

Detection and Identification of *Stachybotrys chartarum* Microbial Volatile Organic Compounds (MVOCs) On-Site Using a Person-Portable Gas Chromatograph/Mass Spectrometer (GC/MS) with a Thermal Desorption Accessory

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Background

- **Exposure to molds and fungi can cause health issues that can become severe**
- **Water damage in residential and commercial buildings provides an optimal environment for mold and fungal growth**
- **The ability to quickly analyze and identify toxigenic molds and fungi will facilitate an efficient remediation process**



Background

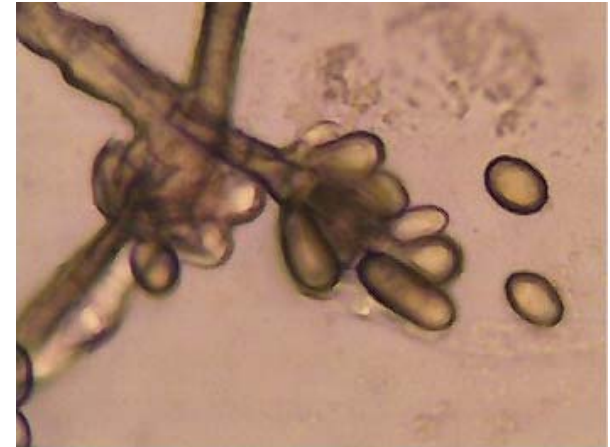
- **Sick Building Syndrome and Damp-Building Related Illnesses contribute to not only allergies but also more serious conditions for individuals with compromised immune systems**
 - **Sick Building Syndrome**
 - Symptoms associated with acute discomfort, such as headache, eye, nose, or throat irritation, dry cough, dry or itchy skin, dizziness and nausea, difficulty in concentrating, fatigue, and sensitivity to odors
 - The cause of the symptoms are unknown
 - Relief occurs soon after leaving the building

Background

- **Damp-Building Related Illness**
 - **Symptoms such as cough, chest tightness, fever, chills and muscle aches**
 - **Symptoms can be clinically defined and have clearly identifiable causes**
 - **Complainants may require prolonged recovery times after leaving the building**

Background

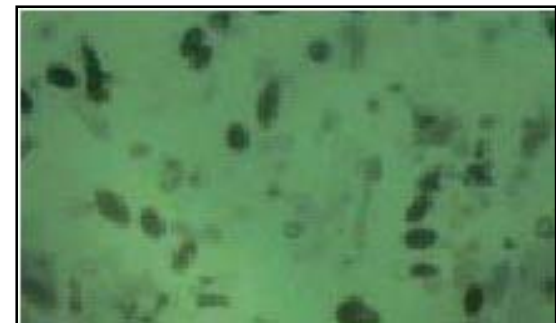
- ***Stachybotrys chartarum***
 - **Saprophytic fungus grows rapidly on water-damaged, cellulose-rich materials**
 - **Two main chemotypes¹**
 - **One chemotype produces highly toxic trichothecene satratoxins G and H, and various proteinases**
 - **Satratoxins G and H are protein synthesis inhibitors; together with the proteinases produced by this mold allows for the breakdown of vascular and cellular walls**
 - **Exposure to this chemotype can cause severe illnesses such as Sick Building Syndrome and Damp Building-Related Illnesses**
 - **The second chemotype produces less hazardous mycotoxins**



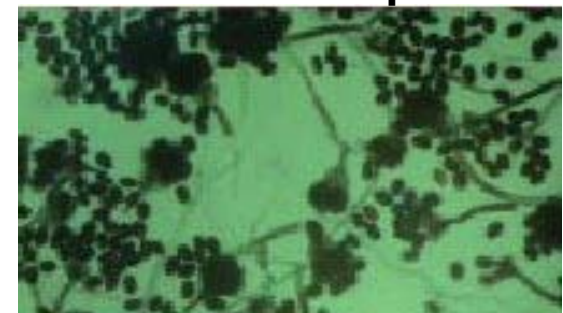
Current Mold Sampling Techniques

- **Airborne samples from the sampling environment are collected then sent to laboratories for analysis of spore types**
 - **Spore Trap Analysis**
 - The individual spores from collected samples are analyzed under a microscope
 - Mold is considered to be present when these spores are found
 - **Mold cultures**
 - Samples are collected, transferred to growth media, and incubated
 - Colonies that form after incubation are viewed under a microscope and analyzed

