GAS CHROMATOGRAPHY-MASS SPECTROMETRY/SOLID PHASE MICROEXTRACTION: A NEW TECHNIQUE FOR THE IDENTIFICATION OF MVOCS FROM MOLDY BUILDING MATERIALS

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ABSTRACT
Gas chromatography-mass spectrometry/solid phase microextraction (GC-MS/SPME) was applied to identify microbial volatile organic compounds (MVOCs). Aqueous reference mixtures of commonly reported MVOCs were studied. Building materials (gypsum board papers, mineral wool, and masonite) and cultivated molds (*Aspergillus penicillioides*, *Stachybotrys chartarum*, and *Chaetomium globosum*) were investigated using both electron impact and chemical ionization. Three different SPME fibers (65 μm PDMS-DVB, 75 μm Carboxen-PDMS, and 70 μm Carbowax-DVB stableflex) designed for automated injection were used. The 70 μm Carbowax-DVB stableflex fiber showed better performance than the other fibers. Several MVOCs were identified in the building materials and the fungal spp. Interestingly, methyl benzoate was identified in both *S. chartarum* and *A. penicillioides* cultures and in the mould-infested materials. SPME combined with GC-MS is a useful method to determine MVOCs emitted from moldy building materials.

INDEX TERMS
Building materials, fungi, GC/MS, MVOCs, SPME

INTRODUCTION
The residential indoor environment is considered to be a significant route for human exposure to air contaminants. Exposure to low levels of VOCs might lead to a variety of symptoms (headache, irritation in the eyes) (Elke et al., 1998; Rylander et al., 1994). Dust particles present in indoor air adsorb several gases and microbial VOCs (Cooley et al., 1998) including alcohols, aldehydes, and terpenes (Zlatkis et al., 1981; Niwa, 1986) Solid phase microextraction (SPME) is a rapid technique for identification of VOCs. SPME utilizes a short fused silica fiber coated with a polymeric organic material that is housed inside a syringe needle. The syringe first penetrates the vial septum, then the fiber exposed to the sample headspace. VOCs concentrate on the fiber surface and desorb in the hot injector of the gas chromatograph (GC) (Pawliszyn, 1997; Scheppers et al., 1999). In the present study we introduce SPME/GC-MS for the identification of MVOCs in building materials.

MATERIALS AND METHODS
Building materials and molds
Three building materials contaminated with mold were used: (i) Two gypsum board papers; (ii) one sample of mineral wool; (iii) one sample of masonite. Cultivation was commenced from the most affected areas. Parts of the same pieces were used for the GC-MS analysis.

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Fungal colonies were grown on DG18 (Oxoid CM729) and malt extract agar without sugar (Oxoid, CM59). Both media contained (g/L) chloramfenicol (0.05) and chlortetracycline (0.05) to prevent bacterial growth. Mold were counted after 7 days incubation at 25ºC. Subcultivation of mould fungi (*A. penicillioides* from mineral wool, *S. chartarum* (n=2) from the gypsum board papers, and *C. globosum* from masonite) was done on MEA as above. Subcultures were grown at 25°C for 10 days before SPME/GC-MS analysis.

SPME fibers

65 μm PDMS-DVB, 75 μm Carboxen-PDMS, and 70 μm Carbowax-DVB stableflex fibers (Supelco, Bellefonte, PA, USA) were used. Fibers were conditioned with helium at 260^{o}C/5 min prior to use. After each extraction cycle, fibers were re-conditioned with helium at 200^{o}C/1 min.

