EVALUATION OF *STACHYBOTRYS CHARTARUM* IN THE HOUSE OF AN INFANT WITH PULMONARY HEMORRHAGE: QUANTITATIVE ASSESSMENT BEFORE, DURING, AND AFTER REMEDIATION

STEPHEN VESPER, PHD, DORR G. DEARBORN, MD, PHD, IWONA YIKE, PHD, TERRY ALLAN, MPH, JOHN SOBOLEWSKI, BS, SIMON F. HINKLEY, PHD, BRUCE B. JARVIS, PHD, AND RICHARD A. HAUGLAND, PHD

ABSTRACT *Stachybotrys chartarum* is an indoor mold that has been associated with pulmonary hemorrhage cases in the Cleveland, Ohio, area. This study applied two new quantitative measurements to air samples from a home in which an infant developed PH. Quantitative polymerase chain reaction and a protein synthesis inhibition assay were used to determine the level of *S. chartarum* spores and their toxicity in air samples taken before, during, and after a remediation program was implemented to remove the fungus. Initial spore concentrations were between 0.1 and 9.3 spores/m^3 of air, and the toxicity of air particulates was correspondingly low. However, the dust in the house contained between 0.4 and 2.1×10^3 spores/mg (as determined by hemocytometer counts). The remediation program removed all contaminated wallboard, paneling, and carpeting in the water-damaged areas of the home. In addition, a sodium hypochlorite solution was used to spray all surfaces during remediation. Although spore counts and toxicity were high during remediation, air samples taken postremediation showed no detectable levels of *S. chartarum* or related toxicity. Nine isolates of *S. chartarum* obtained from the home were analyzed for spore toxicity, hemolytic activity, and random amplified polymorphic DNA banding patterns. None of the isolates produced highly toxic spores (>90 μg T2 toxin equivalents

Drs. Vesper and Haugland are from the US Environmental Protection Agency, National Exposure Research Laboratory; Drs. Dearborn and Yike are from the Department of Pediatrics, Rainbow Babies and Children Hospital, Case Western Reserve University. Mr. Allan and Mr. Sobolewski are from the Cuyahoga County Board of Health, Cleveland; Drs. Hinkley and Jarvis are from the Department of Chemistry and Biochemistry, Joint Institute for Food Safety and Applied Nutrition (JIFSAN), University of Maryland.

Correspondence: Steve Vesper, PhD, US EPA, 26 West Martin Luther King Drive, ML 314, Cincinnati, OH 45268. (E-mail: Vesper.Stephen@EPA.gov)

per gram wet weight spores) after growth for 10 and 30 days on wet wallboard, but three isolates were hemolytic consistently. DNA banding patterns suggested that at least one of these isolates was related to isolates from homes of infants with previously investigated cases.

KEY WORDS Mold, Pulmonary Hemorrhage, Remediation, *Stachybotrys*.

INTRODUCTION

Pulmonary hemorrhage (PH) is usually a rare disorder in infants.[1] However, some infants living in water-damaged homes in Cleveland, Ohio, developed unexplained PH.[2] An investigation by the Centers for Disease Control and Prevention (CDC) concluded that *Stachybotrys chartarum* likely was connected to this disease.[3–5] *S. chartarum* (Ehrenb. ex Link) Hughes (= *S. atra* Corda) is a toxigenic fungus that grows on wet, cellulose-based products such as paper, cardboard, and wallboard[6]; in addition to PH, it has been associated with other human health problems.[7–10] Recently, it was demonstrated that *S. chartarum* can grow and produce a hemolytic agent at 37°C, suggesting that, under some circumstances, this fungus may be an opportunistic pathogen.[11]

Since 1993, there have been more than 40 PH cases in the Cleveland metropolitan area, with the majority of cases clustered in a small geographic area. These cases have resulted in a 30% mortality rate.[12] For a 3-year period (1993–1995), 12% of all sudden infant death syndrome deaths in the cluster area were actually PH deaths.[12] The PH problem is not limited to Cleveland. In an informal survey, more than 140 cases of PH have been reported in infants across the US during the past 5 years.[13]

In this study, the home of an infant with PH was investigated extensively. The air in the home was monitored for *S. chartarum* spores and toxicity before, during, and after remediation to remove mold-infested wallboard and other building materials. This is the first report of the parallel use of quantitative polymerase chain reaction (PCR) for identification and enumeration of *S. chartarum*[14] and a quantitative protein translation assay for trichothecene toxicity.[15] In addition, analyses for several mycotoxins were performed on mold samples taken from the home. Cultures of nine *S. chartarum* isolates from the home were tested for hemolytic activity, trichothecene toxicity, and genetic relatedness.

