, and dramatically improved detection specificity. Our method combines NICI analysis of HFB derivatives[26,46] with MSMS. In the present study we show that it is possible to apply GC-MS and GC-MSMS for demonstrating TRID in settled dust in *S. chartarum*-contaminated dwellings.

Conclusions

In conclusion, a previously developed screening method for detection of *S. chartarum* mycotoxins was optimized and successfully applied on indoor environment samples. For the first time, to our knowledge, we demonstrate the presence of TRID in settled house dust collected in the breathing zone.

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Our plans are now to incorporate an internal standard in the method for allowing accurate quantification, and to apply the method in investigations of indoor environments in moisture damaged buildings in relation to reported health effects.

Acknowledgements

References

1 K. F. Nielsen, U. Thrane, T. O. Larsen, P. A. Nielsen and S. Gravesen, *Int. Biodeterior. Biodegrad.*, 1998, **42**, 9.
2 S. Gravesen, P. Nielsen, A. R. Iversen and K. F. Nielsen, *Environ. Health Perspect. Suppl.*, 1999, **107**, 505.
3 A. Nevalainen and M. Seuri, *Indoor Air*, 2005, **15**, 58.
4 K. H. Kilburn, *Adv. Appl. Microbiol.*, 2004, **55**, 339.
5 B. B. Jarvis, *Adv. Exp. Med. Biol.*, 2002, **504**, 43.
6 K. F. Nielsen, *Fungal Genet. Biol.*, 2003, **39**, 103.
7 B. B. Jarvis, *Phytochemistry*, 2003, **64**, 53.
8 B. B. Jarvis, *Nat. Toxins*, 1995, **3**, 10.
9 S. Vesper, D. Dearborn, I. Yike, T. Allan, J. Sobolewski, S. Hinkley and B. Jarvis, *J. Urban Health*, 2000, **77**, 68.
10 D. G. Dearborn, I. Yike, W. G. Sorenson, M. J. Miller and R. A. Etzel, *Environ. Health Perspect. Suppl.*, 1999, **107**, 495.
11 J. F. Grove, *Nat. Prod. Rep.*, 1993, **10**, 429.
12 B. B. Jarvis, W. G. Sorenson, E.-L. Hintikka, M. Nikulin, Y. Zhou, J. Jiang, S. Wang, S. Hinkley, R. A. Etzel and D. Dearborn, *Appl. Environ. Microbiol.*, 1998, **64**, 3620.
13 B. Andersen, K. F. Nielsen and U. Thrane, *Mycologia*, 2003, **95**, 1227.
14 B. Feinberg and C. McCaughlin, in *Trichothecene mycotoxins: pathophysiologic effects*, ed. V. R. Beasley, FL CRC Press, Boca Raton, 1989, vol. 1, pp. 161–170.
15 G. H. Yang, B. B. Jarvis, Y. J. Chung and J. J. Pestka, *Toxicol. Appl. Pharmacol.*, 2000, **164**, 149.
16 B. Andersen, K. F. Nielsen and B. B. Jarvis, *Mycologia*, 2002, **94**, 392.
17 W. A. Croft, B. B. Jarvis and C. S. Yatawara, *Atmos. Environ.*, 1986, **20**, 549.
18 M. J. Hodgson, M. Philip, L. Wing-Yan, L. Morrow, D. Miller, B. B. Jarvis, H. Robbins, J. F. Halsey and E. Storey, *J. Occup. Environ. Med.*, 1998, **40**, 241.
19 S. M. Flappan, J. Portnoy, P. Jones and C. Barnes, *Environ. Health Perspect.*, 1999, **107**, 927.
20 E. Johanning, R. Biagini, H. DeLon, P. Morey, B. B. Jarvis and P. Landbergis, *Int. Arch. Occup. Environ. Health*, 1996, **68**, 207.
21 M. A. Andersson, M. Nikulin, U. Köljalg, M. C. Andersson, F. Rainey, K. Reijula, E.-L. Hintikka and M. Salkinoja-Salonen, *Appl. Environ. Microbiol.*, 1997, **63**, 387.
22 T. Tuomi, K. Reijula, T. Johnsson, K. Hemminki, E.-L. Hintikka, O. Lindroos, S. Kalso, P. Koukila-Kähkölä, H. Mussalo-Rauhamaa and T. Haahtela, *Appl. Environ. Microbiol.*, 2000, **66**, 1899.
23 T. Tuomi, L. Saarinen and K. Reijula, *Analyst*, 1998, **123**, 1835.
24 T. L. Brasel, J. M. Martin, C. G. Carriker, S. C. Wilson and D. C. Straus, *Appl. Environ. Microbiol.*, 2005, **71**, 7376.
25 S. Hinkley and B. Jarvis, *Methods Mol. Biol.*, 2001, **157**, 173.
26 K. F. Nielsen and U. Thrane, *J. Chromatogr., A*, 2001, **929**, 75.
27 K. F. Nielsen, M. O. Hansen, T. O. Larsen and U. Thrane, *Int. Biodeterior. Biodegrad.*, 1998, **42**, 1.
28 B. J. Kelman, C. A. Robbins, L. J. Swenson and B. D. Hardin, *Int. J. Toxicol.*, 2004, **23**, 3.
29 G. Fischer and W. Dott, *Arch. Microbiol.*, 2003, **179**, 75.
30 G. Wichmann, O. Herbarth and I. Lehmann, *Environ. Toxicol.*, 2002, **17**, 211.
31 L. N. Johannessen, A. M. Nielsen and M. Løvik, *Clin. Exp. Allergy*, 2005, **35**, 782.
32 R. L. Górny, T. Reponen, K. Willeke, E. Schmechel, E. Robine, M. Boissier and S. A. Grinshpun, *Appl. Environ. Microbiol.*, 2002, **68**, 3522.
33 W. G. Sorenson, D. G. Frazer, B. B. Jarvis, J. Simpson and V. A. Robinson, *Appl. Environ. Microbiol.*, 1987, **53**, 1370.
34 J. Kildesø, H. Wurtz, K. F. Nielsen, P. Kruse, K. Wilkins, U. Thrane, S. Gravesen, P. A. Nielsen and T. Schneider, *Indoor Air*, 2003, **13**, 148.
35 S. H. Cho, S. C. Seo, D. Schmechel, S. Grinshpun and A. T. Reponen, *Atmos. Environ.*, 2005, **39**, 5454.
36 K. Huttunen, J. Pelkonen, K. F. Nielsen, U. Nuutinen, J. Jussila and M. R. Hirvonen, *Environ. Health Perspect.*, 2004, **112**, 659.
37 W. Smoragiewicz, B. Cossette, A. Boutard and K. Krzystyniak, *Int. Arch. Occup. Env. Hea.*, 1993, **65**, 113.
38 C. I. Szathmary, C. J. Mirocha, M. Palyusik and S. V. Pathre, *Appl. Environ. Microbiol.*, 1976, **32**, 579.
39 B. Harrach, C. J. Mirocha, S. V. Pathre and M. Palyusik, *Appl. Environ. Microbiol.*, 1981, **41**, 1428.
40 B. B. Jarvis, C. S. Yatawara, S. L. Greene and V. M. Vrudhula, *Appl. Environ. Microbiol.*, 1984, **48**, 673.
41 A. Bata, B. Harrach, K. Ujsza'szi, A. Kis-Tomas and R. Lasztitz, *Appl. Environ. Microbiol.*, 1985, **49**, 678.
42 T. Krishnamurthy, M. B. Wasserman and E. Sarver, *Biomed. Environ. Mass Spectrom.*, 1986, **13**, 503.
43 T. Krishnamurthy, E. Sarver, W. S. L. Greene and B. B. Jarvis, *J. Assoc. Off. Anal. Chem.*, 1987, **70**, 132.
44 O. M. O. El-Maghraby, G. A. Bean, B. B. Jarvis and M. B. Aboul-Nasr, *Mycopathologia*, 1991, **113**, 109.
45 B. B. Jarvis, Y. Zhou, J. Jiang and S. Wang, *J. Nat. Prod.*, 1996, **59**, 553.
46 R. Kostiainen and A. Rizzo, *Anal. Chim. Acta*, 1988, **204**, 233.

