

1 **Detection of Airborne *Stachybotrys chartarum* Macrocylic**
2 **Trichothecene Mycotoxins in the Indoor Environment**

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18 **Running Title:** Detection of Indoor Airborne *Stachybotrys* Mycotoxins

19

1 **Abstract**

2 The existence of airborne mycotoxins in mold-contaminated buildings has long
3 been hypothesized to be a potential occupant health risk. However, little work has been
4 done to demonstrate these compounds in such environments. The presence of airborne
5 macrocyclic trichothecene mycotoxins in indoor environments with known *Stachybotrys*
6 *chartarum* contamination was therefore investigated. In seven buildings, air was
7 collected using a high volume liquid impaction bioaerosol sampler (SpinCon PAS 450-
8 10) under static or disturbed conditions. An additional building was sampled using an
9 Andersen GPS-1 PUF sampler modified to separate and collect particulates smaller than
10 conidia. Four control buildings (i.e., no detectable *S. chartarum* growth or history of
11 water damage) and outdoor air were also tested. Samples were analyzed using a
12 macrocyclic trichothecene-specific enzyme-linked immunosorbent assay (ELISA).
13 ELISA specificity was tested using phosphate buffered saline extracts of the fungal
14 genera *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Memnoniella*, *Penicillium*,
15 *Rhizopus*, *Trichoderma*, five *Stachybotrys* strains and the indoor air allergens Can f 1,
16 Der p 1, and Fel d 1. In test buildings, results showed that detectable toxin
17 concentrations increased with sampling time and short periods of air disturbance.
18 Trichothecene values ranged from less than 10 to greater than 1300 pg/m³ of sampled air.
19 The control environments demonstrated statistically significant (P<0.001) lower levels of
20 airborne trichothecenes. ELISA specificity experiments demonstrated a high specificity
21 for the trichothecene-producing strain of *S. chartarum*. Our data indicate that airborne
22 macrocyclic trichothecenes can exist in *Stachybotrys*-contaminated buildings and this
23 should be taken into consideration in future indoor air quality investigations.

1

2 **Keywords:** *Stachybotrys chartarum*, trichothecenes, airborne, indoor, detection

1 **Introduction**

2

3 For the past 25 years, there has been a growing concern about the presence of
4 fungi and adverse human health effects in indoor environments. Several genera of fungi,
5 including *Stachybotrys*, *Chaetomium*, and *Aspergillus*, have raised particular concern
6 because of their associated toxin production. In 2002, Dr. Stephen Redd of the Centers
7 for Disease Control and Prevention made a formal statement before the United States
8 House of Representatives concerning the relationship between indoor molds and human
9 illnesses (42). In this, Dr. Redd stated that “molds can be harmful [and] it is important to
10 maintain buildings, prevent water damage and mold growth, and clean up moldy
11 materials.” Additionally, “there remain many unresolved scientific questions”
12 concerning indoor mold contamination, but current research is expanding to include
13 “methods, development, and testing, specifically with regard to state-of-the-art
14 techniques for assessing indoor air quality-related exposures.” To date, the majority of
15 indoor air quality investigations have focused on the analysis of mold growth on building
16 materials (1, 19, 38, 44) and measurement of airborne particulate matter including dust,
17 fungal conidia (6, 21) and animal danders (14). These types of investigations cannot
18 directly assess occupant exposure to mycotoxins. Exposure to these factors can influence
19 allergic hypersensitivity responses (2, 10, 32) and symptoms of asthma in certain
20 individuals (12, 33), but most likely does not account for the often reported symptoms
21 such as nausea, dizziness, nose bleeds, physical and mental fatigue, and neurological
22 disorders (31, 43) seen in subjects occupying sick buildings.

1 Among the many fungi isolated from contaminated indoor environments,
2 *Stachybotrys chartarum* is one of the most well known. *S. chartarum* is a known
3 producer of a number of potent mycotoxins, in particular, the macrocyclic trichothecenes
4 verrucarins B and J, roridin E, satratoxins F, G, and H, and isosatratoxins F, G, and H
5 (23, 29). It has been proposed to be associated with human adverse health effects (9, 13,
6 15, 26, 27). The members of the macrocyclic trichothecene family of mycotoxins are
7 known to be potent inhibitors of protein synthesis in eukaryotes (16, 41, 53). Goodwin et
8 al., (17) and Murphy et al., (36) showed that when the non-macrocyclic trichothecene
9 mycotoxin diacetoxyscirpenol (also known as anguidine) was injected into human beings,
10 the observed symptoms were nausea, vomiting, low blood pressure, drowsiness, ataxia,
11 and mental confusion. These symptoms, among others, are commonly reported by
12 occupants of *Stachybotrys*-contaminated buildings.

13 *S. chartarum* airborne mycotoxins have been studied in various laboratory
14 settings (4, 37, 49, 52) and are known to be detrimental in several animal models (35, 40,
15 54). However, research that effectively demonstrates the presence of airborne *S.*
16 *chartarum* trichothecene mycotoxins in native indoor environments is lacking. Studies
17 have mostly focused on detecting these mycotoxins on bulk material (52) or in settled
18 dust (48). The aim of this study was to determine if airborne macrocyclic trichothecene
19 mycotoxins exist in indoor environments contaminated with *Stachybotrys chartarum* with
20 an emphasis on the development of simple and rapid collection/detection techniques.

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Materials and Methods

Indoor Test Environments Eight mold-contaminated buildings encompassing 16 tested rooms and 40 air collections throughout the state of Texas were chosen for sampling. These were labeled as Test Buildings 1-8. Buildings ranged in age from 10 years (Buildings 1-3) to greater than 20 years old (Buildings 4-8). Total visible fungal growth and water damage ranged from less than 1 ft² to greater than 500 ft². Further fungal growth was noted upon invasive inspection (i.e., hidden mold contamination). Self-reported occupant complaints, obtained from personal communication with the occupants, ranged from minor (musty odors) to severe (loss of mental awareness, dizziness, persistent headaches, etc.). Prior to air sampling, fungi growing on visible surfaces were sampled using the adhesive tape technique (50). Microscopic identification was based on morphology and was performed by a trained technician in our laboratory. Rooms chosen for testing demonstrated the highest degree of visible fungal contamination, water damage, and/or self-reported occupant complaint present in the building. Buildings 1, 2, 7, and 8 were unoccupied. For the remaining buildings, occupants were instructed not to enter or disturb the test rooms during sampling. To prevent cross-contamination, sampled rooms were isolated from the rest of the building by shutting doors or, in the case of heavy contamination, sealed with plastic sheeting and duct tape. Basic descriptions of each test building are outlined in Table 1.

1

2 **Air Sampling**

3

4 *Air Samplers* Two samplers were employed for the collection of airborne
5 trichothecene mycotoxins: the SpinCon PAS 450-10 bioaerosol sampler (Sceptor
6 Industries, Inc., Kansas City, MO) and the Andersen GPS-1 Polyurethane Foam (PUF)
7 High Volume Air Sampler (Thermo Electron Corporation, Cheswick, PA). The SpinCon
8 has been evaluated in the outdoor environment and it has been determined to be a highly
9 effective air sampling device (3, 5). From October 2001 to September 2002, the SpinCon
10 was employed for the collection of airborne *Bacillus anthracis* in buildings throughout
11 the Washington D.C. area following the well-known bioterrorism attack on that area (22).
12 For our purposes, the machine was operated at a manufactured set flow rate of 450 LPM.
13 Entrained solids were collected and concentrated in a phosphate buffered saline (PBS)
14 solution (pH 7.4) to a final volume of 10 ml. Over the collection period, evaporated PBS
15 was replaced by water in a standardized manner.

16 High volume samplers incorporating polyurethane foam are generally designed
17 for the collection of airborne pesticides and organic pollutants in the outdoor environment
18 (30, 34, 46). To our knowledge, the Andersen PUF sampler has never been used for
19 indoor air applications. For our purposes, the apparatus was modified to separate and
20 collect particulates smaller than *S. chartarum* conidia through the incorporation of two
21 sterile glass fiber filters of decreasing pore sizes placed in series (Figure 1). We have
22 been able to show, using a similar filtration setup in a controlled setting, that conidia can
23 be separated from minute particles that carry trichothecene mycotoxins (4). Large

1 particles were captured using a 90 mm GF/D glass fiber filter (Whatman, Clifton, NJ)
2 with a pore size of 2.7 μm in the upper chamber of the sampling module. Air leaks were
3 prevented by using custom made rubber gaskets (85 and 110 mm inner and outer
4 diameters, respectively). To collect remaining particulate matter, a 90 mm Whatman
5 EPM-2000 glass microfiber filter was fit immediately before the lower chamber of the
6 sampling module. EPM filter material was selected by the United States Environmental
7 Protection Agency as the standard for use in high volume air sampling. According to the
8 manufacturer, this material is 99.99% efficient for 0.3 μm -sized dioctyl phthalate
9 particles (standard particles for testing filter efficiency) at a 5 cm/sec flowrate. A metal
10 screen was placed immediately after the filter to disperse the air pressure and prevent
11 puncture and/or tearing. The machine was adjusted to collect at a flow rate of 150 LPM.
12 The flow rate was based on a calibration curve that was determined with the filters in
13 place using a manometer attached to the sampling module.

14
15 *Controlled SpinCon Setup* Prior to building sampling, controlled trials were
16 performed to determine if the SpinCon bioaerosol sampler was capable of collecting
17 airborne macrocyclic trichothecene mycotoxins. To do this, we constructed a setup
18 whereby air was pulled over cellulose-containing ceiling tile with confluent *S. chartarum*
19 growth directly into the collection chamber of the SpinCon (Figure 2). We have
20 described the conditions and methods for growth of *S. chartarum* on ceiling tile
21 previously (4). For sampling, a 24-inch section of polyvinyl chloride (PVC) pipe filled
22 with *S. chartarum*-contaminated ceiling tile (representing approximately 500 cm^2 of
23 growth) was attached to the air inlet of the SpinCon. Potential leaks surrounding the inlet

1 were covered by securely taping several layers of aluminum foil around the PVC pipe at
2 that vicinity. All controlled trials were run in the outdoor environment. Sampling was
3 performed for 10 and 30 minutes (N=3 replicates for each time interval). For comparison
4 purposes, collection was also performed using an equal area of sterile ceiling tile in the
5 same manner.

