NIOSH HEALTH HAZARD EVALUATION REPORT

HETA # 2004-0005-3024
Grove Park Inn Resort and Spa
Asheville, North Carolina

November 2006

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

PREFACE

The Hazard Evaluation and Technical Assistance Branch (HETAB) of the National Institute for Occupational Safety and Health (NIOSH) conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health (OSHA) Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employers or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

HETAB also provides, upon request, technical and consultative assistance to federal, state, and local agencies; labor; industry; and other groups or individuals to control occupational health hazards and to prevent related trauma and disease. Mention of company names or products does not constitute endorsement by NIOSH.

ACKNOWLEDGMENTS AND AVAILABILITY OF REPORT

This report was prepared by Melissa Finley, Elena Page, Kenneth Wallingford, and Nancy Clark Burton of HETAB, Division of Surveillance, Hazard Evaluations and Field Studies (DSHEFS). Analytical support was provided by Microbiology Specialists Inc. (Houston, Texas), P&K Microbiology Services, Inc. (Cherry Hill, New Jersey), and DataChem Laboratories, Inc. (Salt Lake City, Utah). Stachylysin™ analysis was performed by Jerome Smith, Raymond Biagini, and Deborah Sammons of the NIOSH Division of Applied Research Technology (DART). Field assistance was provided by Deborah Sammons and Barbara MacKenzie of DART. Desktop publishing was performed by Robin Smith. Editorial assistance was provided by Ellen Galloway.

Copies of this report have been sent to employee and management representatives at the Grove Park Inn Resort and Spa and the OSHA Regional Office. This report is not copyrighted and may be freely reproduced. The report may be viewed and printed from the following internet address: www.cdc.gov/niosh/hhe/hhesearch.htmL. Copies may be purchased from the National Technical Information Service (NTIS) at 5825 Port Royal Road, Springfield, Virginia 22161.

For the purpose of informing affected employees, copies of this report shall be posted by the employer in a prominent place accessible to the employees for a period of 30 calendar days.

Highlights of the NIOSH Health Hazard Evaluation

The National Institute for Occupational Safety and Health (NIOSH) received a health hazard evaluation request from employees of the Grove Park Inn Resort and Spa. We evaluated reports of chronic bronchitis and pneumonia, headaches, hoarseness, cough, sore throats, burning/watery eyes and nose, red and flaky nose, dizziness, nosebleeds, shortness of breath, nausea, inability to concentrate, sneezing, excess fatigue, fever, chills, muscle aches and dry, itchy skin, that workers believed may have been related to exposure to mold and fungus in the treatment rooms and gas released from pools. NIOSH investigators conducted site visits in November and December 2003 to look at these issues.

What NIOSH Did

- We checked the Spa for evidence of water damage and microbial contamination.
- We did a ventilation assessment.
- We took bulk samples of wall material to look for fungus and bacteria.
- We took water samples to look for fungus and bacteria.
- We tested the air near the pools for chlorine.
- We talked confidentially to employees about their jobs, their exposures, and their symptoms.
- We collected blood samples for Stachylysin™ a possible indicator of exposure to *Stachybotrys chartarum*.

What NIOSH Found

- There was water damage and visible mold growth in Room 18 and the women's restroom.
- The ventilation in the treatments rooms was adequate.
- Mycobacterium and Gram-negative bacteria were detected in pool and fountain water at levels higher than suggested guidelines.
- No chlorine was detected in the air.
- Stachylysin™ was detected in the blood of a few employees, but did not correlate with exposure to *Stachybotrys chartarum*.

What Spa Managers Can Do

- Remove mold in Room 18 and women's restroom.
- Take steps to prevent recurrent mold growth.
- Monitor moisture levels in treatment room walls.
- Reduce levels of microbial contamination in pool and fountain water.
- Add moisture barrier between the steam room and adjacent areas.
- Implement an indoor environmental quality management plan.
- Improve communication between management and staff.

What the Spa Employees Can Do

- Report work-related symptoms to Spa management.
- Get evaluated by a physician trained in occupational medicine if you have work-related symptoms.