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Mass Spectrometry-Based Strategy for Direct Detection and Quantification of Some Mycotoxins Produced by *Stachybotrys* and *Aspergillus* spp. in Indoor Environments[▽]

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Dampness in buildings has been linked to adverse health effects, but the specific causative agents are unknown. Mycotoxins are secondary metabolites produced by molds and toxic to higher vertebrates. In this study, mass spectrometry was used to demonstrate the presence of mycotoxins predominantly produced by *Aspergillus* spp. and *Stachybotrys* spp. in buildings with either ongoing dampness or a history of water damage. Verrucarol and trichodermol, hydrolysis products of macrocyclic trichothecenes (including satratoxins), and trichodermin, predominately produced by *Stachybotrys chartarum*, were analyzed by gas chromatography-tandem mass spectrometry, whereas sterigmatocystin (mainly produced by *Aspergillus versicolor*), satratoxin G, and satratoxin H were analyzed by high-performance liquid chromatography-tandem mass spectrometry. These mycotoxin analytes were demonstrated in 45 of 62 building material samples studied, in three of eight settled dust samples, and in five of eight cultures of airborne dust samples. This is the first report on the use of tandem mass spectrometry for demonstrating mycotoxins in dust settled on surfaces above floor level in damp buildings. The direct detection of the highly toxic sterigmatocystin and macrocyclic trichothecene mycotoxins in indoor environments is important due to their potential health impacts.

Microorganisms are thought to be involved in health problems connected to damp buildings. However, the causative microbiological agents are unknown (22). Many molds that thrive in damp indoor environments are potent mycotoxin producers and may play a role in the reported adverse health effects (1, 5, 17, 23, 24, 26, 30). Mycotoxins are secondary metabolites, e.g., produced to give molds strategic advantages over encroaching organisms. Examples are sterigmatocystin (STRG), a carcinogenic mycotoxin produced mainly by *Aspergillus versicolor*; satratoxin G (SATG) and satratoxin H (SATH), which are cytotoxic mycotoxins produced by *Stachybotrys chartarum*; and citrinin, gliotoxin, and patulin, produced by, e.g., *Aspergillus* spp. and *Penicillium* spp. The latter three mycotoxins have been shown to be immunomodulatory, causing a polarization in cytokine production towards a Th2 phenotype (36), and citrinin caused depletion of intracellular glutathione at nontoxic concentrations (18). Based on spore counts, the airborne mycotoxin concentrations found in damp buildings have been estimated to be insufficient for causing adverse health effects (20). However, indoor molds may fragment into very small airborne mycotoxin-containing particles, resulting in up to a 500-fold larger exposure than assumed previously (4, 11, 21, 32). In addition, Cho et al. (7) showed that the respiratory deposition of *S. chartarum* fragments was over 200-fold higher than that of spores in adults and an additional 4 to 5 times higher in infants. These aerosolized fragments could potentially also be the source of allergens (13).

S. chartarum and *A. versicolor* are two commonly encountered molds in buildings with moisture problems (9, 12, 15, 28) and are prominent mycotoxin producers. Thin-layer chromatography, high-performance liquid chromatography (HPLC), and enzyme-linked immunosorbent assay are techniques that have been applied for detecting some of these mycotoxins, e.g., in ceiling materials (8, 10, 15), in paper materials (19, 26, 29), in a gypsum board liner (2), in airborne dust (4, 6), and in airborne particles in a home where an infant developed pulmonary hemorrhage (35). However, many have preferred to use mass spectrometry (MS)-based methods, especially tandem MS (MSMS), because of the high analytical specificity offered. Thus, HPLC-MSMS was used to demonstrate SATs and STRG in mold-affected interior materials and carpet dust from buildings with a history of water damage (9, 33, 34). Gas chromatography (GC)-MS and GC-MSMS were used to detect verrucarol (VER) and trichodermol (TRID), hydrolysis products of, respectively, macrocyclic trichothecenes and trichodermin of *S. chartarum*, in mold-affected building materials (3, 14, 27) and settled house dust (3).

In the present study, we used GC-MSMS for determining the amounts of VER and TRID and HPLC-MSMS for determining the amounts of SATG, SATH, and STRG in samples from water-damaged indoor environments. The goal was to apply state-of-the-art MS technology to direct analysis of building materials, settled dust, and cultivated airborne fungal particles for some mycotoxins mainly produced by *S. chartarum* and *A. versicolor*. We demonstrate that, by applying these com-

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TABLE 1. Mycotoxins detected in the building material and dust samples studied[a]

Mycotoxin(s)	No. of samples of the indicated building material in which mycotoxin(s) was detected					No. of samples of dust in which mycotoxin(s) was detected	
	Gypsum paper (n = 39)	Wood based (n = 8)	Concrete/stone (n = 3)	Paper (n = 6)	Other[b] (n = 7)	Settled dust (n = 8)	Airborne dust culture (n = 8)
TRID	1	ND	1	1	ND	ND	3
VER	ND	1	ND	ND	2	1	ND
STRG	2	4	ND	1	ND	ND	ND
TRID, VER	10	ND	1	2	ND	1	1
TRID, STRG	6	ND	ND	ND	ND	1	ND
VER, STRG	ND	ND	ND	ND	ND	ND	1
TRID, VER, STRG	6	1	ND	1	ND	ND	ND
TRID, VER, SATG, SATH	1	ND	ND	ND	ND	ND	ND
TRID, VER, STRG, SATG	1	ND	ND	ND	ND	ND	ND
TRID, VER, STRG, SATG, SATH	3	ND	ND	ND	ND	ND	ND

[a] ND, not detected; n, number of samples analyzed.
[b] Includes five linoleum samples and two synthetic material samples.

plementary MS methods, mycotoxins may be detected not only in building materials, but also in cultivable airborne fungal particles and settled house dust in damp buildings.

MATERIALS AND METHODS

Chemicals and standards. The solvents and reagents were of analytical or HPLC grade and used without any further purification. The buffers were degassed and filtered through 0.45-μm filters (Millipore, Bedford, MA) before use. Water was distilled and deionized. Methanol, dichloromethane, and sodium hydroxide were purchased from Fischer Chemicals (Leicester, United Kingdom) and acetonitrile, toluene, and acetone from Lab Scan (Dublin, Ireland). N-Heptafluorobutyrylimidazole (HFBI), STRG, and VER were purchased from Sigma (Schnelldorf, Germany). 1,12-Dodecanediol, ammonium acetate, and sodium acetate were purchased from Fluka (Schnelldorf, Germany). Reserpine (5 ng/μl) was purchased from Varian, Inc. (Walnut Creek, CA). The trichodermin was a kind gift from Poul Rasmussen (Leo-Pharma, Denmark), and TRID was derived from trichodermin by hydrolysis. Crude SATG and SATH mycotoxin standards were kindly provided by Bruce B. Jarvis (Dept. of Chemistry and Biotechnology, University of Maryland).

Building material and dust samples. Pieces (4 to 6 cm²) of paper (n = 39) collected from gypsum boards at 31 different locations were analyzed (Table 1). *Stachybotrys* was identified in all gypsum paper samples by conventional microscopic examination (31). Other materials with visible mold growth that were sampled were wood (n = 8), concrete (n = 3), paper (n = 6), masonite, linoleum, carpet, and tile (n = 7 altogether). These samples were found to be positive for *Stachybotrys* spp. and/or *Aspergillus* spp. by using a combination of microscopy and culture (on malt extract agar) identification.

Settled dust (n = 8) was sampled in four homes with histories of water damage (Table 2). In one home (object 1), the damage was caused by flooding in the apartment above and further worsened by leaky waste pipes. Growths of S.