6

7 *Volumetric Spore Traps* In each tested environment, airborne conidia counts
8 (based on five-minute samples) were evaluated prior to or during sampling using either
9 an Allergenco MK-3 non-viable volumetric collector (Allergenco, San Antonio, TX) or a
10 Burkard Personal Volumetric Air Sampler (Spiral Biotech, Inc., Norwood, MA). The
11 collection characteristics of the Allergenco and Burkard have been shown to be similar
12 (39). One sample (referred to as a spore trap from here onward) was collected per air
13 sampling condition as described below. For reference purposes, one spore trap was
14 collected in the outdoor environment just prior to sampling for each building. An
15 outdoor spore trap was not collected for Building 7 as wet weather conditions did not
16 allow for proper sampling. For each collection period, the samplers were placed at an
17 elevation of 3-4 feet above the ground. Conidia and other airborne particulates were
18 collected on glass microscope slides that had been coated with a thin layer of petroleum
19 grease using a foam makeup applicator. Following sampling, conidia were identified to
20 the genus level by a trained technician in our laboratory using an Olympus BH2-RFCA
21 optical light microscope (Olympus America, Inc., Melville, NY). Airborne conidia
22 concentrations were calculated using the following equation:

23

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Conidia Counted

((Conversion # for Microscopic Field [mm] & Trace Width [mm]) x (Sampling Flow Rate [m³/min]) x (Sampling Time [min]))

2

3 The conversion number was defined as the microscope objective diameter divided by the
4 trace width. For our case, this number, for the 400x objective, was set at 0.409 (0.45
5 mm/1.1 mm). The sampling flow rate for both volumetric spore traps was 0.015 m³/min
6 and the sampling time was five minutes. For simplicity, airborne conidia concentrations
7 were determined by dividing the conidia counts by 0.031. In addition to conidia
8 characterization, levels of debris (non-identifiable particles) were qualitatively measured
9 based on the approximate percentage of the viewed field covered by such particles: very
10 light (< 20%), light (21-40%), medium (41-60%), heavy (61-80%), and very heavy (>
11 80%; approaching unreadable). Semi-quantification of debris was important because it
12 was composed mostly of highly respirable particles (< 2 µm in diameter) and was viewed
13 as a potential source of mycotoxins (i.e., the particles could serve as carriers of
14 macrocyclic trichothecenes).

15

16 *Sampling Conditions* The use of personal protective equipment was observed for
17 all test areas. This included impervious full-body suits (Sunrise Industries, Inc.,
18 Guntersville, AL), full face piece respirators (3M, St. Paul, MN) equipped with organic
19 vapor/acid gas cartridge/P100 filter cartridges (3M 60923) or a combination of the two.

20 Buildings 1-7 were sampled using the SpinCon PAS 450-10 bioaerosol sampler.
21 Collection was performed at an elevation of 3-4 feet above the ground. Air was sampled

1 in each room under static and/or disturbed conditions for 10 minutes (N=7 rooms, 9
2 samples under disturbed conditions), 20 minutes (N=4 rooms, 4 samples under disturbed
3 conditions), 30 minutes (N=6 rooms, 6 samples under disturbed conditions), and/or 120
4 minutes (N=14 rooms, 14 samples under static conditions, 4 samples under disturbed
5 conditions). For each room, collection was first performed under static conditions
6 followed by collection with air disturbance in order of sampling time (longest to
7 shortest). Following each sampling interval, rooms were left undisturbed for 20 to 30
8 minutes to allow the room air to return to pre-collection conditions. To prevent cross-
9 contamination between samples, the SpinCon was run through a rinse cycle, as instructed
10 by the manufacturer, using a 5% (v/v) bleach solution followed by pure water. We have
11 shown that rinsing with a dilute bleach solution is effective for the removal/inactivation
12 of trichothecene mycotoxins (55). For those areas sampled with disturbance, 20-inch box
13 fans (Lasko Products, Inc., West Chester, PA) set on “high” were placed in a manner that
14 would circulate the air in the room (one in each corner of the room). The fans were
15 allowed to run for five minutes prior to collection for initial generation of particulate
16 matter. One five-minute volumetric spore trap was obtained for each test as already
17 described. For static conditions, these samples were taken just prior to sampling. For
18 disturbed conditions, the spore traps were collected five minutes prior to the end of the
19 collection. In addition, four control buildings (i.e., no visible fungal contamination or
20 history of water incursion events) were sampled in a similar manner under static and/or
21 disturbed conditions for 10 minutes (N=6 rooms, 6 samples under disturbed conditions)
22 and 120 minutes (14 rooms, 24 samples under static conditions). These buildings were
23 labeled as Control Buildings 1-4. For reference, four outdoor air samples were collected

1 using the SpinCon for 30, 60, 90, and 120 minutes (N=1 for each sampling period).
2 Spore traps for these outdoor samples were taken just prior to sampling for the sole
3 purpose of determining if *S. chartarum* conidia were present.

4 Building 8 was sampled using the Andersen GPS-1 PUF High Volume Air
5 Sampler modified as already described. The sampling inlet was at a manufactured set
6 height of approximately five feet. Air was sampled in a heavily mold-contaminated
7 closet (Figure 1) under static conditions for 24, 48, and 72 hours (N=1 for each time
8 period). A five-minute Allergenco spore trap was taken prior to each sampling interval.
9 In addition, one room in Control Building was sampled for 24 hours under static
10 conditions. Volumetric spore traps were collected for each control area as described for
11 test buildings.

12
13 **Sample Preparation** Following collection, SpinCon samples (all 10 ml) were
14 filtered using Fisherbrand 13 mm diameter nylon syringe filters with a 0.45 μm pore size
15 (Fisher Scientific, Hampton, NH). Loss of sample due to filter absorbance was minimal.
16 The filtered fluid was sterilely transferred to 15 ml polypropylene conical centrifuge
17 tubes, frozen at -80°C , and lyophilized using a VirTis Freezmobile (SP Industries, Inc.,
18 Gardiner, NY). The dried samples were individually resuspended in 1 ml of room
19 temperature (25°C) pyrogen-free water for immediate testing.

20 Filters obtained from the Andersen PUF Sampler were removed immediately after
21 testing and transferred individually to 50 ml polypropylene centrifuge tubes on-site. The
22 filters were suspended in 40 ml of PBS, vortexed vigorously for 60 seconds, removed
23 from the tubes using sterile forceps, and then discarded. The PBS extracts were filtered

1 (as described above) into new 50 ml tubes. These were frozen at -80°C, lyophilized, and
2 resuspended in 1 ml pyrogen-free water for immediate testing.

3

4 **Macrocytic Trichothecene Analysis** Samples were analyzed for
5 macrocytic trichothecenes using the QuantiTox Kit for trichothecenes (EnviroLogix,
6 Portland, ME) as outlined by the manufacturer. This competitive ELISA kit incorporates
7 trichothecene-specific antibodies immobilized on polystyrene microtiter wells (7). We
8 have previously demonstrated that this assay is highly specific for macrocytic
9 trichothecene mycotoxins, particularly those produced by *Stachybotrys chartarum* (4).
10 All reagents and antibody-coated wells were allowed to equilibrate to room temperature
11 before use. For testing, samples or control mixtures were added to wells in triplicate.
12 Following the incubation, wells were read at 450 nm using an EL-312 microtiter plate
13 reader (Bio-Tek Instruments, Winooski, VT). To ensure that the ELISA ran correctly,
14 the macrocytic trichothecene roridin A at a concentration of 50 ng/ml in PBS was used
15 as a positive control for each set of tests. PBS alone was used as negative control.

16

17 **Fungal Conidia and Indoor Allergen Crossreactivity** The composition of indoor air
18 is complex in that numerous types of particulates (fungal conidia, bacteria, animal
19 dander, etc.) are present at any given time. Because of this, we tested our detection
20 method (ELISA) against some of the most common indoor air constituents. The
21 following fourteen strains of fungi were tested: one macrocytic trichothecene-producing
22 *Stachybotrys chartarum* strain (ATCC 201212), four atranone-producing *Stachybotrys*
23 *chartarum* strains (IBT 9633, IBT 9757, IBT 9293, IBT 9290; IBT Culture Collection of

1 Fungi, Mycology Group, BioCentrum DTU, Technical University of Denmark),
2 *Alternaria alternata*, *Aspergillus niger* (ATCC 10575), *Chaetomium globosum* (ATCC
3 16021), *Cladosporium cladosporioides*, *Fusarium sporotrichioides* (ATCC 24630),
4 *Memnoniella echinata* (ATCC 11973), *Penicillium chrysogenum*, *Trichoderma viride* and
5 one species of *Rhizopus*. Out of the five *S. chartarum* strains tested, only strain 201212
6 produced macrocyclic trichothecenes (28). Fungi not purchased from a supplier were
7 collected from outside samples, purified, and identified in our laboratory by a trained
8 technician according to Sutton et al., (51) and de Hoog et al., (11). All fungi were
9 maintained on potato dextrose agar (BD Diagnostic Systems, Sparks, MD) on 90 mm
10 plastic Petri dishes in a controlled 25°C incubator (Fisher Scientific Isotemp Incubator,
11 Model 304).

12 For testing purposes, conidia were collected from plates that had reached
13 confluence (approximately 7-14 days) using sterile cotton swabs. To collect the conidia,
14 swabs were gently rolled over the surface of the fungal growth. The amount of culture
15 swabbed was variable as certain fungi do not sporulate as readily as others (i.e.,
16 *Alternaria* versus *Aspergillus*). The cotton tips of the swabs were placed in 1 ml of
17 sterile room temperature (25°C) PBS in 1.5 ml microcentrifuge tubes and vortexed for
18 approximately one minute to remove conidia. The conidia were then counted using a
19 hemacytometer and diluted in PBS (pH 7.4) to concentrations that were based on what
20 we observed in sampled buildings (Table 2). For example, the highest concentration of
21 *Cladosporium* conidia collected in any given test building was approximately 1.2×10^6
22 total conidia (1871 conidia/m^3 of sampled air collected at 150 LPM for 72 hours). Two
23 10-fold dilutions were made in PBS (pH 7.4) from these solutions resulting in three test

1 concentrations. Fungi that were in low abundance ($< 1 \times 10^4$ total collected conidia) in
2 the indoor environments were tested at 1×10^6 , 10^5 , and 10^4 conidia/ml.

3 For ELISA testing, the fungal extracts were centrifuged at 14,500 RPM for 1
4 minute to pellet the conidia. Care was taken not to disturb the conidia pellet and only the
5 top 80% of the supernatant was used for ELISA testing. Each sample was run in
6 duplicate wells on two separate occasions.