Spore trap sample



Culture sample



Current Mold Sampling Techniques

▪ Concerns

- The accuracy and precision of the results of both of these methods are questionable due to frequent (day-to-day or hour-to-hour) fluctuations of spore concentration
- Not all toxigenic molds have airborne spores that can be collected and analyzed
- Even “rush” analysis can take 1-2 days

Current Mold GC/MS Sampling Techniques

- **For *S. chartarum*, a fungus that does not have airborne spores, analysis of unique MVOCs is more accurate for identification**
 - **Samples are collected on-site via a thermal desorption tube, then sent to a remote site for GC/MS analysis**
 - **More accurate than individual spore analysis, however potential for sample contamination is constantly present**
 - **Samples still take 1-2 days even for rush analysis**

On-site GC/MS Sample Analysis

- **Utilizing a person-portable GC/MS, samples can be collected, desorbed onto the GC/MS, and analyzed on-site**
 - **Eliminates the possibility of sample contamination in transportation**
 - **Samples can be collected and analyzed within minutes**

Sampling Equipment

- HAPSITE ER person-portable GC/MS equipped with the Thermal Desorber Sampling System



Experimental

- **Studies by Gao et al² introduced the concept of detecting unique MVOCs as a method of confirming the presence of microorganism growth in buildings**
- **Studies by Betancourt et al³ and Mason et al⁴ list unique MVOCs produced by various toxigenic strains of *S. chartarum* grown on gypsum wall board and sugar plates**

Experimental

- **Based on information found in mold characterization literature, the following MVOCs were obtained for sampling**

- **Standard 1**

- Propanoic acid methyl ester
- Acetoin
- 3-Furanmethanol
- Styrene
- Anisole
- 3-Octanone
- 3- and 4-methylanisole
- Napthalene
- 3,5-dimethoxytoluene

- **Standard 2**

- Geosmin
- 2-Methylisoborneol
- α -Terpineol

Experimental

- For initial characterization of the MVOC mixture, 0.2 μL of a 1000 $\mu\text{g}/\text{mL}$ standard was injected into a 1 L polytetrafluoroethylene (Teflon) bag containing Ultra High-Purity nitrogen
- 250 mL of analytes were collected at a rate of 150 mL/min onto a carbon-based thermal desorption tube
 - Supleco Carboxen thermal desorption tube



Experimental

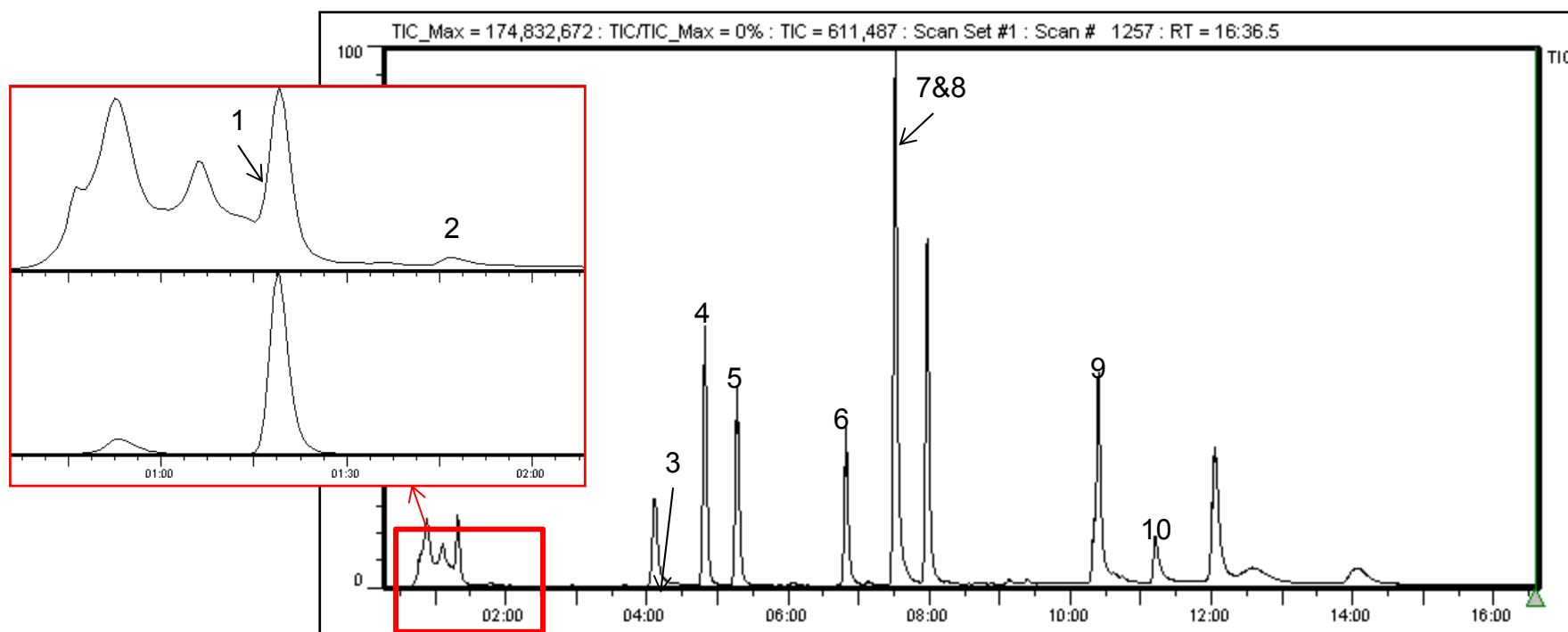
- Samples were desorbed from the thermal desorber tube at 300 °C for 5 minutes
- The sample was then transferred to an on-board concentrator within the HAPSITE ER via an external desorption accessory attached to the HAPSITE ER