INFANT AND HOME DESCRIPTION

Pulmonary Hemorrhage Case History and Treatment

In November 1998, a 2-month-old African-American male infant was transferred to the intensive care unit of Rainbow Babies and Children's Hospital, Cleveland,

because of acute respiratory failure related to PH. He had suffered from respiratory congestion and coughing for the previous 3 weeks, corresponding to the time he had been exposed to an open ceiling, where plumbing repairs were being performed, about 3 m from his crib. He was not being breast fed, and there was environmental tobacco smoke in the home. He did not have any noted seizure activity, infection, or hemoglobinuria, which have been seen in some of the other infants suffering PH in Cleveland.[13] Objective evaluation for evidence of abuse or other causes of alveolar hemorrhage[12] were negative. Prussian blue staining of alveolar macrophages obtained by bronchoalveolar lavage 40 days after the PH indicated only trace continued alveolar bleeding (iron index = 10/300; for this index, 100 alveolar macrophages are scored 0 to 3 for iron staining, for a maximum score of 300). Since his PH, he has not returned to the original house or been exposed to environmental tobacco smoke. Other than having viral bronchiolitis and related reactive airways disease, he has remained in good health. Because this infant fits the profile of a typical PH case[5], the house was investigated for mold contamination.

House Description

This infant lived in a three-story, wood-frame colonial house built in 1921. The layout of the basement and first floor is shown in Figure 1. The house has balloon-

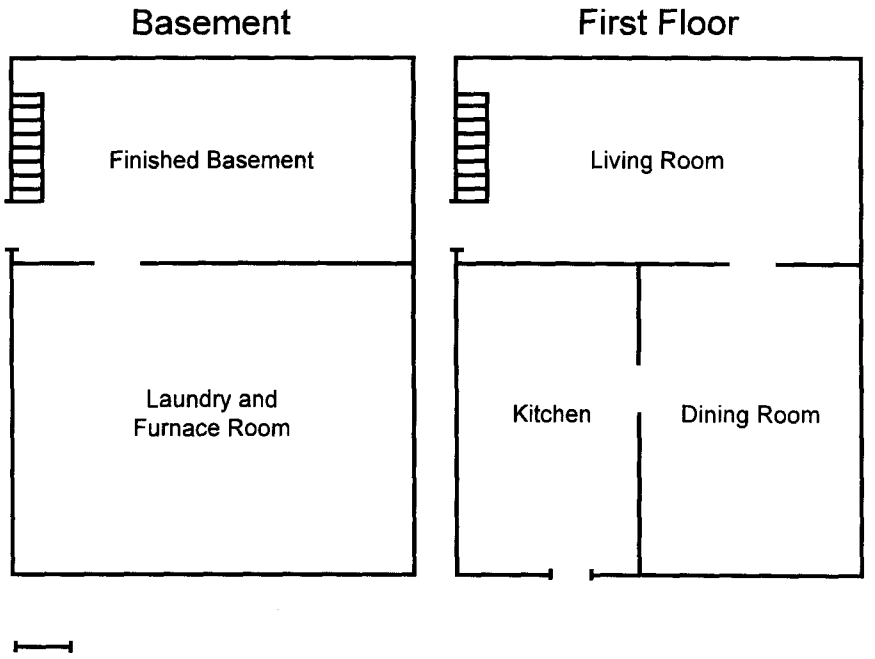

FIGURE 1 Floor plan of basement and first floor for subject house. Scale bar is 1 m.

frame construction on a red clay tile foundation. The entire home is approximately 400 m^2. The property is elevated slightly with respect to the street. Storm and sanitary drainage is to the front of the property.

After construction, modifications included the following: exterior aluminum siding, blown-in fiberglass insulation, and the "finishing of the basement walls," which occurred in the 1970s. The finishing of the walls consisted of installing wood paneling on top of wallboard over wood furring strips directly on the lower exterior foundation walls on the southern half of the basement. A similarly constructed wood-framed dividing wall was built between the laundry/furnace room and the finished south side of the basement.

The heating system consisted of a "Cleveland drop" forced-air furnace configuration, which to our knowledge, has been observed only in northeast Ohio. All return air was drawn directly from the basement space. Cold air from the dining and living rooms was returned to the basement space primarily through cold air returns that not connected directly to the furnace. To a lesser degree, air may also be returned to the basement via stairways and wall cavities. The open cold air returns were located on the west and south sides of the house. The furnace was installed within the past 10 years and is 80% efficient, but it was being operated with no filter. The cold air return located on the south side of the basement was enclosed in the finished wall.

Home Investigation

On receiving the referral from Rainbow Baby and Children's Hospital PH Prevention Program, a preliminary mold and moisture investigation was conducted on December 1, 1998. The investigation revealed a water-damaged ceiling in the northwest corner of the living room. The ceiling consisted of cellulose ceiling tiles installed directly over the existing plaster and lath. This water damage was determined to be the result of leakage from the second floor bathroom located directly overhead.