7 Three common indoor allergens (Indoor Biotechnologies, Charlottesville, VA)
8 were also tested: *Dermatophagoides pteronyssinus* allergen 1 (dust mite allergen, Der p
9 1), *Felis domesticus* allergen 1 (cat hair extract; Fel d 1), and *Canis familiaris* allergen 1
10 (dog hair extract; Can f 1). Allergens were received in 50% glycerol and, for ELISA
11 testing purposes, were individually diluted in PBS to 50 ng/ml, 5 ng/ml, and 500 pg/ml.
12 Samples were tested in triplicate wells.

13

14 **ELISA Interpretation** ELISA results from building samples were
15 converted into percent inhibitions and relative trichothecene concentrations based on the
16 raw data (absorbance readings at 450 nm). The percent inhibition represents the degree
17 of inhibition the test sample had on the capability of the satratoxin G-HRP conjugate to
18 bind to the immobilized antibody. They were calculated as done by Schick et al., (45)
19 using the following equation:

20

21 $\% \text{ Inhibition} = 100 \times 1 - [(O.D._{450} \text{ sample} - \text{background}) / (O.D._{450} \text{ control} - \text{background})]$

22

1 A higher percent inhibition corresponds to a greater concentration of trichothecenes in the
2 sample.

3 To obtain relative trichothecene concentrations, an ELISA-based macrocyclic
4 trichothecene standard curve was developed and previously described by our laboratory
5 (4). Briefly, a mixture of four macrocyclic trichothecenes (satratoxins G and H, roridin
6 A, and verrucarin A) in equal amounts was diluted to 12 test concentrations (500 to 0.1
7 ng/ml) and tested via the ELISA as already described. Satratoxins G and H were purified
8 as described by Hinkley and Jarvis (25) in our laboratory. Roridin A and verrucarin A
9 were purchased from Sigma-Aldrich Co. (St. Louis, MO). Average ELISA absorbance
10 readings at 450 nanometers were plotted against toxin concentrations (N=3 replicates per
11 concentration) to generate a standard curve. Using this curve, an approximate
12 trichothecene amount was determined for each sample (in ng/ml). Taking into account
13 the collection rate of the air samplers and assuming 100% air sampling efficiency, a
14 semi-quantitative estimate of the amount of airborne trichothecenes for each tested area
15 was then determined (in pg/m³). Cross-reactivities to fungal extracts and allergens were
16 expressed only as percent inhibitions.

17

18 **Statistical Analysis** Statistical analyses were performed using Sigma Stat 2.0
19 software (Systat Software, Inc., Point Richmond, CA). Controls were grouped based on
20 sampling time. Test samples were individually compared to control groups (e.g., each
21 120-minute static test was compared to the 120-minute static control group) using a
22 Student's *t*-test. Statistical significance was defined as having a P-value of less than 0.05.
23 All conditions of normality were met for these analyses. Because 20, 30, and 120 minute

1 disturbed sampling was not performed in control environments, these samples were
2 compared to environments sampled for 120 minutes under static conditions.

3 Additionally, test samples were grouped and compared to control groups. Test
4 buildings where *Stachybotrys chartarum* was not clearly identified using our survey
5 methods were excluded from statistical analyses as they were not applicable for
6 trichothecene determination. These represented five rooms (all rooms in Building 7 and
7 the upstairs bedroom in Building 1). Thirty-minute samples (N=15 data points) and two
8 hour samples (N=27 data points under static conditions, 9 data points under disturbed
9 conditions) were compared to two hour static controls (N=69 data points) using a
10 Kruskal-Wallis One Way Analysis of Variance (ANOVA) on Ranks. Ten-minute test
11 samples taken under disturbed conditions (N=24 data points) were compared to similarly
12 sampled controls (N=9 data points) using a Mann-Whitney Rank Sum Test. Statistical
13 significance for these analyses was reported as having a P-value of <0.001.

14 Data obtained from samples using the Andersen PUF sampler (N=3 data points
15 for each filter type and collection period) were normalized and compared using a one-
16 way ANOVA followed by Tukey post-hoc analysis. Test filters were compared to
17 corresponding filter types that were used for 24-hour sampling in a control environment.
18 Statistical significance was defined as having a P-value of less than 0.05.

19 All ELISA cross-reactivities (as percent inhibitions) for fungal extracts and
20 allergens were compared to PBS alone using a one-way ANOVA (P<0.05).

21

22 **Results**

23

1 **Airborne Conidia Types and Counts** Airborne conidia types and concentrations
2 for test and control buildings are shown Tables 3 and 4, respectively. Marked differences
3 were seen between buildings, individual rooms within the same building, sampling times
4 and disturbance conditions. Overall airborne conidia types and counts were higher in test
5 buildings than in control buildings. In control buildings and outdoor environments,
6 airborne conidia types and counts, in a generalized order of prevalence, were as follows:
7 *Penicillium/Aspergillus*-like, *Cladosporium*, *Alternaria*, Ascospores (likely *Chaetomium*,
8 but unable to confirm due to the absence of fruiting bodies), *Bipolaris*, *Nigrospora*, and
9 *Curvularia*. *Fusarium*, *Memnoniella*, and *Stachybotrys* conidia were not isolated from
10 these environments. In test buildings, airborne conidia types and counts, in order of
11 prevalence, were as follows: *Penicillium/Aspergillus*-like, Ascospores, *Cladosporium*,
12 *Alternaria*, *Stachybotrys*, *Memnoniella*, *Fusarium*, *Nigrospora*, and *Curvularia*.
13 *Bipolaris* conidia were not isolated from test buildings. Accurate counts could not be
14 made in Buildings 2-4 under some of the disturbed sampling conditions/aggressive
15 sampling. This was due to an overwhelming amount of collected debris. However,
16 attempts were made to assess the airborne *Stachybotrys* conidia concentrations in
17 Building 3 during aggressive sampling. Overall, higher airborne conidia counts were
18 observed under static versus disturbed conditions in test and control buildings, though
19 concentrations were higher under shorter periods of disturbance (i.e., 10 and 20 versus 30
20 and 120 minutes of sampling). The same trend was seen with debris counts where static
21 conditions and shorter disturbance intervals resulted in the highest counts.
22

1 **Controlled SpinCon Setup** Results from the controlled SpinCon setup are shown in
2 Table 2. For reference, the lower limit of ELISA detection for purified samples was 100
3 pg/ml (4). As can be seen, collection of airborne trichothecene mycotoxins from
4 *Stachybotrys chartarum*-contaminated ceiling tile in the sampler was successful.
5 Concentrations were higher after 30 minutes compared to 10 minutes of sampling (2700
6 versus 1500 picograms/m³ of sampled air). Sterile ceiling tile alone demonstrated ELISA
7 reactivity but values were much lower (P<0.05) than when *Stachybotrys* was present in
8 the system. Trichothecene values for 10 and 30 minutes using sterile ceiling tile were 62
9 and 64 pg/m³ of sampled air, respectively.

10

11 **Building Analyses** Percent inhibitions and relative trichothecene concentrations for
12 each SpinCon-sampled test and control room are shown in Tables 5 and 6, respectively.
13 Values were higher with shorter sampling periods and disturbance times, regardless of
14 the nature of the building (test or control). Statistically significant airborne trichothecene
15 equivalents were found in all rooms in Buildings 1-6 under at least one sampling
16 condition. Values obtained for Building 7 were not significantly different from control
17 buildings. Estimated airborne trichothecene concentrations from all of the SpinCon-
18 sampled buildings are summarized in Figure 3. Overall, detectable levels of airborne
19 macrocyclic trichothecene mycotoxins were significantly (P<0.001) higher in *S.*
20 *chartarum*-contaminated buildings versus control buildings. The median values were
21 3.9, 9.0, 7.5, and 61.5 pg/m³ of air for two-hour static controls and tests, two-hour
22 disturbed tests, and 30-minute disturbed tests, respectively. The median values were 44.9
23 and 248.5 pg/m³ of air for the 10-minute controls and tests, respectively. Outdoor air,

1 regardless of the sampling time, was negative (Table 6). No *Stachybotrys* conidia were
2 collected in the outdoor air for any of the sampling times [data not shown].

3 Table 7 shows results from the two buildings that were sampled using the
4 Andersen PUF high volume sampler. Data are presented similarly to Table 2, but with an
5 additional column noting the pore size of the glass microfiber filter used. For each
6 sampling time in the test building, the 2.7 µm pore size filter in the setup demonstrated
7 higher ELISA reactivity than the second (<0.3 µm pore size) filter. Values were highest
8 for the 24-hour sampling period, followed by the 72 and 48-hour collection intervals,
9 respectively. A general trend was not observed but statistically higher trichothecene
10 concentrations were seen on the filters from the environment with known *Stachybotrys*
11 *chartarum* growth versus the control building. Percent inhibitions and trichothecene
12 equivalents observed in the one control room tested with the PUF sampler were lower
13 than controls tested with the SpinCon (Table 6).

14

15 **Fungal Conidia and Indoor Allergen Cross-reactivity** ELISA cross-reactivity data
16 to 14 frequently isolated indoor fungi and three common indoor allergens are shown in
17 Table 8. Data are expressed as percent inhibitions. *S. chartarum* strain 201212 was
18 significantly (P<0.05) different than the extracting solvent (PBS) alone at all three tested
19 conidia concentrations. Of the remaining fungi tested, only *S. chartarum* strain 9633
20 demonstrated significant (P<0.05) ELISA cross-reactivity, and then, only at the highest
21 concentration (1 x 10⁶ conidia/ml). Can f 1, Der p 1, and Fel d 1 demonstrated no cross-
22 reactive ELISA binding.

23

1 **Discussion**

2 In this study, through the use of two types of high volume air samplers in
3 conjunction with sensitive and specific ELISA technology, we were successful in
4 demonstrating airborne macrocyclic trichothecene mycotoxins in a controlled situation
5 and eight native *Stachybotrys chartarum*-contaminated indoor environments. It should
6 be noted that the observed positive results were likely influenced, albeit slightly, by non-
7 specific binding to other airborne constituents. This is based on the false positive results
8 observed in control buildings (Table 6). We hypothesize this non-specificity to be
9 inherent to the antibody. The antibody used for detection in our analyses was raised
10 against satratoxin G, a 500 MW compound. Cross-reactivity and/or non-specific binding
11 to other similarly structured airborne compounds (e.g. volatile organic compounds, other
12 small molecular weight microbial toxins, protein haptens, etc.) is expected, particularly
13 when considering the complex composition of indoor air. Further support for this
14 observed phenomenon lies in the fact that we were testing with a polyclonal antibody,
15 which, in itself, has an associated degree of non-specificity. Nonetheless, we have
16 previously demonstrated the highly specific nature of the ELISA (4) and because other
17 common indoor fungi demonstrated no ELISA cross-reactivities (Table 8), our results
18 likely reflect the presence of airborne *S. chartarum* macrocyclic trichothecene
19 mycotoxins in the sampled buildings contaminated with this organism.