TABLE 2. Mycotoxin contents in the settled dust samples studied

Building	Sampling date	Sampling location	Mycotoxin content (pg/mg sample)[a]		
			VER	TRID	STRG
Object 1	4 Oct.	Top of a doorframe	ND	3.4	17
Object 1	4 Oct.	Floor	ND	ND	ND
Object 2	5 Oct.	Floor	ND	ND	ND
Object 2	5 Oct.	Surfaces above floor level	ND	ND	ND
Object 2	5 Oct.	Air duct leading from inside to outside of house	ND	ND	ND
Object 2	5 Oct.	Air duct leading to inside from outside of house	ND	ND	ND
Object 3	4 Jan.	Outlet of an air ventilation duct	43	ND	ND
Object 4	4 Jan.	Top of a bedroom skirting board	19	2.4	ND

[a] ND, not detected.

chartarum (approximately 2 to 3 m²) were found on indoor wall surfaces. One dust sample from the top of a doorframe (460 mg) and another from the floor (560 mg) were collected by using a vacuum cleaner (3). In another house (object 2), moisture load from an outer wall caused water damage inside the construction. One dust sample each from the floor (1,020 mg), from surfaces above floor level (420 mg), and from the inlet (50 mg) and outlet (390 mg) of an air ventilation duct where dust had previously been found to be culture-positive for *S. chartarum* was collected by using a vacuum cleaner (3). Dust samples were collected on cotton swabs from two additional dwellings; one sample was collected from the outlet of an air ventilation duct in a school (object 3) where *Stachybotrys* spp. were found both in air samples and inside the wall construction, and the other sample from the top of a bedroom skirting board in a private home (object 4). The latter swab contained large amounts of dark-pigmented fragments of hyphae and spores, mainly of *Chaetomium* spp. and *Stachybotrys* spp. (unpublished results).

Cultures (n = 8) of airborne cultivable fungal particles collected by using a Reuter centrifugal sampler (RCS; Folex-Biotest-Schleussner Inc., Farfield, NJ) during a 4-min sampling period (40 liters/min) were analyzed. The rose bengal agar strips (agar strip HS; Biotest-Serum Institute GmbH, Frankfurt/Main, Germany) were cultivated at 25°C for approximately 12 days before microscopic examination and kept in plastic bags at 4°C before sample preparation and chemical analysis. The sampling sites included two private homes (apartments), a shop, an office, a room in a municipal hall, a kindergarten, a school, and an indoor ice rink. The numbers of cultivable airborne fungal particles in these locations ranged from 31 to 281 CFU/m³ air (Table 3).

Sample preparation, extraction, and purification. Pieces of agar cultures (approximately 5 cm²), dust samples (~0.4 g), and building material samples (0.3 to 3 g) were prepared for chemical analysis as described elsewhere (3). In brief, the samples were covered with methanol (3 to 5 ml) in 10-ml glass test tubes with Teflon-lined screw caps and stored in the dark for 72 h at room temperature. After the extraction, the samples were centrifuged (3,200 rpm, 5 min) and the supernatants were decanted into new tubes. One-hundred-microliter amounts of sterile water were added, and the mixtures were extracted twice with 2 ml heptane. The methanolic phases were evaporated under a gentle stream of nitrogen, dissolved in dichloromethane, and applied to polyethyleneimine (1 ml)-bonded silica gel columns (JT Baker, Phillipsburg, NJ) that had been preconditioned with 4 ml each of methanol and dichloromethane. The samples were eluted with 5 ml dichloromethane, evaporated under nitrogen, redissolved in 1 ml methanol, filtered through 0.45-μm Millex syringe filters (polytetrafluoroethylene; Millipore, Bedford, MA) into new Teflon-capped analysis vials, and kept at −20°C until HPLC analysis or further preparation.

HPLC-MS. A ProStar HPLC/1200L triple-quadrupole MSMS system (Varian Inc., Walnut Creek, CA) was used. Twenty microliters of each sample was injected, using an autosampler (model 410; Varian), into a Polaris 5-μM C₁₈-A 150- by 2.0-mm RP-18 column equipped with a MetaGuard 2.0-mm Polaris 5-μM C₁₈-A precolumn (Varian). Reserpine was used as the internal standard. The column was maintained at 25°C, and the flow rate was 0.2 ml/min. A supplement of 10 mM ammonium acetate and 20 μM sodium acetate was added to the methanol-aqueous buffer to increase the cationization in the electrospray ionization mode. An initial methanol concentration of 20% methanol was held for

TABLE 3. Mycotoxin contents in cultures of the airborne dust samples studied

Building	Sampling date	CFU/m^3	Mycoflora in culture[a]	Mycotoxin in culture (pg/cm^2 agar)[b]		
				VER	TRID	STRG
Municipal hall	4 Jan.	106	88% *Stachybotrys* spp., 12% *Mycelia sterila*	ND	330	ND
Private home 1	4 Jan.	281	36% *Penicillium* spp., 24% *Mycelia sterila*, 20% *Chaetomium* spp., 18% *Stachybotrys* spp., 2% *Cladosporium* spp.	ND	ND	ND
Kindergarten	3 Jan.	44	43% *Stachybotrys* spp., 29% *Mycelia sterila*, 14% *Aspergillus* spp., 14% *Penicillium* spp.	ND	1,500	ND
Private home 2	14 Feb.	44	44% yeasts, 14% *Stachybotrys* spp., 14% *Cladosporium* spp., 14% *Penicillium* spp., 14% *Mycelia sterila*	2,900	790	ND
School	13 Feb.	19	34% *Stachybotrys* spp. 33% *Geomyces* spp., 33% *Mycelia sterila*	ND	1,900	ND
Office	8 Mar.	38	83% *Mycelia sterila*, 7% *Stachybotrys* spp.	ND	ND	ND
Shop	2 Mar.	31	40% *Penicillium* spp., 20% *Aspergillus* spp., 20% *Cladosporium* spp., 20% *Mycelia sterila*	ND	ND	ND
Indoor ice rink	18 Jan.	100	38% *Mycelia sterila*, 19% *Cladosporium* spp., 19% *Penicillium* spp., 12% *Aspergillus* spp., 12% *Stachybotrys* spp.	250	ND	130

[a] *Mycelia sterila* is a nonsporulating mycelium.
[b] ND, not detected.

1 min, after which it was raised linearly (9 min) to 70% and held for 8 min before it was again raised linearly (1 min) to 95% and held for 5 min. At the end of the run, the concentration of methanol was linearly lowered again (1 min) to 20% and kept there for 12 min for stabilization. Ten microliters of methanol was injected in between samples to minimize cross-contamination. Nitrogen from a nitrogen generator (Domnick Hunter, Ltd., Tyne and Wear, United Kingdom) was used as both the nebulizing gas (50 lb/in^2) and the drying gas (20 lb/in^2), and argonium (1.75 mTorr) was used for collision-induced dissociation. The capillary temperature was 310°C, the capillary voltage 40 V, the needle voltage 5,000 V, and the electron multiplier voltage 2,000 V. The MS spectra were collected as centroid data from m/z 100 to 800, with a scan time of 0.5 s and a scan width of 0.7 s.

The MS was tuned through direct injection of polypropylene glycol tuning solution with a syringe, according to the manufacturer's protocol. Standards and reserpine were included in each batch of samples analyzed in order to assure instrument performance. Two calibration curves were constructed by injecting STRG ($n = 3$) (0, 25, 50, 100, 250, 500, and 1,000 pg and 0.5, 1, 2.5, 5, 10, and 25 ng) together with reserpine (1 and 10 ng). The coefficient of variation was calculated by dividing the standard deviation by the mean peak area ratio of the STRG standard (1-ng injections) to the internal standard ($n = 9$), and the recovery value was calculated by dividing the mean peak area from 1-ng injections of the STRG standards ($n = 9$) that had passed the sample preparation procedure by the corresponding STRG standards that did not pass this procedure.

GC-MS. The sample preparation was performed essentially as described previously (3). In brief, 200 μl of the methanolic sample extracts were mixed with 500 pg of internal standard (1,12-dodecanediol), evaporated, hydrolyzed in 0.2 M methanolic NaOH, and extracted with water and dichloromethane. The organic phases were transferred to new tubes, evaporated to dryness, and placed in a desiccator overnight. The dried extracts were then subjected to derivatization by adding 80 μl of acetonitrile-toluene (1:6, vol/vol) and 20 μl of HFBI followed by heating at 70°C for 60 min. Then, samples were left standing in an excess of derivatizing agent at room temperature for a minimum of 4 h before analysis. The derivatives were analyzed by using MSMS in negative-ion chemical ionization mode, at an energy of 70 eV and an ion source temperature of 150°C, and with ammonia as the ionization gas (0.4 kPa). Sample volumes of 1 to 2 μl were injected in the splitless mode. The injector syringe was washed five times with acetone and toluene, before and after, respectively, each sample injection. A mix of HFBI and acetone (1:3, vol/vol) was injected in between samples to eliminate any trace of un- or semiderivatized VER/TRID. The performance of the instrument was assured by including TRID/VER standards and 1,12-dodecanediol (internal standard) in each batch of samples analyzed. Two calibration curves were constructed by injecting VER/TRID ($n = 3$) (0, 25, 50, 100, 250, 500, and 1,000 pg and 0.5, 1, 2.5, 5, 10, and 25 ng) together with the internal standard (250 pg and 2.5 ng). The coefficient of variation was calculated by dividing the standard deviation by the mean peak area ratio of the VER/TRID standard to the internal standard ($n = 9$), and the recovery value was calculated by dividing the mean peak area from 1-ng injections of VER/TRID standards ($n = 9$) that had passed the sample preparation procedure by corresponding VER/TRID standards that did not pass this procedure.