Standard solutions

Three aqueous mixtures of the reference MVOCs (0.1 μl in 10 ml water) were prepared in 20 ml vials. Mixture I contained 2-methylpentane, 2-pentanol, 3-methyl-1-butanol, and 3-octanone. Mixture II contained 3-methyl-2-butanol, 2-methyl-1-butanol, 1-pentanol, and 1-octen-3-ol. Mixture III contained 2-methyl-1-propanol, 2-hexanone, and 2-heptanone (all purchased from Fluka, Steinheim, Germany). Vials were flushed with nitrogen and sealed with metal caps (Microliter Analyzed Supplies, Inc.); all mixtures were analyzed using all three fibers. The SPME syringe penetrates the vial septum and the fiber is exposed to the headspace. Pre-incubation was done for 1 min at 50^{o}C, extraction for 3 min at 50^{o}C, and desorbtion for 2 min at 220^{o}C. Nitrogen-flushed vials containing 10 ml water were used as blanks.

Subcultured fungi and building materials

Pieces (1 cm^{2}) of agar with visible growth were transferred to 20 ml vials. Sterile agar pieces were analyzed as blanks. Analysis conditions for the fungi and the MVOC mixtures were similar except there was no pre-incubation and the extraction time was 5 min. The 70 μm Carbowax-DVB stableflex fiber was used. A piece (1-2 cm^{2}, 200-300 mg) of each building material was inserted into a 20 ml vial. The pre-incubation time was 1 min (70^{o}C) and the desorption time 2 min (220^{o}C). The extraction conditions varied (times of 5, 10, 15, 20 min and temperatures of 45, 50, 60, 70, 90^{o}C) to determine optimum analysis conditions. Sterile pieces of gypsum board were used as blanks.

GC-MS

A Saturn 2000 ion-trap GC/MS instrument (Varian, Palo Alto, CA, USA) equipped with a fused-silica capillary column (CP-Sil 8CB-MS, 0.25 μm film thickness, 30 m × 0.25 mm i.d.) (Chrompack, Middelburg, The Netherlands) was used. Samples were injected with closed split using a Combi Pal SPME autosampler (Walnut Creek, CA, USA). Helium was the carrier gas, and the temperature of the column was programmed from 45 to 280^{o}C at a rate of 6 ^{o}C/min. The temperature of the injector, transfer line and ion trap was 220, 280 and 230^{o}C, respectively. Samples were analyzed both in electron impact (EI) and chemical ionization (CI, isobutane) modes.

RESULTS

Fungi present in building materials

S. chartarum was isolated from both gypsum board samples and one of them also contained *Penicillium* spp (only *S. chartarum* strain from the first sample was available). *Chaetomium* spp., *Aspergillus* spp., and *Penicillium* spp. were isolated from the masonite, and *A. penicillioides*, *Penicillium* spp, and *Cladosporium* spp were isolated from the mineral wool.

Identification of MVOCs from subcultured molds

The 70 μm Carbowax-DVB stableflex fiber was superior over the other fibers for detecting the MVOCs in the standard solutions. Subcultures of *S. chartarum, A. penicillioides*, and *C. globosum* were studied. Several compounds present in the MVOCs mixtures were identified in the chromatograms of the isolated fungi including 2-pentanol, 2-heptanone, 3-octanone, 1-octen-3-ol, and 2-methyl-1-butanol. 2-Pentanol was only observed in EI mode and 2-methyl-1-butanol was found only in CI, the other compounds mentioned were detected both by CI and EI (Figure 1).