20 As Table 1 shows, there was a considerable range in water damage and mold
21 contamination in the eight test buildings. Similarly, self-reported occupant health
22 complaints varied from building to building. This was expected because no widely
23 accepted methods for measuring indoor mold related health issues currently exist.

1 Animal models have demonstrated that lethal doses for trichothecenes are relatively low
2 (8) and toxicity is greater via the respiratory route (53). These studies focused primarily
3 on non-macrocytic trichothecenes which are known to be less toxic than the macrocytic
4 trichothecenes produced by *S. chartarum* (53). We estimated airborne trichothecene
5 levels ranging from approximately 4 to 250 picograms/m³ of sampled air (median
6 quantified concentrations). For these example purposes, 100 picograms/m³ of air will be
7 assumed. With a minute ventilation of 6 liters per minute (under moderate activity), a
8 human inhales approximately 0.36 m³ of air per hour (47). In an environment with an
9 airborne concentration of 100 picograms/m³, an individual potentially inhales
10 approximately 300 picograms of trichothecenes in an eight-hour period. The LD₅₀ of
11 satratoxin H in mice administered the toxin intravenously is 5.7 mg/kg body weight (41).
12 Based on studies with the non-macrocytic trichothecene mycotoxin T-2 toxin (53), it can
13 be assumed that the LD₅₀ value via the respiratory route is 10-fold less or approximately
14 600 µg/kg body weight for satratoxin H. With the above scenario, this concentration far
15 exceeds what could possibly exist in contaminated buildings. However, when discussing
16 exposure to *S. chartarum* trichothecene mycotoxins in indoor environments, immediate
17 lethal doses are not an issue. Instead, the concentrations that may lead to the commonly
18 reported symptoms of nausea, diarrhea, headaches, dizziness, hemorrhage, and others are
19 of interest. Theoretically, these concentrations could be hundreds to thousands of times
20 less than the reported LD₅₀ values. Inhalation of 300 picograms per eight hours would
21 result in approximately a 10 nanogram exposure over a period of a month. These
22 concentrations certainly have the potential for the chronic development of adverse health
23 effects in humans. This is of particular interest when considering that the air inside

1 fungal-contaminated buildings is likely composed of numerous other mycotoxins,
2 allergens, and immunosuppressants. To date, however, there have been no described
3 methods or standards relating human health and airborne macrocyclic trichothecene
4 exposure in indoor mold-contaminated environments.

5 Seven of the eight *Stachybotrys*-contaminated environments were tested using the
6 SpinCon PAS 450-10 high volume wet concentration bioaerosol sampler. In general, our
7 data demonstrated an increase in airborne mycotoxin concentrations in relation to
8 increased *Stachybotrys* conidia and debris counts. This was an expected result since the
9 two primary mycotoxins produced by *S. chartarum* (satratoxins G and H) are known to
10 be associated primarily with conidia (20) and consequently, fungal fragments (4, 18). In
11 a similar manner, invasive inspection techniques and remediation results in extensive
12 release of airborne particulate matter including conidia and mycotoxins. This poses a
13 potential health risk to remediators and emphasizes the need for PPE while working in
14 mold-contaminated areas.

15 The mechanism used to disturb the air for these experiments was a means to
16 shorten the overall sampling times. Such an intense disturbance and consequent release
17 of particulates inclusive of *S. chartarum* conidia would not be expected to occur in native
18 environments. However, subtler disturbance mechanisms (human and mechanical
19 vibrations, ceiling fans, air conditioning units, etc.) are hypothesized to cause the
20 persistent release of such particulate matter over a longer period of time. This supports
21 the idea that adverse human health effects in mold-contaminated buildings are a result of
22 chronic more often than acute exposure. Longer disturbance under intense conditions
23 resulted in lower airborne trichothecene concentrations. The cause for this phenomenon

1 is not completely clear, but here we hypothesize two reasons. First, although rooms were
2 closed off from the rest of the building during sampling, they were not completely sealed.
3 During fan operation, we likely created a positive pressure environment. Particulate
4 matter and associated mycotoxins could have exited the sampling area via the path of
5 least resistance (e.g., under doors, through wall sockets, wall cracks, etc.). Second,
6 operation of the fans caused an intense wind velocity in the rooms that could have been
7 large enough to overpower the collection capacity of the SpinCon. In other words,
8 particles of interest could have been rendered unattainable by our sampling method.
9 These issues aside, we believe we have developed a means to collect and analyze
10 aerosolized *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in buildings
11 contaminated with this organism. Additionally, our sampling techniques could be
12 modified to test for other airborne constituents such as allergens, endotoxin, and/or
13 mycotoxins produced by other fungi. Based on our data, this would involve an initial
14 two-hour or longer static sample to evaluate airborne contamination in the native setting.
15 This would be followed by a much shorter sampling period (ideally 10 minutes) under air
16 disturbance conditions to assess the potential for trichothecenes to become airborne in
17 that environment.

18 Our testing methods were highly specific for buildings contaminated with *S.*
19 *chartarum*. The specific nature of our methods was seen in both controlled and natural
20 settings. For controlled analyses, ELISA cross-reactivity was tested with 13 strains of
21 fungi (frequently isolated from indoor environments) that do not produce macrocyclic
22 trichothecenes. Only *S. chartarum* strain 9633 demonstrated significant cross-reactivity,
23 and then, only at the highest conidia concentration tested (1×10^6 conidia/ml). This

1 could have been due to a basal level of trichothecene production not detectable by
2 previously described analytical methods (23, 24, 29). Regarding the specificity of our
3 testing in natural settings, certain areas (Bedroom in Test Building 1 and all of Building
4 7) were heavily contaminated with fungi; however, no *Stachybotrys* growth sites were
5 observed and, consequently, airborne *Stachybotrys* conidia counts were zero. Results
6 obtained from sampling in these areas were similar to negative controls. The Bedroom in
7 Building 1 demonstrated significantly ($P < 0.05$) higher values after 30 minutes of
8 collection under disturbed conditions likely because these values were compared to those
9 obtained following two-hour sampling under static conditions. Even when high
10 concentrations of other fungi were present (such as *Chaetomium* and/or *Memnoniella*),
11 positive results were only seen in environments contaminated with *Stachybotrys*
12 *chartarum* during the time of sampling.

13 Building 8 was unique in that we tested the hypothesis that airborne trichothecene
14 mycotoxins were present on particulates smaller than fungal conidia. This is important
15 because in the indoor environment, fragments and other highly respirable particles greatly
16 outnumber intact fungal conidia (18). Many widely used techniques such as bulk
17 sampling (e.g., adhesive tape technique, surface swabs, collection of bulk materials, etc.)
18 and viable/non-viable airborne conidia assessments (e.g., volumetric spore traps,
19 Andersen impaction devices, etc.) are not designed for the collection and analysis of
20 these potential health hazards. Previously, in a controlled filtration setup (similar to the
21 one depicted in Figure 1), we were able to demonstrate *S. chartarum* trichothecene
22 mycotoxins on particles smaller than conidia (4). In the current study, we were able to
23 show this same phenomenon after 24, 48, and 72 hours of high volume air sampling in a

1 native mold-contaminated building. These findings indicate the need to collect and
2 analyze this class of particulates when conducting indoor air quality investigations.

3 Our study shows that macrocyclic trichothecene mycotoxins from *Stachybotrys*
4 *chartarum* can become airborne in indoor environments contaminated with this organism.
5 Our data suggest the need to test for these potential occupant health risks during indoor
6 air quality investigations. Although we were able to semi-quantitate airborne
7 concentrations, it is still not known what levels of these mycotoxins pose a definitive
8 human health risk. Additionally, normal background levels (if they do exist) have not
9 been characterized. Future research should focus on the relationship between respiratory
10 exposure to airborne trichothecenes in fungal-contaminated buildings and human health
11 issues resulting from such exposures.

12

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3

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Figure 1 – Andersen GPS-1 PUF High Volume Air Sampler setup. Panel A shows the collection module following a 24-hour sampling period. The top filter with a considerable amount of collected particulates is visible here. Panel B is a schematic of the collection module with a top view on the left and a side view on the right. The module was modified to collect and separate particles using glass microfiber filters. Large particles, including most fungal conidia, were collected on 90 mm diameter 2.7 μ m pore size GF/D filters (1) while remaining particles able to pass through the first filter were collected on highly efficient EPM filters of the same diameter (2). A heavily mold-contaminated storage closet adjacent to the source of the water damage in Test Building 8 (shown in panel C) was chosen for the sampling. Water-saturated air and ensuing fungal contamination was a result of major damage to the air conditioning unit. The degree of the damage was evident by growth near the air exit grates throughout the building (panel D).

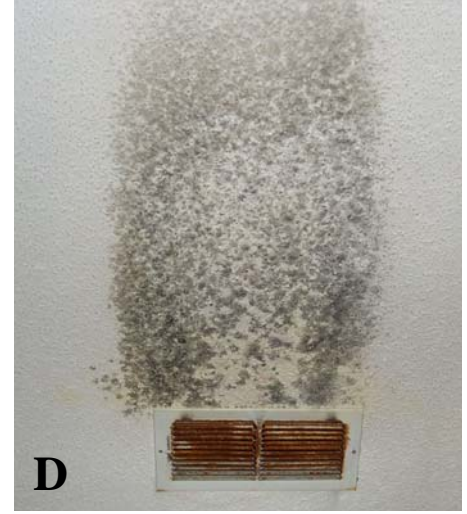
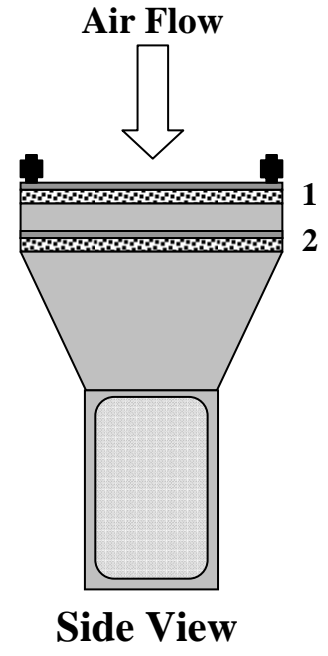
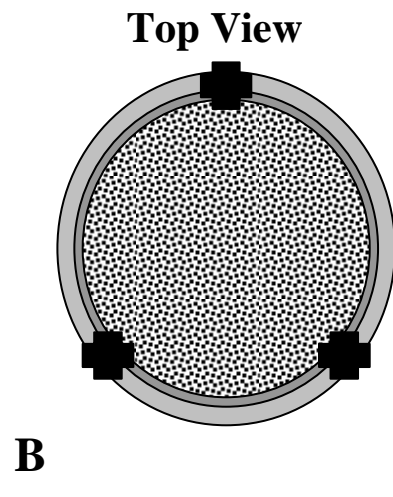
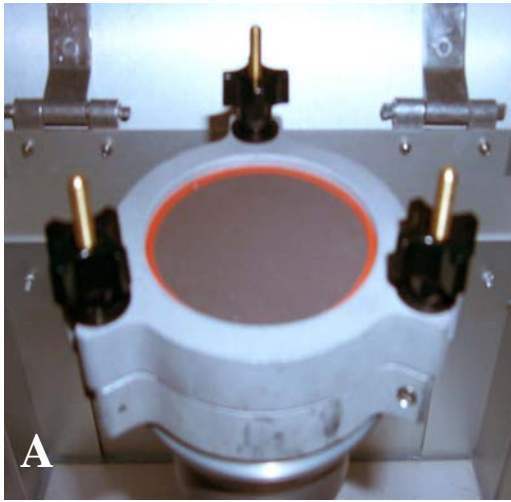