The full extent of water problems and mold growth in this home were not apparent until demolition began. The water sources included exterior water infiltration through faulty footer drains and downspouts; leakage through at-grade basement windows; backup of a laundry drain located behind the finished wall; missing structural elements in the foundation wall; and leakage from the sanitary stack through the vertical length of the house.

A follow-up investigation was conducted on December 9, 1998, to perform further environmental sampling. In addition to water damage in the living room, water damage was also present on the paneling in the southeast corner of the

basement and on the framed paneled wall dividing the basement space. The reverse side of the wallboard on the divider was covered by an estimated 15 m^2 of the black mold. The extent of mold growth triggered the development a remediation program.

Remediation and Repair

Remediation began January 19, 1999. The agency in charge of designing the remediation specifications utilized the 1993 "New York City guidelines"[16] as the guidance document for formulation of the remediation plan. The furnace blower was turned off. A prefilter was placed on the forced-air furnace, and a high-efficiency particulate arrester (HEPA) air filtering device (HAFD), which created negative pressure in the basement space, was placed on the north unfinished side of the basement. The air was exhausted out the north window. A partial visquine door was placed in the kitchen doorway that leads to the basement steps. The basement finished walls were demolished. The center dividing wall was removed first, followed by the ceiling, west wall, south wall, and east wall. The walls consisted of paneling over wallboard. All wall surfaces were sprayed with a pressure sprayer, which applied a sodium hypochlorite solution of approximately 3,000 ppm, used to minimize dust and possibly kill microorganisms. Workers wore full Tyvek suits with half-face negative-pressure respirators mounted with piggyback HEPA and organic vapor cartridges. To monitor air for fungal spores in the breathing zone of the worker, a personal monitoring pump (PMP) was worn by one of the workers for the duration of the project. All waste material was contained in 6-mil plastic bags and disposed in a solid waste landfill.

At this time, the full extent of mold contamination became visible in the basement. Approximately 70 m^2 of mold on cellulose materials were present on the reverse side of the paneling and on both sides of the wallboard, as well as on the wood framing.

On January 20, 1999, remediation/demolition continued. Based on the extent of mold contamination in the basement (70 m^2), the same engineering controls were used during the entire remediation. A critical barrier was placed at the head of the stairway to the second floor bedrooms. Another critical barrier was constructed on the south side of the dining room south of the doorway to the kitchen. Air was drawn via an HAFD through this wall and exhausted out the north dining room window. Remediation work consisted of removing the ceiling from the living room and part of the dining room and removing the north, south, and east walls from the living room. The interior walls on both sides of the

archway from the living room to the dining room were removed. The walls were removed down to the studs. The living and dining room floors were covered by sheets of plywood and covered with plastic sheeting to protect the carpet from contamination.

On January 21, 1999, the critical barrier to the second floor was removed. The entryways to the bedrooms and the carpeted stairs were sealed with visquine. The bathroom was gutted by removing the walls, floor, and ceiling. On January 22, 1999, the west wall of the living room was removed. At this time, the decision was made to remove all the carpeting from the house. The basement walls were repaired and spray painted. The stairs to the basement were removed. On April 6, 1999, all rehabilitation work had been completed except for modifying the cold air returns. The cold air returns were modified to eliminate the Cleveland drop so that return air no longer is pulled directly from the basement.

The following actions were instituted to address water problems in the home. The existing basement windows were replaced with glass block, the missing clay tile block and mortar in the basement walls were replaced, and the sanitary stack leakage was also repaired. In addition, several water problems will be addressed prospectively. The faulty downspout may be removed from the footer drains and redirected as applicable under existing local building code, and the area where the driveway abuts the foundation wall will be sealed. The need for full waterproofing will be assessed. Other potential water sources will also be monitored, including occupant moisture sources, plumbing sources, and other water infiltration points. The basement has been rendered free of cellulose-based products and left unfinished to minimize occupant use.

Remediation costs (excluding rehabilitation) were $5,210 for removal of all mold-contaminated material from the house. This cost does not include the drafting of job specifications and oversight of contractors, which were performed by government agencies. These costs do not include any sampling or analysis.