Experimental

- **Samples then were desorbed from the on-board concentrator onto the column where they were separated using a ~16-minute analysis**
 - **Column- HP-1MS, 15 m, 0.32 i.d., 1 μ m**
 - **Hold 60 °C for 2 minutes, 6 °C/min to 80 °C, 10 °C/min to 120 °C, 26 °C/min to 200 °C, hold for 5 minutes**

MVOC mixture in Nitrogen

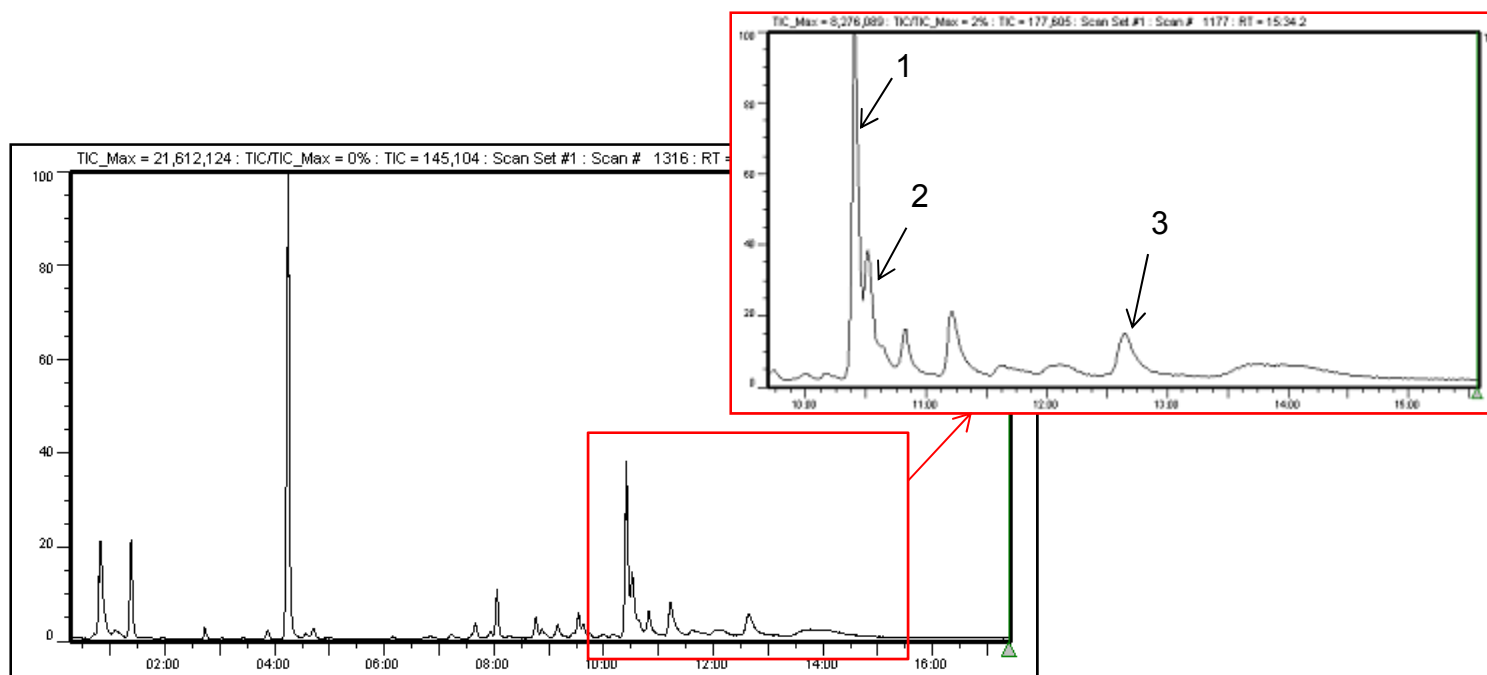


No.	Compound	Conc. (ppbv)	Conc. ($\mu\text{g}/\text{m}^3$)	RT
1	Propanoic Acid Methyl Ester	61	200	1:20
2	Acetoin	61	200	1:48
3	3-Furanmethanol	55	200	4:12
4	Styrene	51	200	4:57
5	Anisole	49	200	5:23
6	3-Octanone	42	200	6:54
7	3-Methylanisole	44	200	7:35
8	4-Methylanisole	44	200	7:35
9	Naphthalene	42	200	10:24
10	3,5-Dimethoxytoluene	35	200	11:12

Experimental

- **A second standard containing the additional MVOCs of interest (geosmin, 2-methylisoborneol, and α -terpineol) in methanol was diluted into a 1 L Teflon bag of nitrogen at a final concentration of 20 $\mu\text{g}/\text{m}^3$ per compound**
 - **Samples were desorbed from the carbon TD tube at 300 °C for 5 minutes and transferred to an on-board carbon-based concentrator in the HAPSITE ER**
- **Analytes were then desorbed from the on-board concentrator to the column and separated using a 16-minute analysis**
 - **Column used: HP-1MS, 15 m, 0.32 i.d., 1.0 μm**
 - **Hold 60 °C for 2 minutes, 6 °C/min to 80 °C, 10 °C/min to 120 °C, 26 °C/min to 200 °C, hold for 5 minutes**