Figure 2 – SpinCon PAS 450-10 bioaerosol sampler controlled setup. PVC pipe was filled with *S. chartarum*-contaminated ceiling tile and attached to the air inlet of the SpinCon. Potential leaks surrounding the inlet were sealed with aluminum foil. Air passing over and through the ceiling tiles was directed into the collection chamber at a rate of 450 LPM. Aerosolized *Stachybotrys chartarum* conidia and other particulate matter were captured by a swirling column of PBS in the collection chamber. These trials were run in the outdoor environment. Sampling was performed for 10 and 30 minutes (N=3 replicates for each time interval). For comparison purposes, collection was also performed using an equal area of sterile ceiling tile in the same manner.

SpinCon PAS 450-10 Bioaerosol Sampler

Collection Chamber

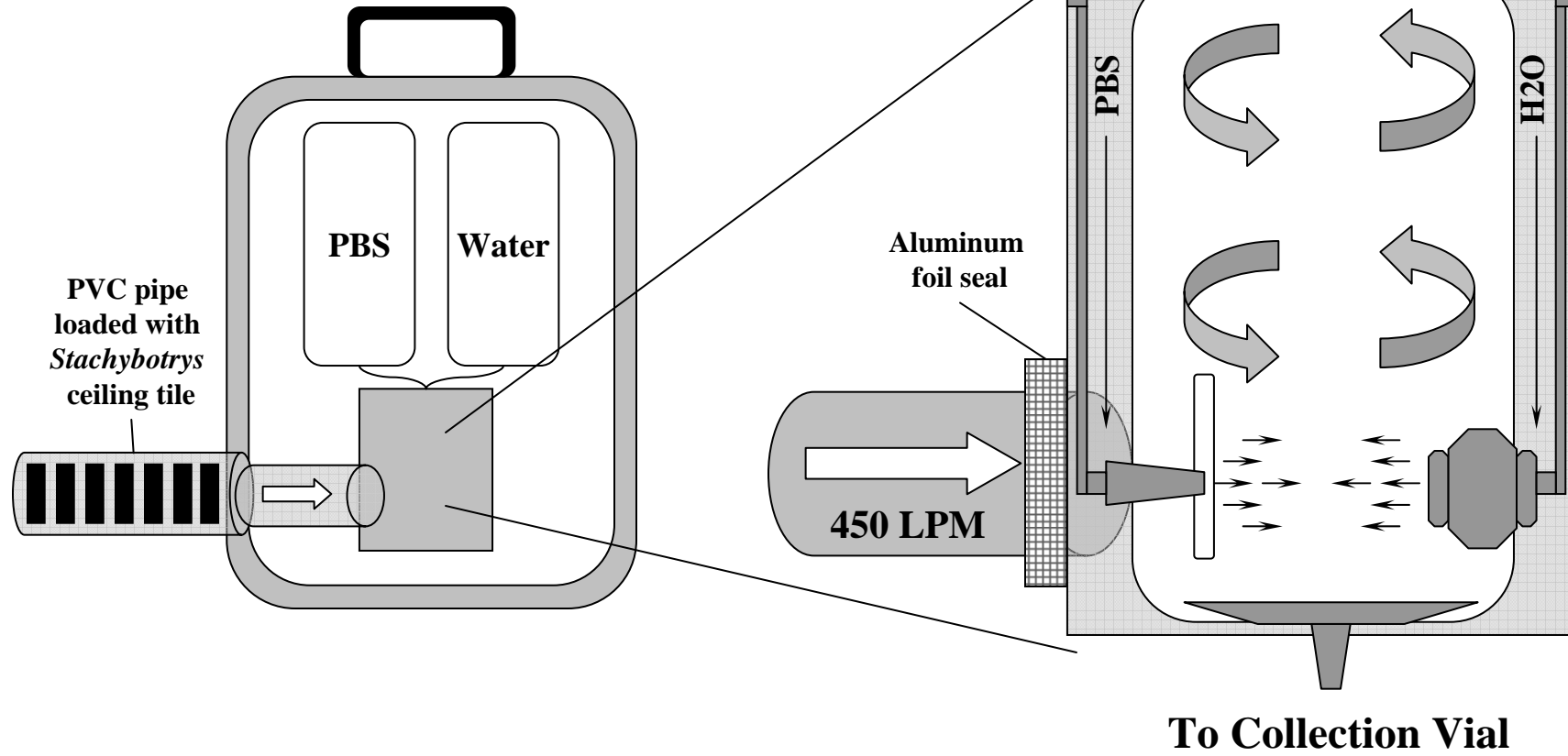
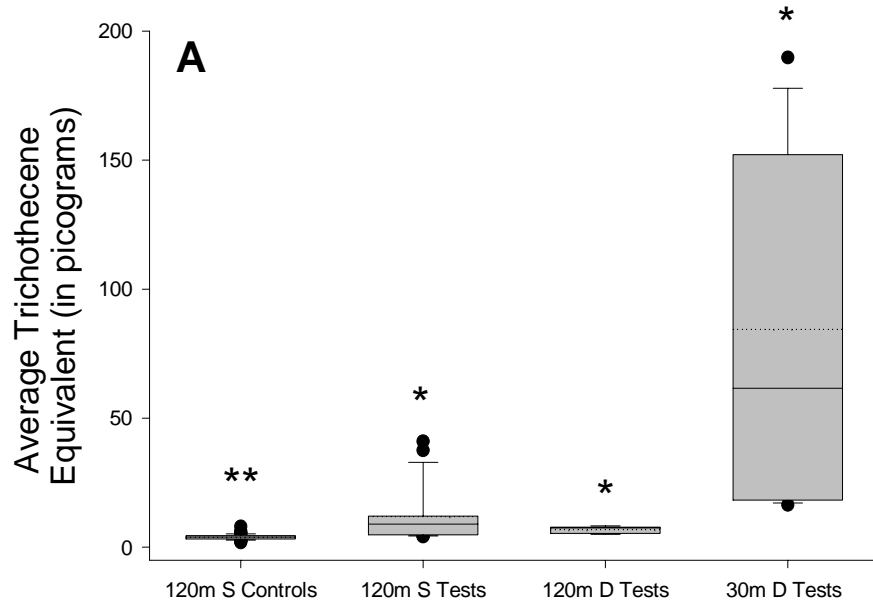


Figure 3 – Box plot data for average trichothecene equivalents per m³ of sampled air in *Stachybotrys*-contaminated and control indoor environments. Trichothecene equivalents (in picograms) were determined using a macrocyclic trichothecene standard curve.

Graph A shows the data distribution from 120 minute (m) control and test samples under static (S) and disturbed (D) conditions and 30-minute disturbed test environments. Medians (solid line) and means (dotted line) are shown. The 10th and 90th percentiles are designated by the bottom and top error bars, respectively. The 25th and 75th percentiles are indicated by the bottom and top of the boxes, respectively. Outliers are designated as the filled circles above and/or below the plot. Test environments were compared to control environments (**) using a Kruskal-Wallis One Way Analysis of Variance on Ranks. Graph B shows the data distribution from control and test environments sampled for 10 minutes under disturbed conditions. Test environments were compared to controls using a Mann-Whitney Rank Sum Test. Statistically significant differences (P <0.05) are indicated by an *.

Average Trichothecene Equivalent per m³ of Sampled Air for 120m and 30m Controls/Tests



Average Trichothecene Equivalent per m³ of Sampled Air for 10m Controls/Tests

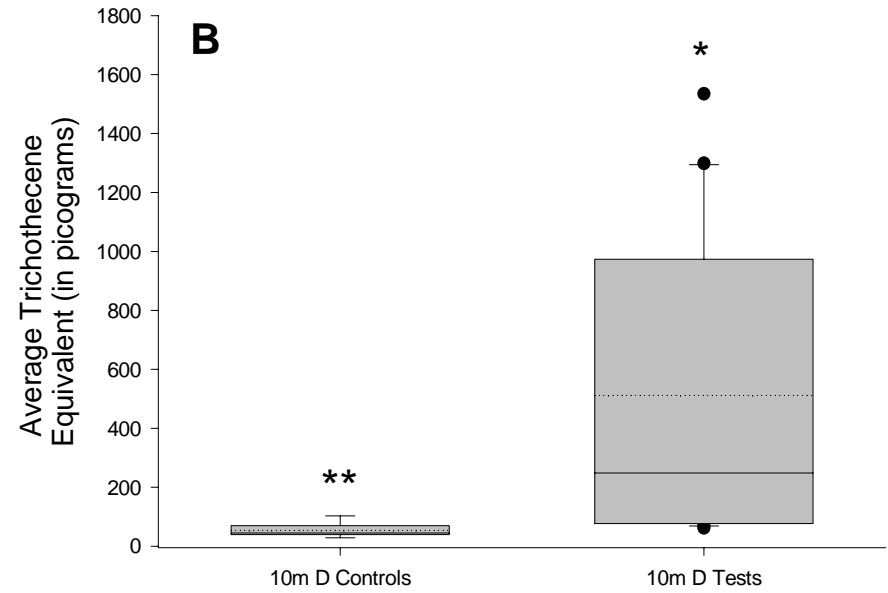


TABLE 1. Test building descriptions

Building	Occupancy	Age ^a	Occupant Complaints ^b	Rooms/ Samples Taken	Visible Water Damage ^c	Total Visible Fungal Contamination ^c	Identified Fungal Genera ^d
1	Empty	10	Musty odor, eye/throat irritation, nosebleeds, rash/itchy skin, headaches, fatigue, loss of mental awareness, dizziness, nausea	3/11	200-300	50-100	<i>Stachybotrys, Chaetomium, Memnoniella, Nigrospora, Cladosporium</i>
2	Empty	10	Musty odor	2/7	>500	>500	<i>Stachybotrys, Aspergillus, Cladosporium, Pencillium, Alternaria, Chaetomium</i>
3	Occupied	10	None Reported	1/4	1-10	10-20	<i>Chaetomium, Stachybotrys</i>
4	Occupied	>20	None Reported	1/1	1-10	<10	<i>Stachybotrys</i>
5	Occupied	>20	Musty odor, eye/throat irritation, nosebleeds, headaches	2/2	50-100	50-100	<i>Stachybotrys, Cladosporium, Aspergillus</i>
6	Occupied	>20	Musty odor, eye/throat irritations, sneezing, sinus congestion	2/4	100-200	20-50	<i>Chaetomium, Aspergillus</i>
7	Empty	>20	Musty odor	4/8	>500	200-300	<i>Memnoniella, Alternaria, Cladosporium, Aspergillus, Fusarium</i>
8	Empty	>20	Musty odor, eye/throat irritations, nosebleeds, headaches	1/3	100-200	100-200	<i>Aspergillus, Alternaria, Stachybotrys, Pencillium, Fusarium, Chaetomium</i>

^aApproximate age (in years) during the time of sampling

^bSelf-reported complaints during the time of occupation.