MATERIALS AND METHODS

Air and Dust Sampling and Spore Quantification

Air samples (Table I) were taken in two ways, using either a cassette filter (37 mm with 0.8-μm filter) or a BioSampler (SKC, Eighty Four, PA) connected to an AirCon-2 high flow sampler pump (Gilian Instrument Co., Clearwater, FL) calibrated at a flow rate of 10 L/min. These samples were taken for a period between 6 and 90 hours at 10 L/min. During the remediation process itself, one worker wore a PMP for about 6 hours a day; the PMP also used a cassette filter (37 mm with 0.8-μm filter). Particulates were recovered from the filters and

TABLE 1 Results of Air Sampling for *Stachybotrys chartarum* (S.c.) Spores

Date	Sample Method	Location	Sampling Time, hours	Flow Rate, L/min	S.c. Spores, no./m^3 air	Toxicity Equivalents, ng T-2/m^3 air
Preremediation						
12/29–12/30	Filter (passive)*	Living room	25.5	10	0.2	0.5
	BioSampler (passive)	Living room	25.5	10	0.3	
12/30–12/31	Filter (active)†	Living room	24	10	9.3	1.8
	BioSampler (active)	Living room	24	10	5.0	
12/31	Filter (active)	Dining room	90	10	0.1	0.5
	BioSampler (active)	Dining room	90	10	1.7	
12/31–1/4	Filter (active)	Basement	90	10	0.6	2.2
During remediation‡						
1/19	Filter	Basement	6.6	6	1.1×10^3	75.1
	BioSampler	Basement	6.6	6	1.6×10^3	
	Filter PMP§	Basement	6.5	2.6	2.0×10^3	86.4
1/20	Filter	Dining room	6.25	10	1.8×10^3	71.0
	BioSampler	Dining room	6.25	10	2.7×10^3	
	Filter PMP	Dining room	5.75	2.9	4.0×10^3	85.4
1/21	Filter	N. bedroom	7.75	10	0.1×10^3	163.8
	BioSampler	N. bedroom	7.75	10	0.3×10^3	
	Filter PMP	N. bedroom	5.75	2.9	1.1×10^3	65.6
1/22	Filter	Dining room	8	10	0.1×10^3	27.2
	BioSampler	Dining room	8	10	0.8×10^4	
	Filter PMP	Dining room	8	3.3	6.3×10^1	67.4
Postremediation‖						
1/28	Filter	Basement	8	10	0.2	4.2
	BioSampler	Dining room	8	10	0	
	Filter	Dining room	8	10		0.3
4/6	BioSampler	Basement	8	10	0	
	Filter	Basement	8	10		8.2
	BioSampler	Dining room	15.75	10	0	
	Filter	Dining room	15.75	10		0.5

*"Passive" means furnace blower off, furnace sealed and inoperable.
†"Active" means furnace blower on, furnace operable.
‡Furnace sealed and inoperable during remediation.
§PMP, personal monitoring pump.
‖Furnace unsealed but inoperable.

BioSamplers as previously described.[14] Quantification of *S. chartarum* spores in the collected particulates was performed using an Applied Biosystems 7700 sequence detector with an internal standard as previously described.[14]

Dust samples were taken from the carpet in the dining room (near the infant's crib) and from the basement floor (near the downspout leak). A 1-m^2 area was

vacuumed with the filter apparatus connected to the vacuum pump described above. The samples were returned to the laboratory, and 10-mg aliquots were added to 1 mL of sterile water. The spores were suspended by mixing, and 10-μL aliquots were counted in a hemocytometer.

Two direct vacuum samples for mycotoxin analysis were obtained from about a section of mold-contaminated basement wallboard about 0.2 × 0.7 m using an open-face 37-mm 0.8-μm polycarbonate filter connected to the Gillian AirCon-2 unit.

TOXICITY OF *STACHYBOTRYS CHARTARUM*

The air particulate samples and isolates of *S. chartarum* were evaluated for their toxicity by the method of Yike et al.[15] This method uses luciferase translation in a mammalian cell-free system to assess protein synthesis inhibition, an activity characteristic of trichothecene mycotoxins. Air particulates collected on cassette filters were extracted with 95% ethanol, and the extracts were assayed for trichothecene toxicity by directly comparing the toxicity of field samples to a parallel assay of the trichothecene T-2 toxin. The results are expressed as toxin equivalents per cubic meter of air by matching the 50% inhibition points of the experimental extracts and the T-2 toxin dose-response curves. The amounts of T-2 toxin causing 50% inhibition were equated to the volume of extracts (microliters) causing 50% inhibition in the luciferase translation. The volume of extract then was converted to the volume of air sampled (cubic meters), and toxin equivalents per cubic meter were calculated.

Isolates were grown on wallboard as described below. After 10 and 30 days of growth on wallboard, spores from each of the isolates were blotted directly onto 9-mm sterile borosilicate filters (Millipore, Bedford, MA). The wet weight of the spores on the filters was determined, and the filters and spores were then freeze-dried and held at 4°C prior to performing toxicity assays.