RESULTS

HPLC-MS standards. The electrospray ionization MS parameters, optimized to achieve maximal detection sensitivity for STRG, SATG, and SATH, have been summarized in Table 4. The mass spectra of the standards are shown in Fig. 1. The spectrum of STRG showed prominent ions of m/z 671 [2M + Na]$^+$, m/z 363 [M + K]$^+$, and m/z 325 [M + H]$^+$. Ion m/z 325 was chosen for fragmentation in MSMS, and the product ions of m/z 310 and m/z 281 were monitored; the detection limit was 0.2 pg (injected amount monitoring m/z 310, signal-to-noise ratio [peak-to-peak value] ≥4). The peak area ratio of the STRG standard/internal standard (reserpine) versus the amounts of STRG standard followed the equations $y = 0.0675x + 0.714$ ($R^2 = 0.992$) for the 0- to 1,000-pg amounts injected and $y = 0.0035x + 1.742$ ($R^2 = 0.992$) for the 0.5 to 25 ng injected; the recovery value was 53% ± 6%, and the coefficient of variation was 11.2%. The SATG mass spectrum showed dominant ions of m/z 1,111 [2M + Na]$^+$, 567 [M + Na]$^+$, and 545 [M + H]$^+$. Ion m/z 567 was chosen for fragmentation in MSMS, and its product ions m/z 263 and m/z 231 were monitored. The dominant ions in the SATH mass spectrum were $m/z = 1,079$ [2M + Na]$^+$, 551 [M + Na]$^+$, and 529 [M + H]$^+$. Ion m/z 551 was used for fragmentation in MSMS, and its product ions of m/z 321 and 303 were monitored. SATH and

TABLE 4. Optimized electrospray ionization MS parameters for the studied mycotoxins and the internal standard

Mycotoxin	Parent ion (m/z)	Product ion(s) (m/z)[a]	Collision-induced dissociation value (V)
SATG	567 [M + Na]$^+$	**263**; 231	−31
SATH	551 [M + Na]$^+$	**321**; 303	−31
STRG	325 [M]$^+$	**310**; 281	−25
Reserpine	609 [M + Na]$^+$	**195**	−45

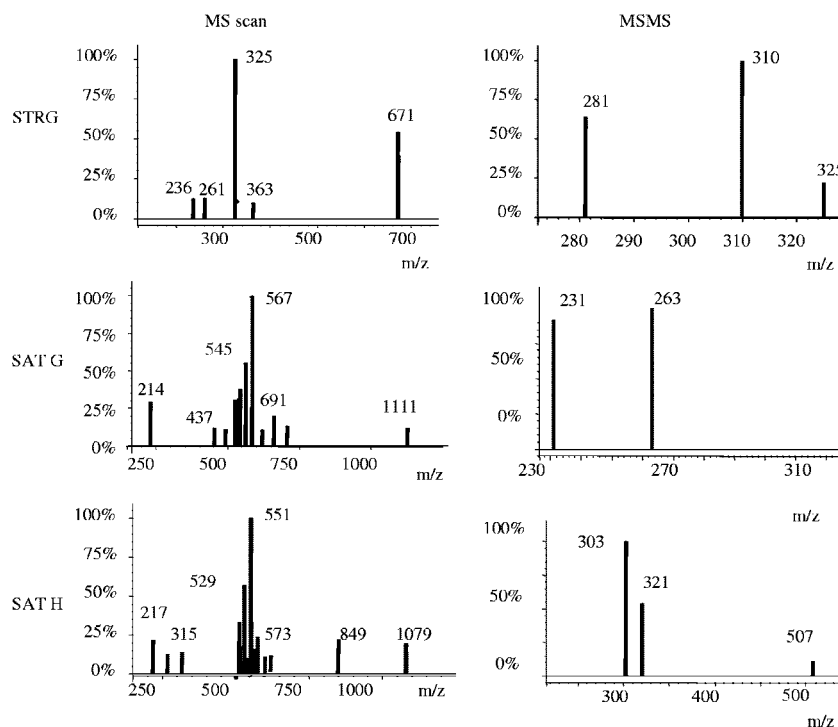[a] The values in boldface represent the main product ions.

FIG. 1. Positive electrospray MS (left) and MSMS (right) spectra of the STRG, SATG, and SATH standards.

SATG could not be quantified, since the purity of these crude mycotoxin preparations was unknown. For reserpine, m/z 609 $[M]^+$ was used as the parent ion in MSMS and its product ion m/z 195 was monitored.

GC-MS standards. The MS characteristics of VER-diheptafluorobutyryl (HFB$_2$) and TRID-heptafluorobutyryl (HFB), including the ions used for monitoring in MSMS and detection sensitivity, have been described previously (3). The peak area ratios of the mycotoxin standards/internal standard versus the amounts of the mycotoxin standards in the samples followed the equations $y = 0.0016x + 0.069$ ($R^2 = 0.991$) for the 0- to 1,000-pg amounts of VER injected and $y = 0.0002x + 0.032$ ($R^2 = 0.995$) for the 0.5- to 25-ng amounts injected. Accordingly, the equations for TRID were $y = 0.0021x + 0.035$ ($R^2 = 0.990$) and $y = 0.0002x + 0.116$ ($R^2 = 0.999$), respectively. The recovery values were 13% ± 1% for VER and 29% ± 4% for TRID, and the coefficients of variation were 5.3% and 3.2% for VER and TRID, respectively.

Building material and dust samples. The mycotoxin analysis results (with amounts adjusted according to recovery values) are summarized in Tables 1 to 3. In the building material samples, the amounts of STRG were 1.9 to 1,100 pg/mg (mean, 110; median, 14); of TRID, 3.4 to 18,000 pg/mg (mean, 660; median, 5.9); and of VER, 7.7 to 600 pg/mg (mean, 16; median, 25). The amounts of STRG (17 pg/mg; 130 pg/cm^2), TRID (2.4 to 3.4 pg/mg; 330 to 1,900 pg/cm^2), and VER (19 to 43 pg/mg; 250 to 2,900 pg/cm^2) in settled dust samples and cultured agar strips, respectively, from RCS samplings are given in Tables 2 and 3.

STRG was detected in 25 of the 62 building material samples studied. It was usually found together with two or more other mycotoxins; in fact, it was the sole mycotoxin found in only seven samples. In particular, STRG was frequently found together with TRID and never with VER, SATG, or SATH in the absence of TRID. A representative chromatogram demonstrating STRG in a paper sample is shown in Fig. 2a. One settled dust sample collected from the top of a doorframe (Fig. 2b) and one dust sample collected with an RCS were positive for STRG.
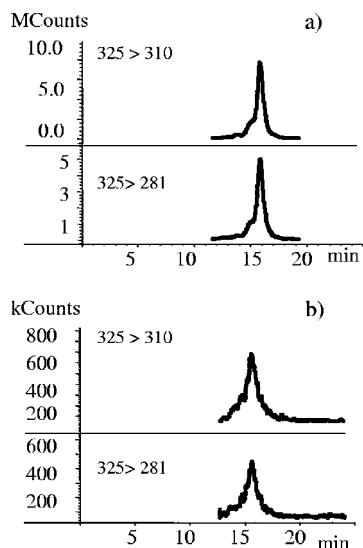