Geosmin, α -Terpineol, 2-Methylisoborneol in Nitrogen

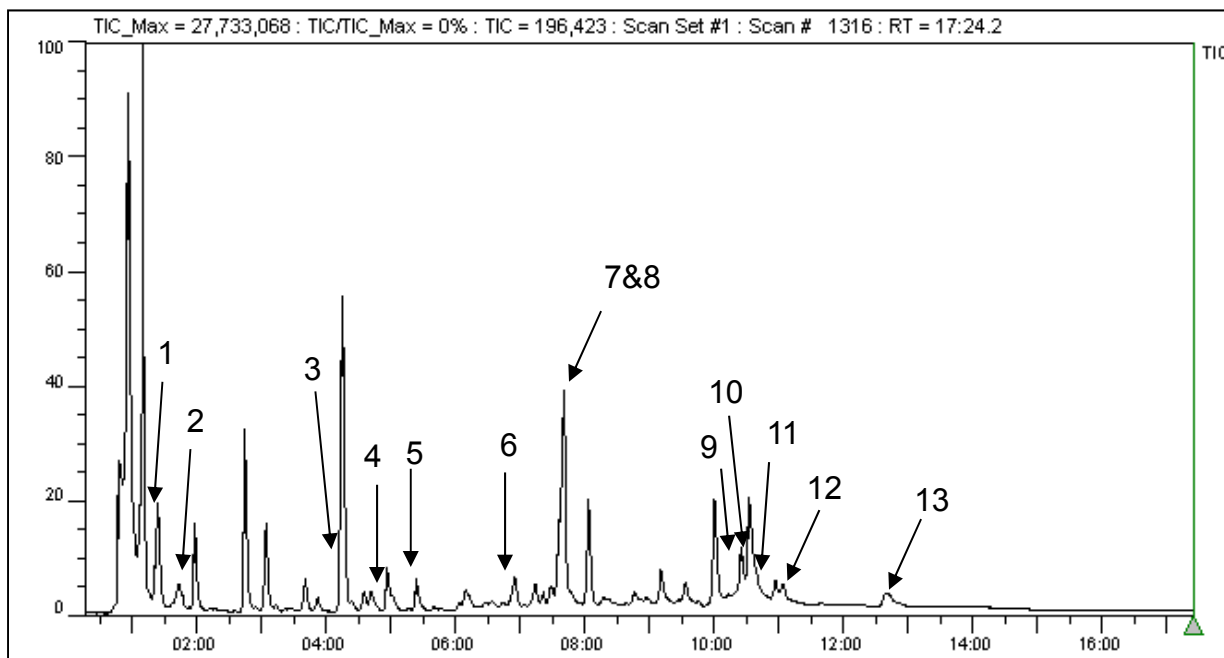


No.	Compound	Conc. (ppbv)	Conc. ($\mu\text{g}/\text{m}^3$)	RT
1	2-Methylisoborneol	2.86	20	10:31
2	α -Terpineol	3.12	20	10:33
3	Geosmin	2.64	20	12:38

Experimental

- **The MVOCs from both standards were injected at 20 $\mu\text{g}/\text{m}^3$ into 1 L Teflon bag containing room air**
 - **A 700 mL sample was collected onto the carbon TD tube**
 - **Sampling rate of 150 mL/min**
- **Analysis was performed using the same parameters as the previous analyses**
 - **Samples were desorbed from the carbon TD tube at 300 °C for 5 minutes and transferred to an on-board carbon-based concentrator in the HAPSITE ER**
 - **Analytes were then desorbed from the on-board concentrator to the column and separated using a 16-minute analysis**