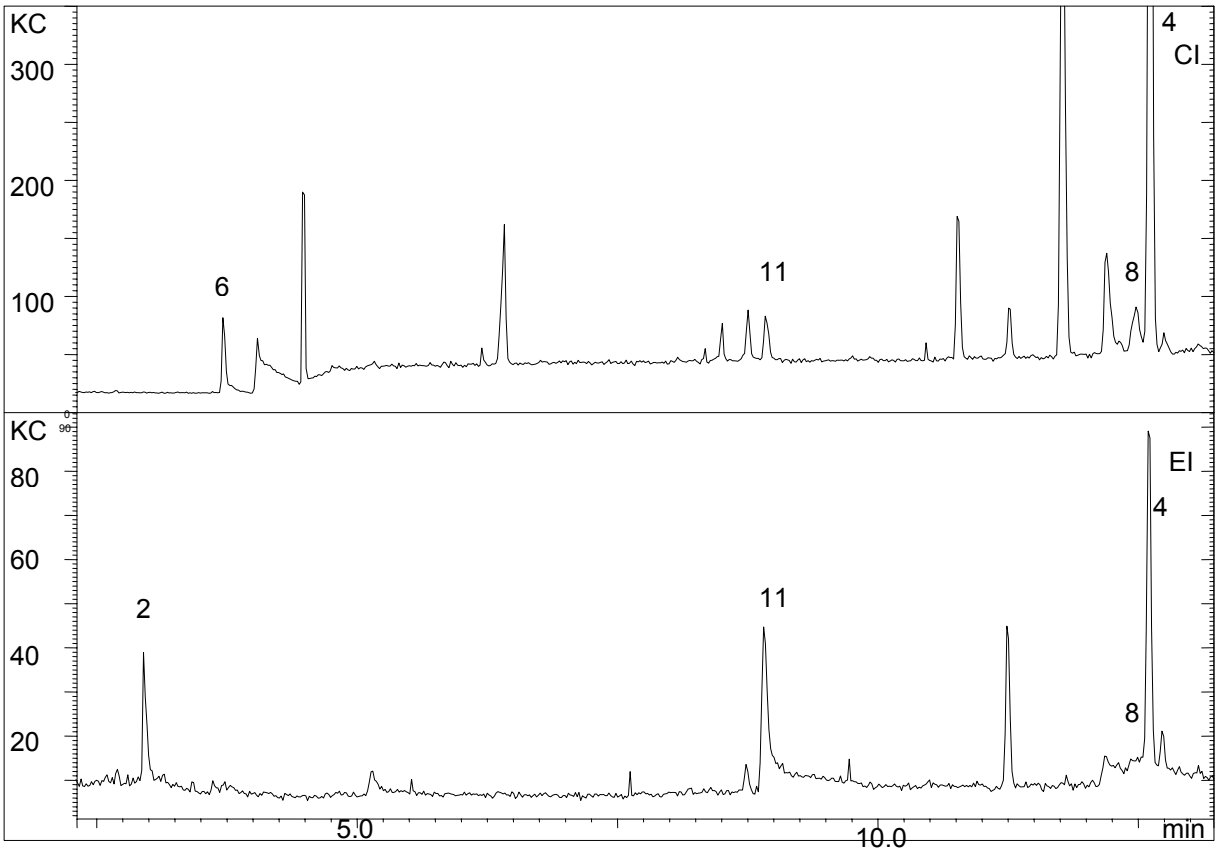

Figure 1. MVOCs identified from the isolated *S. chartarum* strain with both CI and EI modes. Key: (2) 2-pentanol, (4) 3-octanone, (6) 2-methyl-1-butanol, (8) 1-octen-3-ol, and (11) 2-heptanone.

Identification of MVOCs in building materials

Extraction at 70 ^{o}C for 20 min provided optimal conditions. 1-Octen-3-ol, 3-octanone, 2-hexanone and 2-heptanone were detected in the gypsum board. The latter two were also observed in the blank. Both masonite and mineral wool contained 3-octanone, 2-hexanone, and 2-heptanone; blank samples were not available for these materials. The chromatograms of the *Stachybotrys* strain contained a large peak eluting at 16.5 min. Its CI spectrum was

dominated by an ion of *m/z* 137 (M+1) and *m/z* 105 for EI. This compound was identified in *A. penicillioides* in lower amounts than in *S. chartarum.* It was also found in the mold-infested gypsum board samples, masonite, and mineral wool (trace amounts only). The mass spectral characteristics were found to correspond to methyl benzoate (Figures 2 and 3).