FIG. 2. HPLC-positive electrospray MSMS chromatograms demonstrating the presence of STRG (m/z 325 > m/z 310 and m/z 325 > m/z 281) in a paper sample culture positive (>30,000 CFU/m^2) for *Aspergillus* spp., including *A. versicolor* (a), and in a settled dust sample from the top of a doorframe (b).
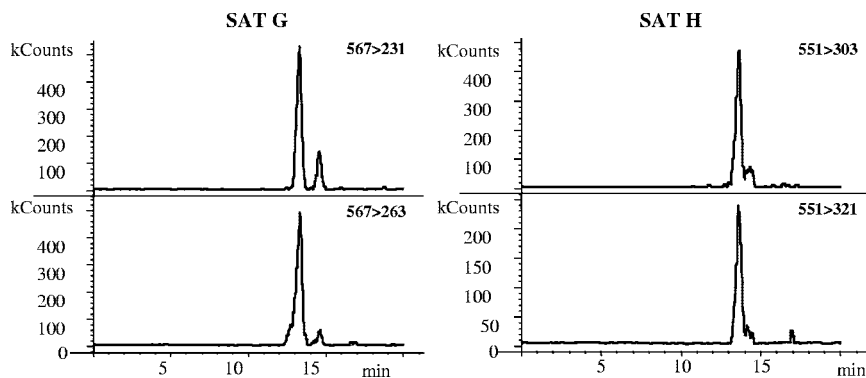
FIG. 3. HPLC-positive electrospray MSMS chromatograms demonstrating the presence of SATG (m/z 567 > m/z 231 and m/z 567 > m/z 263) and SATH (m/z 551 > m/z 303 and m/z 551 > m/z 321) in a gypsum board paper sample.

SATG was found in five and SATH in four building material samples; all were gypsum board papers that were also positive for VER and TRID. Representative chromatograms are shown in Fig. 3. SATG and SATH were not identified in any of the dust samples.

VER was the sole mycotoxin in only 3 out of 29 building material samples. It was usually found together with TRID or a combination of TRID and STRG, and always where SATG and SATH were found. VER was identified in two dust samples, once as the sole mycotoxin and once together with TRID (Table 1). VER was also found in two cultured agar strips from the RCS samplings, once together with TRID and once with STRG.

TRID also was rarely the sole mycotoxin found in the building material samples. In total, TRID was found in 35 of the 62 building materials studied, in two of the eight settled dust samples (one of the latter samples, collected from the top of a doorframe, has been described previously [3]), and in four of

the eight cultured dust samples. Representative chromatograms demonstrating the presence of VER and TRID in building material samples and settled and cultured dust samples are shown in Fig. 4.

DISCUSSION

S. chartarum and *A. versicolor* are water-associated indoor molds in Scandinavia and many other parts of the world (12, 15). Since both of these species are potent mycotoxin producers, they were the foci of the present study. The general criterion for including a building material in this investigation was visible mold growth; the gypsum board-derived paper samples were contaminated with *Stachybotrys* spp., while the other samples, in general, contained a diverse mycoflora where *A. versicolor* (besides *S. chartarum*) was a dominating species. The dust samples included were all collected from indoor environments with severe moisture damage.

Our results demonstrate that molds in these sampled indoor environments regularly produce mycotoxins, since 45 of the total of 62 building material samples (73%) were positive for at least one of the studied mycotoxins. By comparison, Toumi et al. (34) found STRG in 19 of 79 (24%) crude building material samples, plus VER, SATG, and SATH in 5 samples, by using HPLC-ion trap MSMS analysis; the recovery of STRG was in accordance with our results. One reason for the high prevalence of mycotoxins found in our study may be the high detection sensitivity offered by triple-quadrupole mass spectrometers in MSMS mode. This type of instrument offers ready detection of subpicogram amounts of STRG, VER, and TRID (3). While the analysis of underivatized STRG by using HPLC-MSMS is straightforward, we initially experienced several problems with the GC-MSMS analyses. These problems (carryover and ghost peak formation), particularly noticeable for VER, were occurring despite frequent syringe washings, changes of solvents and columns, and cleaning of the MS, and were found to depend largely upon adsorption of non- or semiderivatized VER in the GC injector. The problems were overcome by regular injections of an HFBI-acetone (1:3, vol/vol) mixture, by avoiding washing the preparations with water after HFBI derivatization (to prevent degradation of the derivative), and by injecting a maximum 1-μl sample in order to minimize the risk of injector contamination (unpublished re-
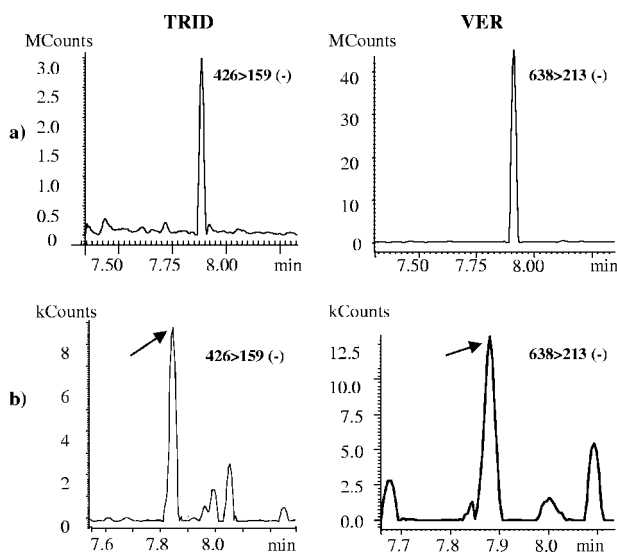

FIG. 4. GC-MSMS (negative-ion chemical ionization) chromatograms of TRID-HFB and VER-HFB$_2$ (indicated by arrows in panel b) in a gypsum board paper sample (a), a settled dust sample collected on a cotton swab (b, left), and a cultured RCS-obtained air sample (b, right).

sults). The GC-MS hydrolysis product method (where VER and TRID are detected) has previously been successfully applied to screen authentic water-damaged building material samples for *S. chartarum* mycotoxins (3, 12, 27).

In most, but not all, cases, the natural producer of a certain mycotoxin was identified in the sample by cultivation and/or microscopy; this was also found in other studies (34). However, *Stachybotrys* was not identified in a small number of building material samples that were positive for VER and/or TRID, viz., in 1 of 28 gypsum papers, in one of two wood-based materials, and in one linoleum sample. Likewise, *Aspergillus* spp. were not identified in all STRG-positive samples, viz., in 4 of 18 gypsum board papers, and in two of five wood-based materials (Table 1).

The CFU counts and mycotoxin contents from the RCS samplings did not correlate, probably because only a small fraction of the molds may have been cultivable. The composition of colonizers and secondary metabolite production may vary over time, even within a single isolate (16), probably due to fluctuations in water activity, nutrition, and coexisting microbial flora. The amounts of mycotoxins present may also have been below the detection limits in certain instances, due to low recovery amounts or instrument limitations.

The five gypsum paper materials that were positive for SATG and SATH were also positive for VER. In addition, VER was identified in an additional 24 building material samples plus two dust samples. It is likely that the detection sensitivity for VER is higher than for SATG or SATH. Also, although VER is thought to derive mainly from SATG and SATH (8, 19), VER is a hydrolysis product also of other macrocyclic trichothecenes and could therefore theoretically represent other SATs or verrucarins, etc.

The STRG/TRID mycotoxin combination was found in six of the building material samples; notably, STRG was never found together with VER only. It can be speculated whether *A. versicolor* has a capability of, or benefits from, growing together with or succeeding *S. chartarum* strains of chemotype A, rather than of chemotype S, due to the strongly cytotoxic mycotoxins produced by the latter. As recent reports have shown synergistic effects in cytotoxicity and apoptosis mechanisms in mouse macrophages challenged by spore extracts from cocultures of *A. versicolor* and *S. chartarum* (25), it is also interesting to speculate whether the two chemotypes play different roles in these mechanisms.

In this study, HPLC-MSMS and GC-MSMS have proven to be complementary analytical tools for detecting some of the most potent mycotoxins produced by molds frequently encountered in damp indoor environments. These methods are so sensitive that STRG, VER, and TRID can be detected not only in mold-affected building materials, but also in house dust. In fact, to the best of our knowledge, this is the first report on the use of MSMS for demonstrating mycotoxins in dust settled on surfaces above floor level in damp buildings. The methods used are important tools for further research aiming to shed some light on the role of molds in building-associated illnesses. In the future, we plan to expand our battery of mycotoxin analytes and to evaluate the health relevance of mycotoxins in indoor environments. Such work is in progress in our laboratory.