ISOLATION OF *STACHYBOTRYS CHARTARUM* CULTURES

Samples from wallboard and paneling were taken from the house and returned in plastic bags to the laboratory. Spores were removed from the samples and placed on 5 × 5 cm pieces of sterile wallboard 9.4 mm (3/8 inch) thick; these were placed in 100 × 15 mm petri dishes containing 20 mL of sterile water and incubated at 23°C. From these samples, four culture isolates were obtained. In addition, the 3.3-m wallboard wall in the basement dividing the laundry and recreation rooms was sampled at about 0.5-m intervals along its length. Spores were collected from the five pieces of wallboard, and one culture isolate was obtained, as described above, from each.

Toxin Analysis

Two filter samples were obtained by directly vacuuming about 0.5 m^2 of moldy wallboard in the basement. The filter and spores were discharged into a screw-top vial, MeOH : $CHCl_3$ (1 : 1, 6 mL) was added, and the mixture was treated by ultrasonic agitation (30 minutes), then left to stand at 4°C overnight. The resultant suspension was filtered, and solvent was removed by rotary evaporation to give a crude extract. A filtration tube (6 mL) was charged with polyethyleneimine silica gel (2 g)[17] and washed with CH_2Cl_2 (3 mL). The crude extract was applied to the cleanup column using two 500-μL portions of CH_2Cl_2. The column was eluted with CH_2Cl_2 (15 mL) to yield the fraction rich in dolabellanes or atranones; a second elution with MeOH (15 mL) produced the spirodrimane-containing fraction.

High-performance liquid chromatography analysis was completed on a Hewlett Packard HP-1100 series instrument with ultraviolet diode array detection (200–400 nm) and with chromatograms quantified at 260 nm. The columns were as follows: the guard column was 15 × 1 mm hand-packed with Rainin C-18, 8 μm (PK-201-H); the main column was 150 × 2 mm Phenomenex sphereclone 3 mm, (OOF-4135-BO). There was a column heater with solvent preheating (40°C) and solvent degassing. Solvents A (water) and B (acetonitrile) each had 0.1% formic acid (88% Fisher). The solvent program was a ramp from 25% to 80% solvent B from 0 to 30 minutes, then ramp to 100% solvent B from 30 to 31 minutes, and maintain 100% until 40 minutes; for the postrun, ramp down to 25% solvent B (over 2 minutes), then 15 minutes column re-equilibration. The flow rate was 200 μL/min; maximum operating pressure was 165 bar (running 25% solvent B). Injection was sample filtration through 0.2-mm polytetrafluoroethylene filters (Phenomenex, 13 mm); automated injection was 5 μL sample volume.

Hemolytic Test of *Stachybotrys chartarum* Isolates on Sheep Blood Agar

Spores of each isolate were inoculated onto and grown on 5 × 5 cm pieces of sterile wallboard 9.4 mm (3/8 inch) thick; these were placed in 100 × 5 mm petri dishes containing 20 mL sterile water and incubated at 23°C. More sterile water was added to the petri dishes after incubation for 4 weeks so that there was always free water around the wallboard pieces during the 8-week incubation.

Every 7 days for 8 weeks, spores from each isolate were recovered with a sterile cotton swab from each wallboard piece. The spores were transferred to plates of 5% sheep blood agar (Becton Dickinson, Sparks, MD) and incubated at 37°C. After 10 days of incubation, the plates were checked for hemolytic activity,

which is the lysis of red blood cells. Clearing of the medium beyond the edge of the fungal growth was considered an indication of hemolytic activity (hemolysis positive). Many microbial pathogens produce hemolysins.

DNA Extraction and Random Amplified Polymorphic DNA Analysis of *Stachybotrys chartarum* Isolates

Each of the nine isolates was grown on potato dextrose agar (Becton Dickinson) for 7 days at 23°C. Spores were collected from each isolate using a sterile cotton swab and were resuspended in sterile water to give a final concentration of 1×10^7 spores per milliliter (as determined by hemocytometer counts). The DNA of each isolate was extracted using a bead beating method.[18]

The recovered DNA was amplified randomly using the R28 primer (5'-ATG-GATCCGC) and PCR protocol described by Fujimori and Okuda.[19] The PCR products were separated on a 0.7% agarose gel. The gels were run at 100 V for 6 hours and stained for 40 minutes with SYBR™ Green (FMC, Rockland, ME) at a concentration of 10 µL per 100 mL of water. The gels were imaged with the FluorImager 595 (Molecular Dynamics, Sunnyvale, CA), and the molecular weight of each band was determined using the associated image analysis program, Fragment NT™. Molecular weight standards consisting of 2,000, 1,200, 800, 400, 200, and 100 base pair fragments (Life Technologies, Grand Island, NY) were run in triplicate on each gel.