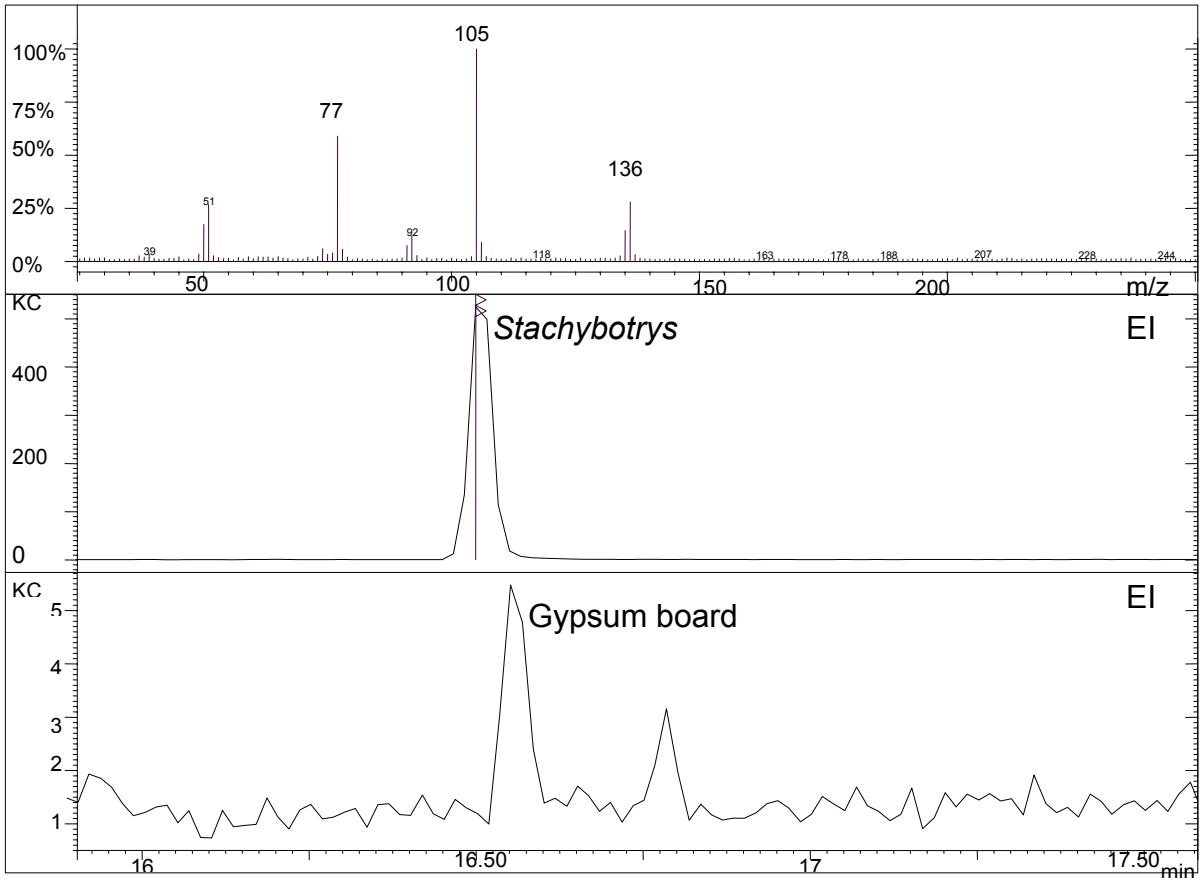

Figure 2. Chromatograms (EI) focusing at *m/z* 105 and related EI mass spectrum of methyl benzoate in a gypsum board paper culture positive for *S. chartarum* and a subculture thereof.