What To Do For More Information:
We encourage you to read the full report. If you would like a copy, either ask your health and safety representative to make you a copy or call 1-513-841-4252 and ask for HETA Report #2004-0005-3024

Health Hazard Evaluation Report 2004-0005-3024
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SUMMARY

The National Institute for Occupational Safety and Health (NIOSH) received a confidential request for a health hazard evaluation (HHE) from employees of the Grove Park Inn Resort and Spa (Spa), Asheville, North Carolina. The request stated that workers were experiencing chronic bronchitis and pneumonia, headaches, hoarseness, cough, sore throats, burning/watery eyes and nose, dizziness, nosebleeds, shortness of breath, nausea, inability to concentrate, sneezing, excess fatigue, fever, chills, muscle aches and dry, itchy skin, that they believed may have been related to exposure to mold and fungi in the treatment rooms and gas released from pools in the facility.

In November and December 2003, NIOSH investigators conducted four site visits to evaluate the issues at the Spa. The environmental component included a moisture assessment, microbial sampling, and measurements of indoor environmental quality (IEQ) indicators (carbon dioxide [CO_2], temperature, and relative humidity [RH]). Water samples were taken from pools and fountains throughout the Spa and tested for bacteria, fungi, mycobacteria, and endotoxin. Chlorine levels in the water and air were measured. The medical component included confidential interviews with employees, administration of a questionnaire, and collection of blood samples for Stachylysin™, a research test that may indicate exposure to *Stachybotrys chartarum*.

The environmental evaluation revealed elevated moisture levels that led to mold growth behind walls and above ceilings of Room 18 and the women's restroom. Microbial sampling identified a variety of fungi including *Stachybotrys chartarum.* Bulk water samples taken from the pool and hot tub systems revealed the presence of Mycobacterium and Gram-negative bacteria. Results of the IEQ monitoring revealed that the ventilation was adequate in supplying air and controlling CO_2 levels, air temperature, and RH to within acceptable ranges.

Massage therapists reported significantly more cough, achiness, sinus problems, dry or sore throat, sneezing and fatigue than did managers, who served as the referent group. Odors may have played a role in the reporting of subjective symptoms by this group of employees. Odors figure prominently in IEQ complaints, have historically guided ventilation practice, and are often used to make judgments on the healthfulness of indoor spaces. Maintenance employees, whose work included cutting into walls and other activities to identify the fungal growth, did not have a significantly higher prevalence of any work-related symptoms when compared to managers.

Regarding the research test we performed, four persons had detectable concentrations of Stachylysin™ in their serum. Three were managers with no known exposure to the Spa or treatment Room 18. One was a maintenance employee who had been working to identify the source of moldy odors in the Spa. No massage therapists had Stachylysin™ detected in their serum. The Stachylysin™ test was performed to determine its usefulness as a biomarker of exposure to *Stachybotrys chartarum,* not to determine whether employees' symptoms were due to mold exposure at the Spa. The lack of detectable Stachylysin™ in the serum of the massage therapists could have reflected an absence of exposure, or that too much time may have elapsed since their exposure, and the Stachylysin™ may have cleared from the serum. It could also reflect poor test sensitivity. The positive findings in three of the managers may reflect an unidentified exposure, or it could reflect cross-reactivity with other antigens, such as common environmental fungi.

NIOSH investigators found localized areas of fungal contamination in building materials in the Spa. The Spa pools and fountains had higher than anticipated levels of microbial contamination. NIOSH investigators recommend remediating the mold found in treatment rooms, monitoring moisture levels in treatment room walls, and adjusting the water disinfection program to reduce microbial levels in pools and fountains.

Keywords: NAICS 721110 (Hotels [except Casino Hotels] and Motels), resort hotel, resort spa, indoor environmental quality, IEQ, microbial contamination, mold, Stachylysin™, *Stachybotrys chartarum,* moisture, pools, bacteria, mycobacteria, cough, achiness, sinus problems, dry throat, sore throat, sneezing, fatigue

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INTRODUCTION

In October 2003, the National Institute for Occupational Safety and Health (NIOSH) received a confidential request for a health hazard evaluation (HHE) from employees of the Grove Park Inn Resort and Spa (Spa), Asheville, North Carolina. The request stated that employees were experiencing chronic bronchitis and pneumonia, headaches, hoarseness, cough, sore throats, burning/watery eyes and nose, dizziness, nosebleeds, shortness of breath, nausea, inability to concentrate, sneezing, excess fatigue, fever, chills, muscle aches and dry, itchy skin, that they believed may have been related to exposure to mold in the treatment rooms and gas released from pools in the facility.