Random amplified polymorphic DNA (RAPD) analysis of the DNA of each isolate was replicated three times. Only bands that appeared in all the analyses were considered positive for that isolate. The results were assembled in a 1-0 matrix depending on whether a band was present (1) or absent (0), as described by Fujimori and Okuda.[19] Phylogenetic relationships of the isolates and isolate distances were inferred from these data using the branch-and-bond option of the Phylogenetic Analysis Using Parsimony (PAUP) Program (version 3.1, Sinauer Associates, Sunderland, MA). A distance analysis was performed on each isolate to determine which was the least similar. This isolate was used as the out group in the phylogenetic evaluation. The PAUP bootstrap search option (1,000 times replication) using the branch-and-bound method was used to estimate the distance between isolates and the levels of support for the branches of the most parsimonious trees.

RESULTS AND DISCUSSION

Results of air sampling with either filters or BioSamplers indicated that the number of airborne *S. chartarum* spores in this house with a resident with PH was low before the remediation began (Table I). The number of *S. chartarum*

spores in the air when the furnace blower was activated (typical condition for the winter months) increased by a factor of 17–47 in the living room. Dust samples collected from the carpeting before remediation began were found to contain up to 2,000 *S. chartarum* spores/milligram dust. This very high number of spores in the dust is comparable to the number of spores found directly on moldy substrates in homes with PH cases described earlier.[4] During demolition, the number of *S. chartarum* spores in the air increased by four orders of magnitude in the basement, about three orders of magnitude in the dining room (where the crib had been located), and about two orders of magnitude in the upstairs bedroom (Table I). Thirty days after demolition, remodeling, and cleaning, airborne *S. chartarum* spores in the house were nondetectable (Table I). Thus, the procedures used in the remediation of this home appear to have been effective in eliminating the *S. chartarum* spores.

A primary pathway for the potential movement of *S. chartarum* spores from contaminated basement areas appears to be the aforementioned Cleveland drop furnace design. The absence of this design does not exclude the possibility of at-risk individuals experiencing negative health effects from living in mold-contaminated housing. The disposition of the home observed in this case stresses the need for critical evaluation of finished basement spaces in homes of at-risk people. In fact, it is the opinion of the authors that basements with chronic moisture problems should not be finished and/or utilized as part of the main living space because of the risk for adverse health effects. The movement of air from contaminated spaces into the main habitable areas should also be evaluated.

The very high number of *S. chartarum* spores observed in the PMP samples taken from the breathing zone of the worker (Table I) suggests that residents should not attempt repairs without the proper protection or preferably should employ a contractor trained in environmental remediation. Farmworkers and others who handle moldy feed have had acute respiratory and systemic toxic effects that must be considered in preparing for remediation. Obviously, any infant should be removed from the home before any disturbance of contaminated materials occurs. Perhaps active cleaning and vacuuming may be sufficient to keep the spore numbers below significant levels, but this remains to be seen. However, repair and remediation, as described in this study, may be the best protection for infants, who appear to be more vulnerable than even older children.

It is unclear whether sodium hypochlorite solution is necessary during remediation. In this particular case, perhaps a water-and-surfactant solution would have been adequate for dust control since all cellulose components were removed during demolition and negative pressure was maintained at all times. When

component remediation is being performed on smaller areas of contamination, a sodium hypochlorite solution may be more appropriate, as described in the New York City guidelines.[16] In either case, a proper respiratory protection program should be in place (29 CFR 1910.134) to ensure worker safety.

The number of spores and the trichothecene toxicity of the air samples were low both before and after remediation. The toxicity values ranged from 0.3 to 8.2 ng T-2 toxin equivalents per cubic meter, resembling those reported earlier[15] for nine fungal-contaminated houses from the Cleveland area. During remediation, the toxicity of air samples increased by two orders of magnitude. The nine cultured isolates of *S. chartarum* from this house also had low toxicity. However, they varied substantially in toxicity (Table II), which may explain why the increases in toxicity do not remain in the same range as the increase in spore counts.

While direct vacuum samples from the contaminated wallboard in the basement contained several mycotoxins, the trichothecene content was low. The two samples (I and II) obtained by vacuuming the mold-covered wallboard directly were extracted with organic solvent and partitioned on a small polyetherimide silica-gel column. High-performance liquid chromatographic analysis of the less polar column fractions revealed the presence of the dolabellane 6β-hydroxydolabella-3*E*, 8*E*, 12-trien-14-one in both filters (sample I, 2.4 μg; sample II, 2.6 μg). Sample II also contained atranone B (1.5 μg) and atranone C (3.2 μg).[20] The more polar fractions contained significant levels of the spirodrimane metabolites.[21,22] Both samples I and II had almost identical chromatograms, except that the levels

TABLE II Toxicity of *Stachybotrys chartarum* Strains Grown at 23°C on Wet Wallboard