^cEstimation in square feet. This estimate includes growth that was observed following invasive inspection.

^dVisible surface growth only. Assessment was made using the adhesive tape technique as described in the text. Fungi are listed by prevalence (highest to lowest).

TABLE 2. Air sampling analyses from controlled SpinCon setup

Sample	Sampling Time	Average ELISA % Inhibition ^a	Average Trichothecene Equivalent ^b	Average Trichothecene Equivalents/m ³ of Sampled Air ^c
Sterile Ceiling Tile	10 minutes	20.8 ± 5.8	0.28 ± 0.06	62.4 ± 12.8
	30 minutes	47.4 ± 7.8	0.87 ± 0.34	64.4 ± 25.1
<i>Stachybotrys</i> Ceiling Tile	10 minutes	76.4 ± 0.6	6.7 ± 0.4	1.48 x 10 ³ ± 91.3*
	30 minutes	87.6 ± 0.2	36.4 ± 1.6	2.69 x 10 ³ ± 120*

^aMeans ± standard deviations of 3 individual runs. Values are based on PBS alone (average of 8 separately run samples).

^bIn ng/ml. Means ± standard deviations of 3 individual runs are shown. Values were derived from an ELISA-based macrocyclic trichothecene standard curve

^cIn picograms. Means ± standard deviations of 3 individual runs are shown. Estimated values are based on the average trichothecene equivalents for the entire collected sample, collection time, and flow rate of the SpinCon. For example, a total of 13.5 m³ of air was collected for each 30 minute sample. Given a final working volume of 1 milliliter, trichothecene concentrations were then estimated from values obtained from the ELISA testing. Values determined as significantly different (P<0.05) than sterile ceiling tile sampled in a similar manner are noted with an *.

TABLE 3. Airborne conidia types and counts isolated from outdoor air and test buildings for each sampling time and condition

Test Building ^a	Room ^b	Sampling Time and Conditions ^c	Airborne Fungal Genera - Conidia Counts per m ³ of Air ^d										Debris Count ^e
			<i>Alt</i>	<i>Asco</i>	<i>Bip</i>	<i>Clado</i>	<i>Curv</i>	<i>Fus</i>	<i>Memn</i>	<i>Nigro</i>	<i>Pen/Asp</i>	<i>Stachy</i>	
1	ODA	NA	774	1065	0	2548	0	0	0	65	12226	0	Medium
	Kitchen	120 min Static	32	5	0	1645	0	0	0	0	11226	0	Very Light
		30 min Disturbed	3710	32	0	161	0	0	0	0	3710	0	Very Light
		10 min Disturbed	97	581	0	1355	0	161	0	0	10677	452	Medium
	TV Room	120 min Static	548	1484	0	1548	65	0	0	0	48613	1226	Heavy
		120 min Disturbed	323	4065	0	1000	0	0	0	65	17129	5032	Medium
		30 min Disturbed	0	0	0	97	0	32	0	32	3935	0	Very Light
		10 min Disturbed	161	2452	0	710	0	226	0	0	28290	2710	Medium
	Bedroom	120 min Static	258	1161	0	161	0	0	0	0	9419	0	Medium
		120 min Disturbed	226	3355	0	1323	0	0	0	32	3968	0	Medium
		30 min Disturbed	258	806	0	1065	0	0	0	0	6065	0	Light
		10 min Disturbed	355	5677	0	2355	97	0	0	0	7935	0	Medium
2	ODA	NA	129	258	0	355	194	0	0	0	3161	0	Medium
	Main Entry	120 min Static	226	1548	0	1000	0	0	0	32	5806	1161	Heavy
		120 min Disturbed	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	THTC
		30 min Disturbed	129	613	0	1387	0	0	0	65	6581	323	Medium
		10 min Disturbed	65	710	0	1065	0	0	0	0	5839	226	Heavy
	Kitchen	120 min Static	226	774	0	194	32	0	0	0	2387	3097	Heavy
		120 min Disturbed	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	THTC
		30 min Disturbed	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	THTC
3	ODA	NA	677	871	0	3129	0	0	0	32	3226	0	Light
	Laundry Room	120 min Static	194	129	0	65	0	0	0	0	4032	8839	Medium
		10 min Agg 1	NA	NA	NA	NA	NA	NA	NA	NA	NA	16968	V. Heavy
		10 min Agg 2	NA	NA	NA	NA	NA	NA	NA	NA	NA	14355	V. Heavy
		10 min Agg 3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	THTC
4	ODA	NA	454	194	65	1452	0	0	0	0	1871	0	Light
	Living Room	30 min Agg	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	THTC
5	ODA	NA	97	129	0	0	0	0	0	0	355	0	Very Light
	Front Bathroom	120 min Static	32	32	0	323	32	0	0	0	1129	0	Light

	Back Bathroom	120 min Static	65	548	0	65	0	0	0	0	355	0	Light
	ODA	NA	129	97	0	1710	0	0	0	32	1645	0	Light
6	Kitchen	120 min Static	0	32	0	226	0	0	0	0	1581	0	Light
		10 min Disturbed	0	419	0	129	0	0	0	0	2548	32	Light
	Garage	120 min Static	0	161	0	419	0	0	0	0	4677	0	Medium
		10 min Disturbed	65	516	0	1065	0	0	0	0	10871	0	Medium
	Hall 1	120 min Static	0	32	0	97	0	0	0	0	1516	0	Light
		20 min Disturbed	0	452	0	161	0	0	4387	0	3548	0	Medium
7	Hall 2	120 min Static	32	65	0	65	0	0	0	0	2774	0	Light
		20 min Disturbed	0	65	0	258	0	0	0	0	13806	0	Light
	Room 253	120 min Static	0	11387	0	0	0	0	12839	0	1258	0	Light
		20 min Disturbed	0	6387	0	0	0	0	18323	0	2226	0	Heavy
	Room 259	120 min Static	97	65	0	129	0	0	0	0	2065	0	Light
		20 min Disturbed	0	65	0	32	0	0	0	0	6935	0	Medium
	ODA	NA	355	258	0	1258	0	0	0	0	2548	0	Light
8	Closet	24 hours Static	32	129	0	1387	0	0	0	0	1867	0	Light
		48 hours Static	161	3710	0	807	0	0	0	0	10774	0	Light
		72 hours Static	32	129	0	1871	0	0	0	0	1677	0	Light

^aBuildings 1-7 were sampled with the SpinCon PAS 450-10 Bioaerosol Sampler. Building 8 was sampled with the Andersen GPS-1 PUF High Volume Air Sampler modified as described in the text.

^bODA (Outdoor air) was considered normal and served as a control for sampling. An ODA sample was not available for Building 7 as wet weather conditions did not allow for the collection of a sample.

^cRooms were sampled under static and/or disturbed conditions for the noted times. Air disturbance was accomplished using 20-inch box fans on a "high" setting. Disturbance was allowed for 5 minutes prior to starting the SpinCon collection. Agg; collections taken during aggressive sampling. NA; not applicable

^d5-minute volumetric spore traps were taken to assess airborne fungal conidia types and concentrations as well as to qualitate the amount of debris present. Calculations are described in the text. Building 5 was sampled using a Burkard Personal Volumetric Air Sampler. All other buildings were tested using an Allergenco MK-3 non-viable volumetric collector. For static conditions, samples were taken just prior to collection. For disturbed conditions, spore traps were started 5 minutes prior to the end of the sampling period. Key: *Alt* - *Alternaria*; *Asco* - Ascospores (most likely *Chaetomium*, but unable to confirm); *Bip* - *Bipolaris*; *Clado* - *Cladosporium*; *Curv* - *Curvularia*; *Fus* - *Fusarium*; *Memn* - *Memnoniella*; *Nigro* - *Nigrospora*; *Pen/Asp* - *Penicillium/Aspergillus*-like (unable to confirm); *Stachy* - *Stachybotrys*. NA; not applicable as debris counts were too high.

^eDebris was defined as non-identifiable particles and were qualitated based on the approximate percentage of the viewed field covered by such particles: Very Light (<20%), Light (21-40%), Medium (41-60%), Heavy (61-80%), and Very Heavy (>80%). THTC; too heavy to count.