An initial site visit was conducted November 3-4, 2003. During the visit, NIOSH industrial hygienists collected bulk samples of wall material for fungal (mold) analysis and water samples from pools and fountains for microbial analysis. They also performed moisture and ventilation assessments of the facility, and the NIOSH medical officer conducted confidential medical interviews with employees of the Spa.

A follow-up site visit was conducted November 10-14, 2003. During this survey, the NIOSH medical officer collected blood samples for Stachylysin™, a research test that may indicate exposure to *Stachybotrys chartarum*. The Stachylysin™ test was performed to determine its usefulness as a biomarker of exposure to *Stachybotrys chartarum,* not to determine whether employees' symptoms were due to mold exposure at the Spa. A third site visit was conducted on December 2, 2003. During this visit, NIOSH industrial hygienists collected air samples for research regarding fungal sampling methodologies, as well as additional water samples from the pools. A fourth visit took place on December 29, 2003, to collect additional water samples from the pools for microbial characterization.

An interim report dated May 20, 2004, summarized the activities of the NIOSH investigators, discussed the most important industrial hygiene and medical findings related to the survey, and offered preliminary recommendations. This final report also contains the results of the bulk, water, and air sampling and medical evaluations, discussions of sampling methods, a review of the potential health effects of agents to which Spa employees are exposed, and recommendations to address identified areas of concern.

BACKGROUND

The Spa is a 40,000-square-foot facility offering a wide range of skin and body treatments and therapies. The facility was added to the resort in February 2001 and houses 18 treatment rooms, four pools, men's and women's locker rooms, saunas, hot tubs, and cold plunge pools. The approximately 120 Spa workers include concierge staff, massage therapists, estheticians, and nail technicians offering treatments including various massages and water therapies, aromatherapy, mud application, and acupressure as well as nail, hair, and beauty salon services. The Spa is open from about 8:00 a.m. to 9:00 p.m. daily, and there are three work shifts. Upkeep of the Spa facilities and ventilation system is managed internally by Spa engineers and maintenance personnel.

METHODS

Environmental Evaluation

Microbial Assessment

During the November 3-4, 2003, visit the Spa was inspected for visible evidence of water damage and microbial contamination. A Tramex Moisture Encounter meter and a Tramex Wet Wall detector were used to qualitatively assess the moisture content of the walls, floors, and ceilings of several treatment rooms. An Optim Model FS-101 boroscope was used to inspect areas behind walls for moisture and microbial contamination.

Bulk Sampling

Nine samples of dust and suspected visible mold growth were collected using sticky tape in several rooms. The tape was then affixed to a

glass slide and analyzed by optical microscopy. Five samples of wall material and insulation from Room 18 and the women's restroom were collected for microbial analysis. These samples were analyzed by optical microscopic examination and cultured for fungal identification and colony counts. Two sterile swabs were also used to collect slime from the bottom of the decorative fountain near Room 18. These samples were analyzed and cultured for fungal and bacterial identification and colony counts.

Air Sampling (Research)

Because of the mold growth discovered during the initial visit, NIOSH industrial hygienists returned on December 2, 2003, to evaluate viable and non-viable fungal air sampling methods for culturable fungi, total spores, and total spore equivalents. Samples were collected in four locations above the ceilings and in the general areas of Room 18 and the women's restroom.

To determine the concentrations of culturable fungi, an Andersen N-6 single-stage impactor was used at a calibrated flow rate of 28.3 liters per minute (Lpm). Samples were collected over sample times of 3, 4, and 5 minutes each on cornmeal agar plates to optimize *Stachybotrys chartarum* growth. Three replicate plates were collected for each sample time at each sampling location. All sample plates were incubated at temperatures consistent with genera-specific growth requirements. The taxa and rank of collected microorganisms were determined by morphology and/or biochemical characteristics.

To determine the concentrations of total spores in air using a non-culturable method, Air-O-Cell® samplers were attached by Tygon® tubing to sampling pumps calibrated at a flow rate of 15 Lpm. Samples were collected over a sample time of 10 minutes. Three replicate samples were collected at each sampling location. Samples were analyzed by optical microscopy for identification, morphological identification, and total number of spores.