REFERENCES

1. **American Academy of Pediatrics Committee on Environmental Health.** 1998. Toxic effects of indoor molds. Pediatrics **101:**712–714.
2. **Andersson, M. A., M. Nikulin, U. Kõljalg, M. C. Andersson, F. Rainey, K. Reijula, E.-L. Hintikka, and M. S. Salkinoja-Salonen.** 1997. Bacteria, molds, and toxins in water-damaged building materials. Appl. Environ. Microbiol. **63:**387–393.
3. **Bloom, E., K. Bal, E. Nyman, and L. Larsson.** 2007. Optimizing a GC-MS method for screening of Stachybotrys mycotoxins in indoor environments. J. Environ. Monit. **9:**151–156.
4. **Brasel, T. L., J. M. Martin, C. G. Carriker, S. C. Wilson, and D. C. Straus.** 2005. Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. Appl. Environ. Microbiol. **71:**7376–7388.
5. **Bush, R. K., J. M. Portnoy, A. Saxon, A. I. Terr, and R. A. Wood.** 2006. The medical effects of mold exposure. J. Allergy Clin. Immunol. **117:**326–333.
6. **Charpin-Kadouch, C., G. Maurel, R. Felipo, J. Queralt, M. Ramadour, H. Dumon, M. Garans, A. Botta, and D. Charpin.** 2006. Mycotoxin identification in moldy dwellings. J. Appl. Toxicol. **26:**475–479.
7. **Cho, S.-H., S.-C. Seo, D. Schmechel, S. A. Grinshpun, and T. Reponen.** 2005. Aerodynamic characteristics and respiratory deposition of fungal fragments. Atmos. Environ. **39:**5454–5465.
8. **Croft, W. A., B. B. Jarvis, and C. S. Yatawara.** 1986. Airborne outbreak of trichothecene toxicosis. Atmos. Environ. **20:**49–552.
9. **Engelhart, S., A. Loock, D. Skutlarek, H. Sagunski, A. Lommel, H. Farber, and M. Exner.** 2002. Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. Appl. Environ. Microbiol. **68:**3886–3890.
10. **Flappan, S. M., J. Portnoy, P. Jones, and C. Barnes.** 1999. Infant pulmonary hemorrhage in suburban home with water damage and mold (Stachybotrys atra). Environ. Health Perspect. **107:**927–930.
11. **Górny, R. L., T. Reponen, K. Willeke, D. Schmechel, E. Robine, M. Boissier, and S. A. Grinshpun.** 2002. Fungal fragments as indoor air biocontaminants. Appl. Environ. Microbiol. **68:**3522–3531.
12. **Gravesen, S., P. A. Nielsen, R. Iversen, and K. F. Nielsen.** 1999. Microfungal contamination of damp buildings—examples of risk constructions and risk materials. Environ. Health Persp. **107**(Suppl. 3):505–508.
13. **Green, B., E. Tovey, J. Sercombe, F. Blachere, D. Beezhold, and D. Schmechel.** 2006. Airborne fungal fragments and allergenicity. Med. Microbiol. **44:**S245–S255. doi:10.1080/13693780600776308.
14. **Hinkley, S. F., and B. B. Jarvis.** 2001. Chromatographic method for Stachybotrys toxins. Methods Mol. Biol. **157:**173–194.
15. **Hodgson, M. J., P. Morey, L. Wing-Yan, L. Morrow, D. Miller, B. B. Jarvis, H. Robbins, J. F. Halsey, and E. Storey.** 1998. Building-associated pulmonary disease from exposure to Stachybotrys chartarum and Aspergillus versicolor. JOEM **40:**241–248.
16. **Jarvis, B. B., J. Salemme, and A. Morais.** 1995. Stachybotrys toxins. Nat. Toxins **3:**10–16.
17. **Jarvis, B. B., and J. D. Miller.** 2005. Mycotoxins as harmful indoor air contaminants. Appl. Microbiol. Biotechnol. **66:**367–372.
18. **Johannessen, L. N., A. M. Nilsen, and M. Løvik.** 2007. Mycotoxin-induced depletion of intracellular glutathione and altered cytokine production in the human alveolar epithelial cell line A549. Toxicol. Lett. **168:**103–112. doi:10.1016/j.toxlet.2006.11.002.
19. **Johanning, E., R. Biagini, H. DeLon, P. Morey, B. B. Jarvis, and P. Landsbergis.** 1996. Health and immunology study following exposure to toxigenic fungi (Stachybotrys chartarum) in water-damaged office environment. Int. Arch. Occup. Environ. Health **68:**207–218.
20. **Kelman, B. J., C. A. Robbins, L. J. Swenson, and B. D. Hardin.** 2004. Risk from inhaled mycotoxins in indoor office and residential environments. Int. J. Toxicol. **23:**3–10.
21. **Kildesø, J., H. Wurtz, K. F. Nielsen, P. Kruse, K. Wilkins, U. Thrane, S. Gravesen, P. A. Nielsen, and T. Schneider.** 2003. Determination of fungal spore release from wet building materials. Indoor Air **13:**148–155.
22. **Mazur, L. J., J. Kim, and the Committee on Environmental Health.** 2006.

Spectrum of noninfectious health effects from molds. Pediatrics **118:**1909–
1926. doi:10.1542/peds.2006-2829.

23. **Miller, J. D.** 1992. Fungi as contaminants in indoor air. Atmos. Environ.
26A:2163–2172.

24. **Müller, A., I. Lehmann, A. Seiffart, U. Diez, H. Wetzig, M. Borte, and O.
Herbarth.** 2002. Increased incidence of allergic sensitisation and respiratory
diseases due to mold exposure: results of the Leipzig Allergy Risk children
Study (LARS). Int. J. Environ. Health **204:**363–365.

25. **Murtoniemi, T., P. Penttinen, A. Nevalainen, and M.-R. Hirvonen.** 2005.
Effects of microbial cocultivation on inflammatory and cytotoxic potential of
spores. Inhal. Toxicol. **17:**681–693.

26. **Nevalainen, A., and M. Seuri.** 2005. Of microbes and men. Indoor Air
15(Suppl. 9)**:**58–64.

27. **Nielsen, K. F., M. O. Hansen, T. O. Larsen, and U. Thrane.** 1998. Production
of trichothecene mycotoxins on water damaged gypsum boards in Danish
buildings. Int. Biodeterior. Biodegrad. **42:**1–7.

28. **Nielsen, K. F., U. Thrane, T. O. Larsen, P. A. Nielsen, and S. Gravesen.** 1998.
Production of mycotoxins on artificially inoculated building materials. Int.
Biodeterior. Biodegrad. **42:**9–16.

29. **Nielsen, K. F., S. Gravesen, P. A. Nielsen, B. Andersen, U. Thrane, and J. C.
Frisvad.** 1999. Production of mycotoxins on artificially and naturally infested
building materials. Mycopathologia **145:**43–56.

30. **Salo, P. M., S. J. Arbes, Jr., M. Sever, R. Jaramillo, R. D. Cohn, S. J. London,
and D. C. Zeldin.** 2006. Exposure to Alternaria alternata in US homes is
associated with asthma symptoms. J. Allergy Clin. Immunol. **118:**892–898.

31. **Samson, R. A., E. S. Hoekstra, J. C. Frisvad, and O. Filtenborg (ed.).** 1995.
Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures,
Baarns, The Netherlands.

32. **Sorenson, W. G., D. G. Frazer, B. B. Jarvis, J. Simpson, and V. A. Robinson.**
1987. Trichothecene mycotoxins in aerosolized conidia of *Stachybotrys atra*.
Appl. Environ. Microbiol. **53:**1370–1375.

33. **Tuomi, T., L. Saarinen, and K. Reijula.** 1998. Detection of polar and mac-
rocyclic trichothecene mycotoxins from indoor environments. Analyst **123:**
1835–1841.