TABLE 4. Airborne conidia types and counts isolated from outdoor air and control buildings for each sampling time and condition

Control Building ^a	Room ^b	Sampling Time and Conditions ^c	Airborne Fungal Genera - Conidia Counts per m ³ of Air ^d										Debris Count ^e
			<i>Alt</i>	<i>Asco</i>	<i>Bip</i>	<i>Clado</i>	<i>Curv</i>	<i>Fus</i>	<i>Memn</i>	<i>Nigro</i>	<i>Pen/Asp</i>	<i>Stachy</i>	
1	ODA	NA	484	194	65	1452	0	0	0	32	1871	0	Light
	Room 1	120 min Static	32	0	0	97	0	0	0	0	226	0	Light
	Room 2	120 min Static	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	Light
	Room 3	120 min Static	0	65	0	32	0	0	0	32	129	0	Very Light
	Room 4	120 min Static	0	65	0	129	0	0	0	32	548	0	Light
	Room 5	120 min Static	0	32	0	0	0	0	0	0	161	0	Very Light
2	ODA	NA	194	32	32	1194	32	0	0	32	1581	0	Medium
	Bedroom	120 min Static	581	0	32	742	0	0	0	0	1548	0	Heavy
	Kitchen	120 min Static	194	0	0	194	0	0	0	0	1452	0	Heavy
	Computer Room	120 min Static	226	32	0	323	0	0	0	0	839	0	Heavy
3	ODA	NA	161	97	0	1516	0	0	0	65	4129	0	Medium
	Computer Room 1	120 min Static	32	32	0	0	0	0	0	0	3226	0	Medium
		10 min Disturbed	32	32	0	97	0	0	0	0	3065	0	Medium
	Computer Room 2	120 min Static	32	0	0	32	0	0	0	0	3452	0	Medium
		10 min Disturbed	32	32	0	32	0	0	0	0	1032	0	Light
	Bedroom	120 min Static	0	0	0	0	32	0	0	0	903	0	Light
	10 min Disturbed	0	0	0	0	0	0	0	0	1194	0	Medium	
4	ODA	NA	0	65	0	32	0	0	0	0	806	0	Light
	Bedroom 1 (Trial 1)	120 min Static	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Bedroom 1 (Trial 2)	120 min Static	0	0	0	97	0	0	0	0	3968	0	Medium
		10 min Disturbed	0	32	0	32	0	0	0	0	806	0	Light

Bedroom 2	120 min Static	65	0	0	161	0	0	0	0	6290	0	Heavy
	10 min Disturbed	32	32	0	32	0	0	0	0	1645	0	Medium
Bedroom 3	120 min Static	65	0	0	97	32	0	0	0	4065	0	Medium
	10 min Disturbed	0	0	0	65	32	0	0	0	710	0	Light

^aBuildings 1-4 were sampled with the SpinCon PAS 450-10 Bioaerosol Sampler. Building 1, room 5 was sampled with the Andersen GPS-1 PUF High Volume Air Sampler immediately following the SpinCon collection.

^bODA (Outdoor air) was considered normal and served as a control for sampling.

^cRooms were sampled under static and/or disturbed conditions for the noted times. Air disturbance was accomplished using 20-inch box fans on a "high" setting. Disturbance was allowed for 5 minutes prior to starting the SpinCon collection. NA; not applicable.

^d5-minute volumetric spore traps were taken to assess airborne fungal conidia types and concentrations as well as to qualitate the amount of debris present. Calculations are described in the text. All control buildings were tested using an Allergenco MK-3 non-viable volumetric collector. For static conditions, samples were taken just prior to collection. For disturbed conditions, spore traps were started 5 minutes prior to the end of the sampling period. Key: *Alt* - *Alternaria*; *Asco* - Ascospores (most likely *Chaetomium*, but unable to confirm); *Bip* - *Bipolaris*; *Clado* - *Cladosporium*; *Curv* - *Curvularia*; *Fus* - *Fusarium*; *Memn* - *Memnoniella*; *Nigro* - *Nigrospora*; *Pen/Asp* - *Penicillium/Aspergillus*-like (unable to confirm); *Stachy* - *Stachybotrys*. NA; not applicable (overwhelming debris count). NT; not taken.

^eDebris was defined as non-identifiable particles and were qualitated based on the approximate percentage of the viewed field covered by such particles: Very Light (<20%), Light (21-40%), Medium (41-60%), Heavy (61-80%), and Very Heavy (>80%). NT; not taken.

TABLE 5. Air sampling analyses from SpinCon-sampled *Stachybotrys chartarum* -contaminated indoor environments

Test Building	Sample Label	Dimensions ^a	Sampling Time and Conditions ^b	Average ELISA % Inhibition ^c	Average Trichothecene Equivalent ^d	Average Trichothecene Equivalents/m ³ of Sampled Air ^e
1	Television Room	20 x 25 x 10	120 min Static	62.5 ± 1.9	2.0 ± 0.3	36.7 ± 4.7*
			120 min Disturbed	32.4 ± 0.5	0.42 ± 0.01	7.7 ± 0.2*
			30 min Disturbed	63.5 ± 2.9	2.2 ± 0.4	159 ± 32.1*
			10 min Disturbed	74.2 ± 1.3	5.3 ± 0.7	1.18 x 10 ³ ± 1.45 x 10 ² *
	Bedroom	20 x 25 x 8	120 min Static	21.0 ± 1.9	0.28 ± 0.02	5.2 ± 0.3
			120 min Disturbed	10.2 ± 3.5	0.20 ± 0.02	3.7 ± 0.4
			30 min Disturbed	18.1 ± 2.0	0.25 ± 0.02	18.8 ± 1.3*
			10 min Disturbed	9.0 ± 4.3	0.19 ± 0.02	42.9 ± 5.1
	Kitchen	25 x 30 x 8	120 min Static	17.2 ± 3.0	0.25 ± 0.02	4.6 ± 0.4
			30 min Disturbed	17.5 ± 0.6	0.25 ± 0.00	18.3 ± 0.4*
			10 min Disturbed	30.2 ± 3.0	0.39 ± 0.04	85.9 ± 9.4
			120 min Static	50.8 ± 3.5	0.98 ± 0.17	18.1 ± 3.2*
2	Kitchen	20 x 20 x 8	120 min Disturbed	30.8 ± 3.6	0.40 ± 0.05	7.4 ± 1.0*
			30 min Disturbed	17.3 ± 4.0	0.25 ± 0.03	18.4 ± 2.4*
			120 min Static	16.8 ± 1.4	0.24 ± 0.01	4.5 ± 0.2
	Main Entry Room	20 x 25 x 8	120 min Disturbed	21.4 ± 1.4	0.28 ± 0.01	5.2 ± 0.3
			30 min Disturbed	49.7 ± 3.8	0.93 ± 0.20	68.8 ± 14.7*
			10 min Disturbed	50.4 ± 0.6	0.95 ± 0.03	210 ± 7.0*
3	Laundry Room	6 x 8 x 8	120 min Static	19.0 ± 2.1	0.26 ± 0.02	4.8 ± 0.3
			10 min Agg 1	95.9 ± 0.4	Above scale	Above scale*
			10 min Agg 2	96.0 ± 0.3	Above scale	Above scale*
			10 min Agg 3	96.1 ± 0.2	Above scale	Above scale*
			10 min Agg 1 (1:10)	59.1 ± 3.1	1.6 ± 0.3	355 ± 65.6*
			10 min Agg 2 (1:10)	69.5 ± 1.5	3.4 ± 0.5	757 ± 99.5*
			10 min Agg 3 (1:10)	75.7 ± 1.0	6.2 ± 0.7	1.37 x 10 ³ ± 1.45 x 10 ² *

4	Enclosed Living Room Area	12 x 4 x 8	30 min Agg	63.6 ± 0.9	2.1 ± 0.2	158 ± 10.8*
5	Front Bathroom	10 x 8 x 8	120 min Static	38.5 ± 2.3	0.54 ± 0.05	10.0 ± 1.0*
	Back Bathroom			39.4 ± 0.9	0.56 ± 0.02	10.3 ± 0.4*
6	Garage	15 x 25 x 8	120 min Static	38.3 ± 5.2	0.54 ± 0.12	10.1 ± 2.1*
			10 min Disturbed	26.1 ± 1.6	0.33 ± 0.02	73.6 ± 4.1
	Kitchen	15 x 20 x 8	120 min Static	31.4 ± 5.3	0.41 ± 0.08	7.6 ± 1.4*
			10 min Disturbed	24.5 ± 3.4	0.32 ± 0.04	70.1 ± 8.3
7	Hall 1	8 x 90 x 8	120 min Static	10.2 ± 2.4	0.20 ± 0.01	3.7 ± 0.3
			20 min Disturbed	7.9 ± 3.1	0.19 ± 0.02	20.7 ± 1.9
	Room 253	15 x 15 x 8	120 min Static	4.3 ± 2.2	0.17 ± 0.01	3.1 ± 0.2
			20 min Disturbed	9.6 ± 2.2	0.20 ± 0.01	21.7 ± 1.4
	Hall 2	8 x 90 x 8	120 min Static	4.5 ± 4.2	0.17 ± 0.02	3.2 ± 0.4
			20 min Disturbed	6.2 ± 0.9	0.18 ± 0.00	19.7 ± 0.5
	Room 259	15 x 15 x 8	120 min Static	15.3 ± 1.5	0.23 ± 0.01	4.3 ± 0.2
			20 min Disturbed	9.7 ± 1.6	0.20 ± 0.01	21.7 ± 1.0

^aLength x width x height in feet

^bRooms were sampled under static and/or disturbed conditions for the noted times. Air disturbance was accomplished using 20-inch box fans on a "high" setting. Disturbance was allowed for 5 minutes prior to starting the SpinCon collection. Buildings 3 and 4 were sampled during aggressive sampling and are noted ("Agg").

^cMeans ± standard deviations. Values are based on PBS alone (average of 8 separately run samples). Values represent triplicate wells.

^dIn ng/ml. Means ± standard deviations are shown. Values were derived from an ELISA-based macrocyclic trichothecene standard curve. Values represent triplicate wells.

^eIn picograms. Means ± standard deviations are shown. Values represent triplicate wells. Estimated values are based on the average trichothecene equivalents for the entire collected sample, collection time, and flow rate of the SpinCon. For example, a total of 54 m³ of air was collected for each 120 minute sample. Given a final working volume of 1 milliliter, trichothecene concentrations were then estimated from values obtained from the ELISA testing. Values determined as significantly different (P<0.05) than control environments sampled in a similar manner are noted with an *. Because 20 and 30 minute disturbed sampling was not performed in control environments, these samples were compared to environments sampled for 120 minutes under static conditions.