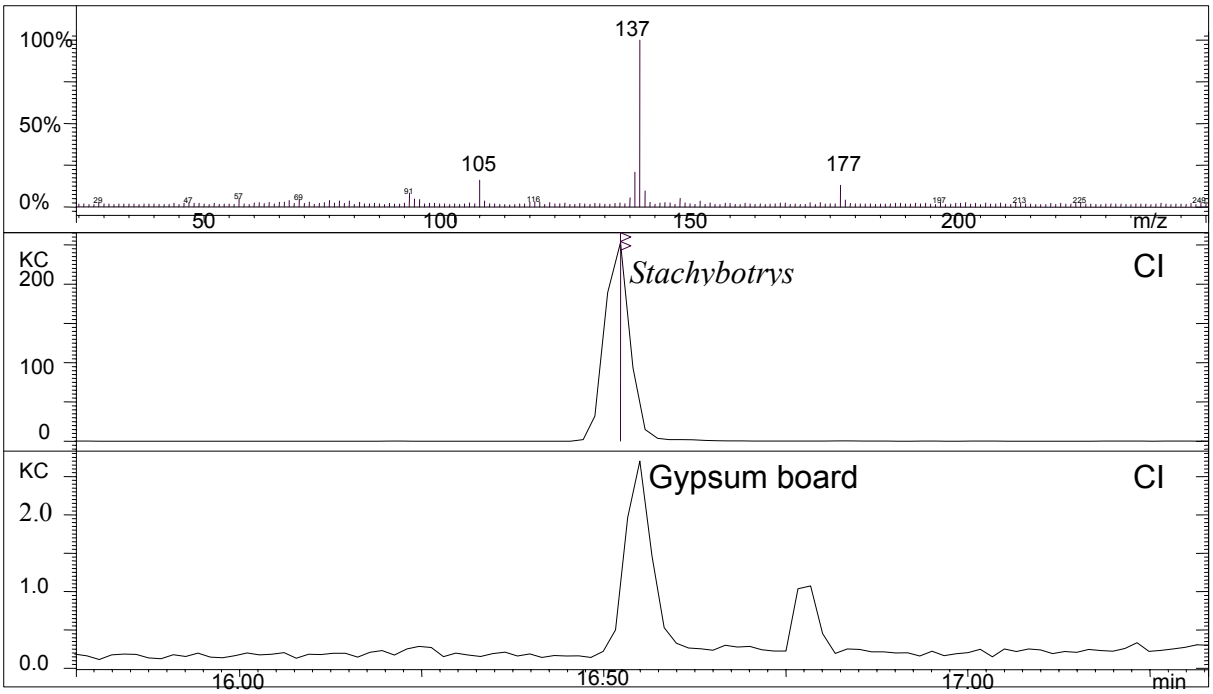

Figure 3. Chromatograms (CI) focusing at *m/z* 137 and related CI mass spectrum of methyl benzoate in a gypsum board paper culture positive for *S. chartarum* and a subculture thereof.

DISCUSSION

The molds isolated from the materials studied in this investigation have all been encountered in indoor environments. *S. chartarum* has been isolated from moist building materials, especially wetted gypsum boards, whereas *C. globosum* was isolated from cellulose containing materials, such as papers (Korpi et al., 1999; Flannigan et al., 1991). Several studies have identified *S. chartarum* and *A. versicolor* in damp and sick residences (Etzel et al., 1998; Hodgson et al., 1998).

Several MVOCs of the three fungal spp were identified, most of them with both EI and CI. However, 2- methyl-1-butanol, was identified only with CI (Figure 1). On the other hand, 2-pentanol was detected only in EI mode (Figure 1); this compound was detected in low sensitivity with both CI and EI. Different SPME fibers were compared to optimize conditions for the detection of the 11 MVOCs (Wilkins et al., 2000; Elke et al., 1999; Guido et al., 1999) in the reference mixture. Best performance was achieved with the 70 μm Carbowax-DVB stableflex fiber. 3-Octanone and 1-octen-3-ol were detected with highest sensitivity and 2-methyl-1-propanol was detected with lowest sensitivity. Differences in the affinity between the fiber materials and the analytes, the vapor pressures, and the analyte's differences in their signals in the MS could explain this. Optimum conditions to extract MVOCs from the building materials were achieved at 70^{o}C/ 20 min. Lower temperatures and shorter extraction times led to lower sensitivity; higher temperature or longer extraction times did not reveal any additional diagnostic peaks. None of the reference MVOCs were detected in the building materials when using EI due to the appearance of several closely eluting compounds. With CI, 1-octen-3-ol, 3-octanone, 2-hexanone, and 2-heptanone were identified in the gypsum board papers, whereas 3-octanone, 2-hexanone, and 2-heptanone were identified in both masonite and mineral wool. A striking finding was the identification of methyl benzoate in two of the studied mould strains. *S. chartarum* produced this compound in abundance whereas *A. penicillioides* produced only trace levels. In most published studies, MVOCs are collected at room temperature; this might not be relevant to detect methyl benzoate. Methyl benzoate was also identified in all mold-infested building materials, both in CI and EI. In conclusion, the SPME technique combined with GC/MS is useful to identify MVOCs. The technique shows great promise for detecting microbial contamination of building materials and further work on this aspect is merited.