Experimental



No.	Compound	Conc. (ppbv)	Conc. ($\mu\text{g}/\text{m}^3$)	RT
1	Propanoic Acid Methyl Ester	5.47	20	1:20
2	Acetoin	5.47	20	1:48
3	3-Furanmethanol	4.91	20	4:12
4	Styrene	4.63	20	4:57
5	Anisole	4.46	20	5:23
6	3-Octanone	3.76	20	6:54
7	3-Methylanisole	3.94	20	7:35
8	4-Methylanisole	3.94	20	7:35
9	Naphthalene	3.76	20	10:24
10	2-Methylisoborneol	2.86	20	10:31
11	α -Terpineol	3.12	20	10:33
12	3,5-Dimethoxytoluene	3.17	20	11:12
13	Geosmin	2.64	20	12:38

Experimental- Sampling with the Air Probe

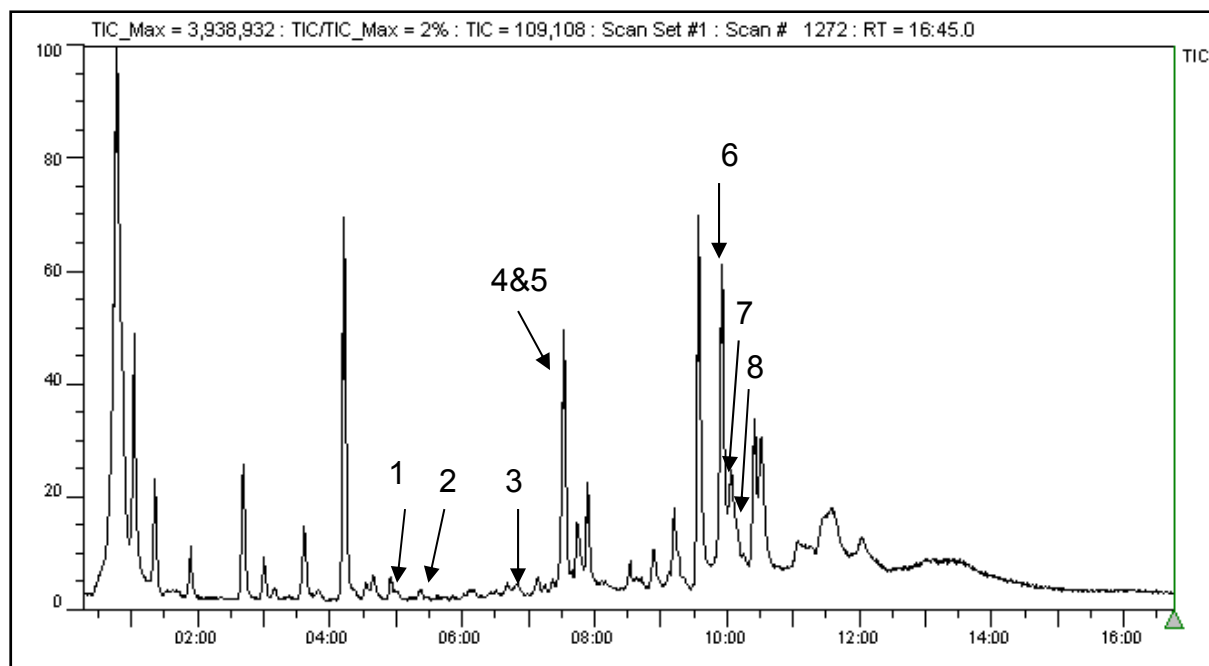
- **A similar study was performed using the HAPSITE ER and sampling directly with the Air Probe**
 - **Eliminates the need for collecting samples and bringing to the GC/MS**
 - **Analysis can be performed while in motion**
 - **Air probe comes standard with the HAPSITE ER**