34. **Tuomi, T., K. Reijula, T. Johnsson, K. Hemminki, E.-L. Hintikka, O.
Lindroos, S. Kalso, P. Koukila-Kähkölä, H. Mussalo-Rauhamaa, and T.
Haahtela.** 2000. Mycotoxins in crude building materials from water-
damaged buildings. Appl. Environ. Microbiol. **66:**1899–1904.

35. **Vesper, S., D. G. Dearborn, I. Yike, T. Allan, J. Sobolewski, S. F. Hinkley,
B. B. Jarvis, and R. A. Haugland.** 2000. Evaluation of Stachybotrys charta-
rum in the house of an infant with pulmonary hemorrhage: quantitative
assessment before, during, and after remediation. J. Urban Health **77:**68–85.

36. **Wichmann, G., O. Herbarth, and I. Lehmann.** 2002. The mycotoxins citrinin,
gliotoxin, and patulin affect interferon-gamma rather than interleukin-4 pro-
duction in human blood cells. Environ. Toxicol. **17:**211–218.

Mögelgifter i inomhusmiljön

Mögel i våra moderna hus har vållat problem i flera decennier och sannolikt ställde mögelsvamparna till det även i forna tider då man i bibeln beskrev att vissa hus hade "spetälska" och var "osunda". Vetenskapligt är problemet med "sjuka hus" fortfarande en olöst gåta. När det finns eller har funnits fuktskador i byggnaden upplever många människor problem med luftvägar, får diffusa besvär (SBS-symtom) och allergikerna reagerar kraftigare på föroreningarna i inomhusluften. De föroreningar man oftast pratar om är partiklar, kemiska ämnen (emissioner) och mögel, de två sistnämnda främst orsakade av fukt.

De senaste decenniernas byggteknik har skapat många alltför fuktiga riskkonstruktioner och olämpliga materialkombinationer. På 1970-talet blev det populärt att bygga med "platta på mark" med uppreglat golv där tryckimpregnerat trä låg direkt på den fuktiga betongplattan. I en sådan konstruktion trivs många olika mögelsvampar och aktinomyceter (bakterier) som alstrar dålig lukt. På 1980-talet uppstod problem med flytspackel samt limmade plast- och linoleummattor direkt på fuktig betong. Detta resulterade i problem med både kemiska emissioner (från plastmattor och lim) och tillväxt av mikroorganismer. Linoleummattans baksida av juteväv är känslig för påväxt av mögelsvamp om den relativa fuktigheten är mer än 75 procent. Valet av linoleumgolv i fuktiga miljöer är kanske inte alltid är det lämpligaste i till exempel daghem, skolor och vårdinrättningar, där man är van att använda våta rengöringsmetoder. Ständig uppfuktning av ytan medför en ökad fuktighet i juteväven på mattans undersida,

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där mögel då trivs. Det vanligaste möglet som återfinns på juteväv, enligt våra undersökningar, är släktet *Aspergillus*, där flera arter är potentiella toxinbildare.

På 1990-talet ansågs det enkelt att bygga med gipsskivor i badrum och i så kallade utfackningsväggar (puts på isolering, utvändig kartonggips, träregelverk med isolering, plastfolie och invändig kartong-
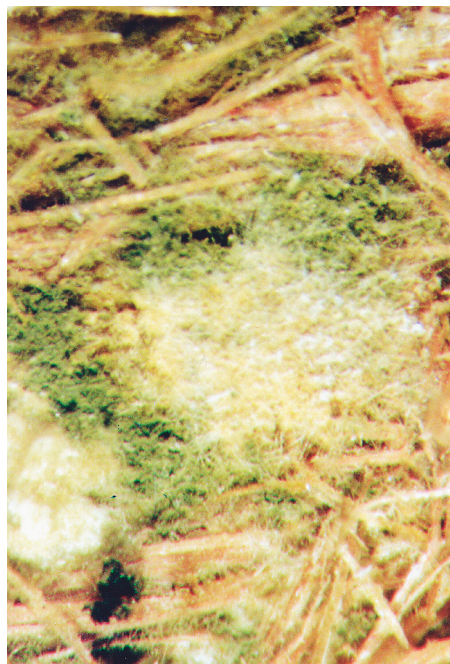
Mögel på baksida av linoleummatta.
FOTO: AIME MUST

gips). Problemet med dessa väggar är att de inte tål vårt fuktiga klimat och även under byggprocessen är de ytterst känsliga för fukt och mögel. Om det kommer in fukt i väggkonstruktionen kan det inte torka ut innan möglet börjar växa på gipsskivan. Gipsskivor med kartong (papper) har visat sig utgöra en särskilt gynnsam miljö för en svart mögelsvamp, Stachybotrys chartarum. Redan i början på 1990-talet påpekade vi (*Aime Must* och *Carl Johan Land*, SLU) att det inte är lämpligt att använda kartonggips i fuktiga konstruktioner, speciellt i badrum, men det har tagit tid för branschen att ta till sig kunskapen. För ett år sedan beslutade det största byggföretaget sig för att inte längre använda kartonggips i våtrum och ytterväggar och nu rekommenderar även våtrumsbranschen att välja annat än kartonggips som underlag för kaklade väggar. Även SP Sveriges Tekniska Forskningsinstitut, Boverket och Fuktgruppen i Lund har konstaterat att kartonggips inte klarar de nya kraven i BBR06 (gäller för all nybyggnation från 1juli 2007).

Svarta mögel växer väl

I flera vetenskapliga artiklar har mykotoxiner från *S. chartarum* avhandlats. Just

på kartonggipsskivor växer detta svarta mögel särskilt väl eftersom det föredrar cellulosainnehållande material (papper) som näring. I tillverkningsprocessen limmas pappkartongen fast med majsstärkelse, vilket är särskilt gynnsamt som näring åt mögelsvampen. Tyvärr skyddas inte gipsskivorna särskilt väl i byggprocessen och det är inte ovanligt att mögelpåväxt förekommer redan innan väggarna monterats (SBUF-rapport 11019). Vid skadeutredningar har man kunnat visa att sporer och fragment från toxiska mögelsläkten, bland annat *Stachybotrys* och *Aspergillus* kan förorena inomhusluften även om det växer dolt inne i väggen. *S. chartarum* producerar flera olika mykotoxiner bland annat makrocykliska trikotecener, atranoner med flera och flera *Aspergillus*-arter bildar sterigmatocystin. Toxinerna påverkar bland annat centrala nervsystemet, immunsystemet och kan orsaka cancer. Från veterinärmedicinen känner man väl till problemet med möjligt foder och strö som kontaminerats med mykotoxiner och där djuren drabbats av olika symptom och där ibland till och med dödsfall förekommit. När det gäller inomhusmiljön har forskningen inom byggsektorn inte ägnat sig särskilt mycket åt denna problematik eftersom det inte har ansetts som troligt att det finns en skadlig exponering, men nyare forskning visar att mögeltoxiner kan spela en större roll än man tidigare trott vid förekomsten av luftvägssjukdomar och andra hälsoproblem. Även om sporhalterna som uppmätts inte är särskilt höga så finns mögeltoxiner i detekterbara mängder även i små fragment, mycket mindre än sporer, vilket pekar på att exponeringen kan vara över 500 gånger större än man tidigare beräknat. Om man exponeras för dessa toxinhalter under lång tid kan man spekulera i hur luftvägar och olika organ påverkas. Enligt vår mening måste hälsorisken vid mögelexponering tas på allvar.

Inga tydliga samband

Fortfarande, trots många miljoner kronors satsning på forskning, kan man inte hitta ett tydligt samband mellan mögeltillväxt och hälsoeffekter, även om det finns flera fall där ökad astma har konstaterats hos personer som exponerats för vissa typer av mögel. Exempelvis har man funnit mer astma hos svenska FN-soldater som under lång tid vistades i mögliga tält och i en annan stor undersökning hos barn om det förekommer "mögellukt" i bostaden. En annan stor grupp som är intressant att studera och som i sitt arbete kan exponeras för höga halter är byggnadsarbetare och saneringspersonal som river/sanerar mögelangripet byggnadsmaterial. De vittnar
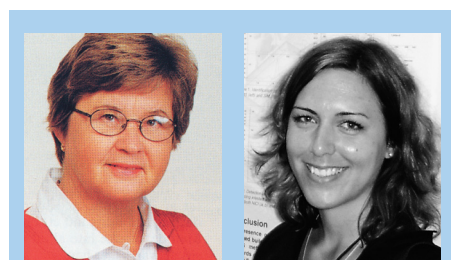
RCS-sampler (Reuter Centrifugal Sampler.
FOTO: AIME MUST

ofta om symptom som de fått efter arbetet och som tyder på kraftig exponering. I våra undersökningar tar vi prover i luften både i extrema och i "normala" miljöer. Med RCS-luftprover (mögelidentifiering) och dammprov på filter (direkt mykotoxinanalys) kan vi få en indikation på hur stora mängder mögel personerna exponeras för.