	Toxicity equivalents (μg T-2 toxin/g wet weight spores) After Incubation	
Strain	10 days	30 days
DDH1	0	10.77
DDH2	0.8	1.39
DDH3	0	6.64
DDH4	0.85	0.98
DDH5	1.53	3.24
DH1	0	2.5
DH2	0	9.3
DH3	0	18.2
DH4	0	1.89

TABLE III Matrix Showing the Presence (1) or Absence (0) of the 26 Bands Produced by Random Amplified Polymorphic DNA Analysis of the Strains of *Stachybotrys chartarum*

	BANDS											
	1 2026– 1970	2 1923– 1889	3 1818– 1750	4 1708– 1657	5 1601– 1592	6 1553– 1517	7 1509– 1451	8 1438– 1416	9 1399– 1367	10 1357– 1347	11 1300– 1251	12 1211– 1153
DDH1	0	1	0	1	0	0	1	0	0	1	0	1
DDH2	1	1	0	1	0	0	1	0	0	1	0	1
DDH3	1	1	0	1	0	1	0	0	0	1	1	1
DDH4	1	1	0	1	0	0	1	0	0	1	1	1
DDH5	1	1	0	1	0	0	0	1	0	0	1	1
DH1	0	1	0	0	1	1	0	1	0	0	1	1
DH2	0	0	1	0	1	0	0	1	0	0	1	1
DH3	1	1	0	0	1	0	0	0	0	1	0	0
DH4	0	0	1	1	0	0	0	0	0	0	1	0

Under each band number, the band width is shown in base pairs, e.g., band 1 goes from 2026 to 1970 BP (base pair), band 2 goes from 1923 to 1889 BP, and so on.

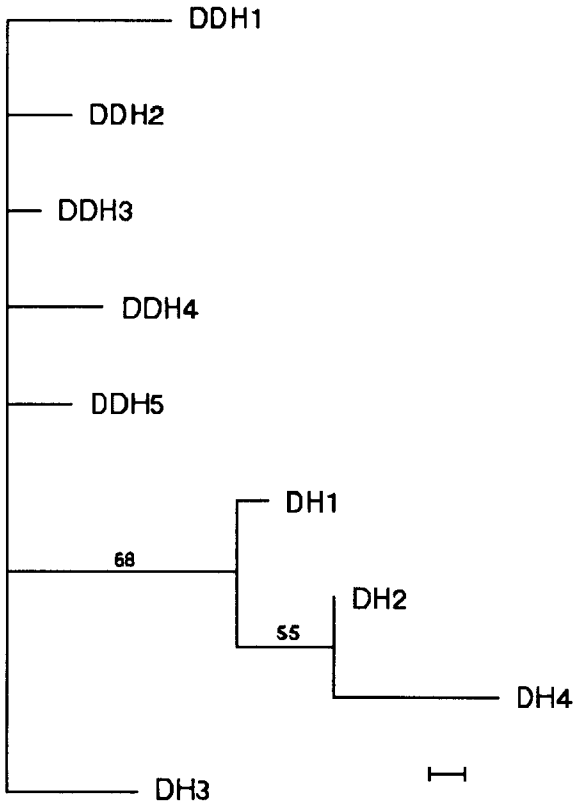

FIGURE 2 The PAUP bootstrap analysis of the nine isolates of *S. chartarum*. Phylogenetic relationships of the isolates were inferred from the binary data (Table II) using the branch-and-bound option of the PAUP program. The scale bar represents the distance resulting from one character change. Only values above 50% are shown.

BANDS													
13 1094–1004	14 995–956	15 931–884	16 872–822	17 754–702	18 700–664	19 656–610	20 599–566	21 534–481	22 469–421	23 402–381	24 367–332	25 310–282	26 223–206
1	0	1	0	1	0	1	0	1	0	0	1	0	1
1	0	1	0	1	0	1	0	1	0	1	0	0	1
1	0	1	0	1	0	1	0	1	0	1	0	0	1
1	0	1	0	1	1	1	0	1	0	1	1	0	1
1	0	1	0	1	0	1	0	1	0	1	0	0	1
1	0	1	0	1	1	1	0	1	0	1	0	0	0
1	0	1	0	1	1	1	0	1	0	0	0	0	0
1	0	1	0	1	0	1	0	1	0	1	0	0	1
1	0	1	0	1	1	1	0	1	0	0	1	0	0

of metabolites in sample I were higher. Sample I had a cumulative total of 180 μg spirodrimane-like compounds, while sample II contained 120 μg.