Sampling with the Air Probe

- **A mix of the two standards of MVOCs was prepared in a 1 L bag filled with room air for a final component concentration of approximately 20 $\mu\text{g}/\text{m}^3$**
- **This mixture was sampled using the air probe and collected on the on-board concentrator for 1 minute**
- **Analytes were then desorbed from the on-board concentrator to the column and separated using a 16-minute analysis**
 - **Column- HP-1MS, 15 m, 0.32 i.d., 1.0 μm**
 - **Hold 60 °C for 2 minutes, 6 °C/min to 80 °C, 10 °C/min to 120 °C, 26 °C/min to 200 °C, hold for 5 minutes**

Sampling with Air Probe



No.	Compound	Conc. (ppbv)	Conc. ($\mu\text{g}/\text{m}^3$)	RT
1	Styrene	4.63	20	4:54
2	Anisole	4.46	20	5:23
3	3-Octanone	3.76	20	6:50
4	3-Methylanisole	3.94	20	7:28
5	4-Methylanisole	3.94	20	7:28
6	Naphthalene	3.76	20	9:57
7	2-Methylisoborneol	2.86	20	10:03
8	α -Terpineol	3.12	20	10:04

Conclusion

- **The person-portable HAPSITE ER allows for sampling and analysis of *S. chartarum* MVOCs to be performed on-site for faster results**
 - **Eliminates questionable sample integrity due to sampling containers and delay in analysis due to transport time**
 - **Focuses on unique signifiers of *S. chartarum***
 - **Two sampling modes:**
 - **Air probe allows for a direct sampling of MVOCs without the need for additional accessories, but the amount of MVOCs detected are limited**
 - **Thermal desorber accessory allows for a broader range of detectable compounds and higher sensitivity**

References

- ¹ Pestka, J. J., I. Yike, D. G. Dearborn, M. D. W. Ward, J. R. Harkema. 2009. *Stachybotrys chartarum*, Trichothecene Mycotoxins, and Damp Building-Related Illness: New Insights into a Public Health Enigma. *Toxicological Sciences* 104(1): 4-26
- ² Gao, P., F. Korley, J. Martin, B. T. Chen. 2002. Determination of Unique Microbial Volatile Organic Compounds Produced by Five *Aspergillus* Species Commonly Found in Problem Buildings. *AIHA Journal* 63:135-140
- ³ Betancourt, D.A., Dean, T.R., Menetrez, M.Y., Moore, S.A. 2006. Characterization of Microbial Volatile Organic Compounds (MVOC) Emitted by *Stachybotrys chartarum*. Proceedings for the AWMA/EPA Indoor Environmental Quality: Problems, Research and Solutions Conference, Research Triangle Park, NC.
- ⁴ Mason, S., D. Cortes, W. E. Horner. 2009. Detection of Gaseous Effluents and By-Products of Fungal Growth that Affect Environments. *HVAC&R Research* 16(3):109-121

QUESTIONS???