Ännu finns inga gränsvärden för luftburet mögel i bostäder, kontor, och vanliga arbetsmiljöer (icke industriell miljö), därför gör vi alltid jämförande mätningar i rum med upplevda problem med "problemfria" rum och utomhusluft. Vår hypotes är att luftburet toxiskt mögel sannolikt kan påverka hälsan och trigga igång inflammationer i luftvägarna.

Vi tar materialprover från verkliga skadefall och analyserar med direktmikroskopi. Mögelsvamparna identifieras till släkte innan de analysera med avseende på mykotoxiner. Vi tar också prover direkt från luften med RCS-sampler (som fångar mögelsporer i luft). Mögelsvampar producerar stora mängder sporer som finns i alla miljöer, men för att kunna finna en avvikande flora måste man ta flera prover och även referensprover utomhus. Genom att identifiera mögelfloran i olika rum och jämföra med referenser i andra delar i byggnaden och med utomhusluften kan man avgöra om det finns en avvikande sammansättning. Det räcker inte att enbart räkna antal kolonier (cfu/m³) eller avläsa så kallade "nedfallsplattor" för att

få en uppfattning om hur exponeringen ser ut. Med en aktiv insamling av en definierad mängd luft kan man ta sporprov i olika delar av byggnaden; i tilluft, rumsluft, och frånluft. Med denna metod kan man ringa in både en avvikande flora och en avvikande mängd mögel jämfört med i ett "friskt" rum och utomhus. På detta sätt har vi identifierat både mögelsvamp och mykotoxiner i luftprov. I vissa fall fann vi mykotoxiner med RCS-analys där det senare visade sig att det fanns fuktskador i konstruktionen men inget "synligt mögel" kunde ses innan provtagningen. Avsikten är att studera förekomsten av mykotoxiner, främst producerade av släktena *Stachybotrys* och *Aspergillus* i fuktskadade byggnader. *Tabell 1* innehåller exempel på sådana fall.

Mykotoxiner i inomhusmiljö

På Lunds universitet, sektionen för medicinsk mikrobiologi, ägnar sig (artikelförfattaren) doktorand *Erica Bloom* åt forskning kring mykotoxiner i inomhusmiljö. När ett miljöprov kommer in dokumenteras det noga och förbereder provet för analys. Provet läggs i metanol och får stå

över natten innan extraktionen görs av olika mykotoxiner. Extraktet renas också i flera steg för att få bort störande orenheter i provet. Efter filtrering analyseras det i en vätskekromatograf med en masspektrometer detektor (så kallad HPLC-MS-analys). Med denna metod kan man bland annat påvisa de cancerframkallande mykotoxinerna *sterigmatocystin* och *aflatoxin*, det cytotoxiska ("vävnadsförstörande") och immunförsvarsnedsättande gliotoxin och de starkt cytotoxiska makrocykliska *satratoxinerna* (G och H). När analysen är klar hydrolyseras återstoden av provextraktet. Vid hydrolys utnyttjas det faktum att alla makrocykliska trikotecener, producerade av framförallt *S. chartarum*, bildar ämnet *verrukarol* och att det inflammationsframkallande ämnet *trikodermin* bildar *trikodermol* när det utsätts för en alkalisk lösning. Dessa två mykotoxiner passar utmärkt för gaskromatografisk och masspektrometrisk analys (så kallad GC-MS). Metoden för *verrukarol* och *trikodermol* (publicerad i *Journal of Environmental Monitoring*) är ytterst specifik och så känslig att man kan upptäcka mindre än en billiondels gram *verrukarol*

Tabell 1: Resultat av provtagning i inomhusluft med RCS-sampler.

Byggnad	cfu/m³	Mycoflora		Mykotoxin pg/cm²		
				VER	TRID	STRG
Bibliotek	106	88 % *Stachybotrys* 12 % MS		*nd*	**330**	*nd*
Daghem	44	43 % *Stachybotrys* 29 % MS 14 % *Aspergillus* 14 % *Penicillium*		*nd*	**1 500**	*nd*
Bostad	44	44 % Yeast 14 % *Stachybotrys* 14 % *Cladosporium* 14 % *Penicillium* 14 % MS		**2 900**	**790**	*nd*
Skola	19	34 % *Stachybotrys* 33 % *Geomyces* 33 % MS		*nd*	**1 900**	*nd*
Ishall	100	38 % MS 19 % *Cladosporium* 19 % *Penicillium* 12 % *Aspergillus* 12 % *Stachybotrys*		**250**	*nd*	**130**

Nd = inte påvisat (not detected)
MS = sterilt mycel

VER = *Verrukarol* (främst *Stachybotrys sp.*)
TRID = *Trikodermol* (främst *Stachybotrys sp.*)
STRG = Sterigmatocystin (*Aspergillus spp.*)

eller *trikodermol* i den tusendels milliliter som injiceras i gaskromatografen.

I de inledande försöken har vi hittat toxiner i 73 procent av de mögelangripna proverna; i gipsskivor 72 procent, i sedimenterade dammprov 38 procent och i luftprov 63 procent (RCS-prov, se tabell 1). Resultaten är nyligen accepterade för publicering i *Journal of Applied and Environmental Microbiology*.

Betydande roll för hälsoeffekter

Mögelsvampar och aktinomyceter i byggnader har i flera vetenskapliga artiklar visat sig ha en betydande roll för hälsoeffekter. Forskning om mykotoxinernas hälsopåverkan och verkningsmekanismer pågår, bland annat vid Folkhälsoinstitutet i Kuopio (Finland) och i USA och intressanta resultat publiceras ständigt. Forskargruppen på medicinsk mikrobiologi i Lund är bland de första att påvisa mykotoxiner direkt i material och luftprov från inomhusmiljöer och är en av få, om inte den enda, grupp som utför denna typ av analyser idag. Tillsammans med Aimex och andra konsulter som tar prover direkt från fuktskadade byggnader bildar detta ett unikt samarbete.

Prover på "måfå"

I många skadeutredningar tas prover alltför ofta på "måfå" utan djupare kunskaper och med metoder som är svåra att utvärdera. Förhoppningsvis kan det snart utarbetas en ny strategi för att praktiskt kunna mäta mykotoxiner i luftprov och bättre kunna avgöra huruvida "mögel" är orsaken till de upplevda hälsoproblemen. Vårt samarbete har ett tydligt mål att från verkliga skadefall spåra mögeltoxiner från väggen (konstruktionen) till näsan (exponeringen) och här fordras ett gott samarbete mellan skadeutredare, analyslaboratorium och forskare. Att avfärda mögel som "inte farligt" har varit enkelt och argumentet att "mögel finns överallt" räcker inte. Mögelproblematiken i våra byggnader är mer komplicerad än så och den tvärvetenskapliga forskningen och kopplingen till verkliga skadefall är mycket betydelsefullt. ■

Referenser

Must, A., *Utfackningsväggar – en riskkonstruktion?*, Bygg & teknik, 1/06.

SBUF-rapport: 11019, *Luftburna mögelsvampar och mykotoxiner i svenska problemhus*.

Bloom, E., K. Bal, E. Nyman, A, Must, and L. Larsson. 2007. *A mass spectrometry-based strategy for the direct detection and quantification of some mycotoxins produced by Stachybotrys and Aspergillus in indoor environments*. Appl. Environ. Microbiology, May 2007. doi: 10.1128/AEM.00343-07.

Bloom, E., K. Bal, E. Nyman, and L. Larsson. 2007. *Optimizing a GC-MS method for screening of Stachybotrys mycotoxins in indoor environments*. J. Environ. Monior. 9:151-156. doi:10.1039/ b613853e.