TABLE 6. Air sampling analyses from SpinCon-sampled control indoor environments and outdoor air

Control Area	Sample Label	Dimensions ^a	Sampling Time and Conditions ^b	Average ELISA % Inhibition ^c	Average Trichothecene Equivalent ^d	Average Trichothecene Equivalents/m ³ of Sampled Air ^e
Building 1	Room 1	15 x 20 x 10	120 min Static	21.5 ± 2.6	0.28 ± 0.02	5.3 ± 0.5
	Room 2 (Trial 1)	30 x 20 x 10		1.7 ± 1.2	0.16 ± 0.01	2.9 ± 0.1
	Room 2 (Trial 2)			5.2 ± 3.2	0.17 ± 0.02	3.2 ± 0.3
	Room 2 (Trial 3)			1.8 ± 3.1	0.15 ± 0.02	2.8 ± 0.4
	Room 2 (Trial 4)			18.4 ± 2.0	0.26 ± 0.02	4.7 ± 0.3
	Room 2 (Trial 5)			0.14 ± 0.24	0.14 ± 0.01	2.6 ± 0.2
	Room 3	20 x 20 x 10		20.0 ± 1.8	0.27 ± 0.02	5.0 ± 0.3
	Room 4 (Trial 1)	20 x 20 x 10		6.6 ± 4.4	0.18 ± 0.02	3.3 ± 0.4
	Room 4 (Trial 2)			13.9 ± 1.6	0.22 ± 0.01	4.1 ± 0.2
	Room 4 (Trial 3)			12.9 ± 1.8	0.21 ± 0.02	4.0 ± 0.2
	Room 4 (Trial 4)			4.6 ± 2.6	0.17 ± 0.01	3.1 ± 0.2
	Room 4 (Trial 5)			10.8 ± 3.2	0.20 ± 0.02	3.8 ± 0.4
	Room 4 (Trial 6)			0.0 ± 0.0	0.12 ± 0.02	2.2 ± 0.4
Room 5	25 x 20 x 10	12.4 ± 1.9	0.21 ± 0.01	3.9 ± 0.2		
Building 2	Bedroom	15 x 12 x 8		11.1 ± 1.8	0.20 ± 0.01	3.8 ± 0.2
	Kitchen	25 x 15 x 10	120 min Static	17.8 ± 3.3	0.25 ± 0.03	4.7 ± 0.5
	Computer Room	20 x 20 x 8		13.2 ± 4.7	0.22 ± 0.03	4.1 ± 0.5
Building 3	Computer Room 1	15 x 12 x 8	120 min Static	17.5 ± 0.5	0.25 ± 0.00	4.6 ± 0.1
			10 min Disturbed	4.3 ± 3.8	0.16 ± 0.03	36.1 ± 6.2
	Computer Room 2	15 x 12 x 8	120 min Static	19.7 ± 12.6	0.29 ± 0.13	5.4 ± 2.4
			10 min Disturbed	23.8 ± 15.3	0.34 ± 0.15	75.8 ± 32.8
	Bedroom	20 x 20 x 8	120 min Static	13.1 ± 2.1	0.22 ± 0.01	4.0 ± 0.3
		10 min Disturbed	14.2 ± 3.4	0.23 ± 0.02	49.9 ± 5.2	

Building 4	Bedroom 1 (Trial 1)	15 x 15 x 8	120 min Static	16.3 ± 6.5	0.25 ± 0.05	4.5 ± 0.9
	Bedroom 1 (Trial 2)		120 min Static	33.0 ± 3.2	0.43 ± 0.05	8.0 ± 1.0
	Bedroom 2	15 x 15 x 8	10 min Disturbed	36.7 ± 9.7	0.54 ± 0.24	120 ± 54.4
			120 min Static	59.5 ± 2.0	1.6 ± 0.2	30.0 ± 3.7
	Bedroom 3	20 x 25 x 8	10 min Disturbed	27.4 ± 4.2	0.35 ± 0.05	78.0 ± 11.4
			120 min Static	47.9 ± 10.5	0.92 ± 0.39	17.1 ± 7.2
			10 min Disturbed	35.8 ± 3.2	0.48 ± 0.06	107 ± 13.7
Outside air	-	-	30 minutes			
			60 minutes	0.0	0.0	0.0
			90 minutes			
			120 minutes			

^aLength x width x height in feet

^bRooms were sampled under static and/or disturbed conditions for the noted times. Air disturbance was accomplished using 20-inch box fans on a "high" setting. Disturbance was allowed for 5 minutes prior to starting the SpinCon collection.

^cMeans ± standard deviations. Values are based on PBS alone (average of 8 separately run samples). Values represent triplicate wells.

^dIn ng/ml. Means ± standard deviations are shown. Values were derived from an ELISA-based macrocyclic trichothecene standard curve. Values represent triplicate wells.

^eIn picograms. Means ± standard deviations are shown. Values represent triplicate wells. Estimated values are based on the average trichothecene equivalents for the entire collected sample, collection time, and flow rate of the SpinCon. For example, a total of 54 m³ of air was collected for each 120 minute sample. Given a final working volume of 1 milliliter, trichothecene concentrations were then estimated from values obtained from the ELISA testing.

TABLE 7. Air sampling analyses from Andersen PUF-sampled test and control indoor environments

Building and Room	Dimensions ^a	Filter Pore Size ^b	Sampling Time	Average ELISA % Inhibition ^c	Average Trichothecene Equivalent ^d	Average Trichothecene Equivalents/m ³ of Sampled Air ^e
Test Building 8 - Garage Storage Closet	3 x 6 x 8	2.7	24 hours	79.2 ± 1.6	1.5 ± 0.5	62.6. ± 12.3
		<0.3		40.1 ± 6.3	0.62 ± 0.04	6.9 ± 2.1
		2.7	48 hours	72.0 ± 1.5	6.2 ± 0.9	14.3 ± 2.0
		<0.3		23.9 ± 3.1	0.77 ± 0.09	1.8 ± 0.2
		2.7	72 hours	80.7 ± 0.4	16.3 ± 0.9	25.1 ± 1.4
		<0.3		52.4 ± 2.5	2.6 ± 0.4	4.1 ± 0.6
Control Building 1 - Room 4	20 x 10 x 10	2.7	24 hours	21.7 ± 1.5	0.41 ± 0.02	1.1 ± 0.1
		<0.3	24 hours	0.0	0.0	0.0

^aLength x width x height in feet

^bIn µm

^cMeans ± standard deviations. Values are based on PBS alone (average of 8 separately run samples). Values represent triplicate wells.

^dIn ng/ml. Means ± standard deviations are shown. Values were derived from an ELISA-based macrocyclic trichothecene standard curve. Values represent triplicate wells.

^eIn picograms. Means ± standard deviations are shown. Values represent triplicate wells. Estimated values are based on the average trichothecene equivalents for the entire collected sample, collection time, and flow rate of the SpinCon. For example, a total of 216 m³ of air was collected for each 24-hour sample. Given a final working volume of 1 milliliter, trichothecene concentrations were then estimated from values obtained from the ELISA testing.

TABLE 8. Competitive ELISA inhibition of commonly isolated indoor fungi and allergens

Sample ^a	Concentration ^b	Average % Inhibition ^c
<i>Stachybotrys chartarum</i> (ATCC 201212)	5 x 10 ⁵ conidia/ml	93.6 ± 0.6*
	5 x 10 ⁴ conidia/ml	92.5 ± 0.2*
	5 x 10 ³ conidia/ml	83.7 ± 1.3*
<i>Stachybotrys chartarum</i> (IBT 9633)	1 x 10 ⁶ conidia/ml	9.17 ± 2.5*
	1 x 10 ⁵ conidia/ml	0.0
	1 x 10 ⁴ conidia/ml	0.0
<i>Stachybotrys chartarum</i> (IBT 9757)	1 x 10 ⁶ conidia/ml	0.0
	1 x 10 ⁵ conidia/ml	0.0
	1 x 10 ⁴ conidia/ml	0.0
<i>Stachybotrys chartarum</i> (IBT 9293)	1 x 10 ⁶ conidia/ml	0.0
	1 x 10 ⁵ conidia/ml	0.0
	1 x 10 ⁴ conidia/ml	0.0
<i>Stachybotrys chartarum</i> (IBT 9290)	1 x 10 ⁶ conidia/ml	0.0
	1 x 10 ⁵ conidia/ml	0.0
	1 x 10 ⁴ conidia/ml	0.0
<i>Alternaria alternata</i>	7 x 10 ⁵ conidia/ml	0.0
	7 x 10 ⁴ conidia/ml	0.0
	7 x 10 ³ conidia/ml	0.0
<i>Aspergillus niger</i> (ATCC 10575)	4.7 x 10 ⁶ conidia/ml	0.0
	4.7 x 10 ⁵ conidia/ml	0.0
	4.7 x 10 ⁴ conidia/ml	0.0
<i>Chaetomium globosum</i> (ATCC 16021)	1.6 x 10 ⁶ conidia/ml	0.0
	1.6 x 10 ⁵ conidia/ml	0.0
	1.6 x 10 ⁴ conidia/ml	0.0
<i>Cladosporium cladosporioides</i>	1.2 x 10 ⁶ conidia/ml	0.0
	1.2 x 10 ⁵ conidia/ml	0.0
	1.2 x 10 ⁴ conidia/ml	0.0
<i>Fusarium sporotrichioides</i> (ATCC 24630)	1 x 10 ⁶ conidia/ml	0.0
	1 x 10 ⁵ conidia/ml	0.0

	1 x 10 ⁴ conidia/ml	0.0
<i>Memmoniella echinata</i> (ATCC 11973)	7 x 10 ⁵ conidia/ml	0.0
	7 x 10 ⁴ conidia/ml	0.0
	7 x 10 ³ conidia/ml	0.0
<i>Penicillium chrysogenum</i>	4.7 x 10 ⁶ conidia/ml	0.0
	4.7 x 10 ⁵ conidia/ml	0.0
	4.7 x 10 ⁴ conidia/ml	0.0
<i>Rhizopus</i> sp.	1 x 10 ⁶ conidia/ml	0.0
	1 x 10 ⁵ conidia/ml	0.0
	1 x 10 ⁴ conidia/ml	0.0
<i>Trichoderma viride</i>	1 x 10 ⁶ conidia/ml	0.0
	1 x 10 ⁵ conidia/ml	0.0
	1 x 10 ⁴ conidia/ml	0.0
Can f 1	50 ng/ml	0.0
	5 ng/ml	0.0
	500 pg/ml	0.0
Der p 1	50 ng/ml	0.0
	5 ng/ml	0.0
	500 pg/ml	0.0
Fel d 1	50 ng/ml	0.0
	5 ng/ml	0.0
	500 pg/ml	0.0

^aCultures were grown in 90 mm petri dishes on potato dextrose agar at 25°C. Strain numbers for purchased fungi are noted. All other fungal samples were obtained from outside sources and identified microscopically in our laboratory.

^bFungal conidia were collected from the surfaces of 14-day-old cultures using sterile cotton swabs. The cotton tips of the swabs were placed in 1 ml of sterile pyrogen-free water and vortexed to remove conidia. Conidia were counted using a hemacytometer and diluted in PBS (pH 7.4) to concentrations similar to what was observed in tested buildings (Table 2). Purified allergens (in 50% glycerol) were diluted in phosphate buffered saline to the mentioned concentrations.

^cMeans ± standard deviations are shown. Negative inhibitions were converted to 0.0% inhibition. Values represent the average of duplicate wells performed on two separate occasions. Values noted with an * were statistically different (P<0.05) than PBS alone as determined using an one-way ANOVA.