It is possible that isolates containing high amounts of toxin were missed in our sampling. However, Jarvis et al.[21] previously found no correlation between the trichothecene toxicity of a strain and its isolation from a case house. In fact, some of the isolates richest in trichothecene came from control houses.[21] This suggests that trichothecene toxicity may not be the only factor in the pathogenesis of PH. Vesper et al.[11] demonstrated that, in addition to previously known toxins, *S. chartarum* can grow and produce a hemolytic agent at 37°C, which could add to the pathogenicity under some circumstances. These studies also described a RAPD banding pattern that seemed to be indicative of potentially virulent strains. The results of RAPD analysis of the nine isolates of *S. chartarum* from this house are shown in Table III. When subjected to PAUP, three of them (DH1, DH2, and DH4) (Fig. 2) were found to have a potential relationship. Each of these three strains was hemolytic consistently during the 8-week test (Table IV). When the RAPD analyses of these three strains are considered together with the five consistently hemolytic strains isolated from Cleveland houses with earlier PH cases,[11] isolate DH4 is most similar to this group of consistently hemolytic strains (Fig. 3), indicating that at least one of the DH strains may be related to the apparent problematic type.[11]

The route of human exposure to *S. chartarum* spores has been assumed to be by inhalation. Therefore, it was somewhat surprising that, in a house so heavily contaminated with *S. chartarum*, so few spores were detected in the air before

TABLE IV Weekly Observations of *Stachybotrys chartarum* Isolates for Hemolytic Activity and Growth at 37°C on Sheep Blood Agar

	Weeks on Wet Wallboard															
	1		2		3		4		5		6		7		8	
	H	G	H	G	H	G	H	G	H	G	H	G	H	G	H	G
DDH1	−	+	−	+	−	+	+	+	+	+	+	+	−	+	−	+
DDH2	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
DDH3	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+
DDH4	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	+
DDH5	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	+
DH1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DH2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DH3	−	+	−	+	−	+	−	+	−	+	+	+	+	+	+	+
DH4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

H = hemolysis; G = growth.

remediation began. These results are consistent, however, with previous results obtained from other homes of infants in Cleveland with PH.[4] However, during the air sampling, no one was present in this house or in the homes in the earlier study. Despite these findings, the very large quantities of *S. chartarum* spores found in the carpet dust samples analyzed in this study suggest that surface dust could have comprised a significant secondary reservoir of spores. The spores in settled dust may have been reaerosolized continually into the air by human activities in the house, resulting in possible inhalation exposure to infants/children.

This extensive evaluation of a PH infant's home heavily contaminated with *S. chartarum* underlines the need to be cautious during remediation. In addition to the New York guidelines, documents available from Health Canada and the American Industrial Hygiene Association are sources that should be consulted. Several unresolved questions arise, such as the following: What was the actual extent of spore and toxin exposure to the infant before remediation? Is more than one route of exposure important? Are there particularly virulent strains of *S. chartarum*? Which toxins are involved in the pathogenesis of PH and other clinical problems? These issues require further investigation. What is clear is that damp/wet basements should not be built out (i.e., finished), especially with materials that support microbial growth.

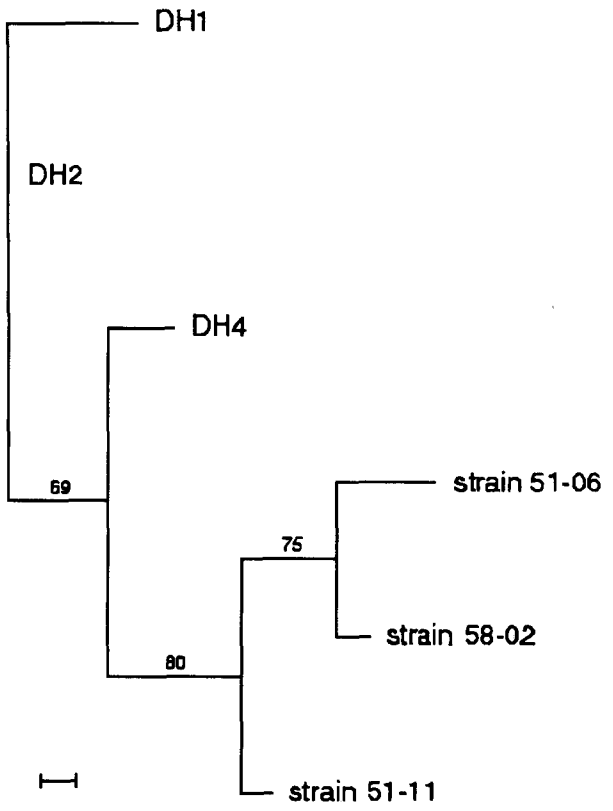

FIGURE 3 The PAUP bootstrap analysis of the three consistently hemolytic isolates of *S. chartarum* from the subject house and three previously isolated from houses with cases of PH. Phylogenetic relationships of the isolates were inferred from the binary data (Table II) using the branch-and-bound option of the PAUP program. The scale bar represents the distance resulting from one character change. Only values above 50% are shown.

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