CONCLUSIONS AND IMPLICATIONS

SPME is a rapid technique for the determination of VOCs. It can be automated, requires a minimum of sample preparation, and is therefore suitable for routine application. Several MVOCs including methyl benzoate were identified in water-affected building materials in the present study. More research is required for evaluating the technique's practical usefulness to identify unhealthy indoor environments.

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REFERENCES

Cooley JD, Wong WC, Jumper CA, *et al*. 1998. Correlation between the prevalence of certain fungi and sick building syndrome. *Occup. Envir. Med*. Vol. 55, pp 579-584.

Elke K, Begerow J, Oppermann H, *et al*. 1999. Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID-a new feasible approach for the detection of an exposure to indoor mould fungi?. *J. Envir. Monit.* Vol. 5, pp 445-52.

Elke K, Jermann E, Begerow J, *et al*. 1998. Determination of benzene, toluene, ethylbenzene and xylen in indoor air at environmental levels using diffusive samplers in combination with headspace solid phase microextraction and high resolution gas chromatography-flame ionization detection. *J. Chrom*. Vol 826, pp 191-200.

Etzel RA, Montana E, Sorenson WG, *et al*. 1998. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. *Arch Ped. Adolesc Med*. Vol 152, pp 757-762.

Flannigan B, Beardwood K, Ricaud PM, *et al*. 1991. Growth and toxin production in moulds isolated from houses. *Biodet. biodeg. Elsevier, Appl. Scien*. pp 487-489.

Graham AM, Walker V, and Hyekal M. 1999. Quantitative determination of trimethylamine in urine by solid phase microextraction and gas chromatography-mass spectrometry. *J. Chrom.* Vol 723, pp 281-285.

Guido F, Regina S, Manfred M, *et al*. 1999. Species-specific production of microbial volatile organic compounds by airborne fungi from a compost facility. *Chemos.* Vol 39, pp 795-810.

Hodgson MJ, Morey P, Leung WY, *et al*. 1998. Building-associated pulmonary disease from exposure to *S. chartarum* and *A. versicolor*. *JOEM.* Vol 40, pp 241-249.

Korpi A, Pasanen AL, and Viitanen H. 1999. Volatile metabolites of *Serpula lacrymans*, *Coniophora puteana*, *Poria placenta*, *S. chartarum* and *C. globosum*. *Building Environ.* Vol 34, pp 205-211.

Niwa T. 1986. Metabolic profiling with GC/MS, and its application to clinical medicine. *J. Chrom.* Vol 379, pp 313-45.

Pawliszym J. 1997. *Solid phase microextraction-theory and practice*. Chichester: Wiley-VCH.

Rylander R, Hsieh V, and Courteheuse C. 1994. The first case of sick building syndrome in Switzerland. *Indoor Envir*. Vol 3, pp 159-162.

Scheppers SC. 1999. *Solid Phase Microextraction, a practical Guide*. New York: Marcel Decker,

Wilkins K, Larsen K, and Simkus M. 2000. Volatile metabolites from mould growth on building materials and synthetic media. *Chemo*. Vol 41, pp 437-446.

Zlatkis A, Brazell RS, and Poole CF. 1981. The role of organic volatile profiles in clinical diagnosis. *Clin. Chem*. Vol 27, pp 789.