To determine the concentrations of total fungal species in air, aerosols were collected using 37-millimeter (mm) diameter polytetrafluoroethylene (PTFE), 0.3-micrometer (µm) pore size filters in three-piece cassettes attached by Tygon® tubing to sampling pumps. One sample was collected at each sample location for 120 minutes with a pump calibrated at a flow rate of 10 Lpm and one sample was collected at each sample location for 300 minutes with a pump calibrated at a flow rate of 4 Lpm. Samples were analyzed for total fungi by quantitative polymerase chain reaction (QPCR). The QPCR analysis panel includes 23 species of fungi commonly associated with water-damaged indoor environments as patented by the United States Environmental Protection Agency (EPA) [http://www.epa.gov/nerlcwww/moldtech.htm].

Ventilation Assessment

Discussions were held with the maintenance managers to obtain information on the operation and maintenance of the heating, ventilating and air conditioning (HVAC) systems serving the Spa. Copies of mechanical plans and a test and balance report were reviewed. A visual inspection was made of the ventilation system, including the air handling units, serving the Spa. To evaluate air flow and distribution in the Spa, carbon dioxide (CO_2), temperature, and relative humidity (RH) measurements were made in four treatment rooms with a TSI Q-Trak monitor Model 8554. Smoke tubes were used to observe air flow patterns in some unoccupied rooms.

Water Assessment

Bulk water samples were collected on November 3-4, December 2, and December 29, 2003 and analyzed for microbial contamination. Three or four samples (totaling approximately 2 liters [L] of water per pool system) were taken from the Spa/mineral pool, lap pool, and men's and women's waterfall pools. Three or four samples (totaling approximately 1.5 L of water per whirlpool system) were also taken from the men's and women's hot and cold whirlpools and the double waterfall decorative fountain system. Sampling locations within each water system included the Accutrol™ monitoring point, the water line, the filter unit, and directly from the pool water. One or two samples (approximately 150 milliliters [mL] total) were taken from each decorative fountain. All samples were collected in sterile plastic containers and analyzed by

optical microscopy. In addition, the samples were analyzed for culturable bacteria, mycobacteria, and fungi.

Twenty-one samples were collected from the pool and hot tub systems and analyzed for endotoxin (a cell wall component of Gram-negative bacteria [GNB]) and free chlorine. Sampling locations within each water system included at the Accutrol ™ monitoring point, from the water line, and directly from the pool water. All samples were collected in 50 mL pyrogen-free conical vials. The samples were analyzed for endotoxin using the *Limulus* amebocyte lysate (LAL) assay. Free chloride was measured by ion chromatography according to EPA Method 300.[1]

Due to concern about offgassing from the various bodies of water, six air samples for chlorine content were collected using direct-reading colorimetric (detector) tubes near the Spa/mineral pool, lap pool, and double waterfall decorative fountain. As chlorine is drawn across a white indicating layer, the layer turns yellowish-orange, and the length of the discoloration indicates the concentration of chlorine in the air.

Temperature, pH, and oxidation-reduction potential were also recorded for each water system from the Accutrol ™ monitor for each pool system at the time of water sample collection. Spa maintenance also provided the results of their routine chemical water tests for free chlorine and total chlorine concentrations, pH, temperature, total alkalinity, and calcium hardness.

Medical Evaluation

Interviews

The NIOSH physician conducted confidential interviews with 29 current and former Spa employees during the first and second site visits. The three former employees were interviewed by telephone. Of the 29, management identified 11 as having reported concerns over exposure to mold in the Spa (seven massage therapists, one esthetician, one concierge, and two administrative personnel). Three others were identified by the HHE requesters (two massage therapists and one nail technician). The rest (15) were randomly selected by the NIOSH investigator from the employee roster (five massage therapists, two nail technicians, three estheticians, two concierge, one programmer, one Spa attendant, and one employee of the retail store). Medical records were reviewed for one person who reported recurrent pneumonia. Medical records were requested from two other employees, but they did not return their release of information forms.

Biological Monitoring and Questionnaire

Preliminary laboratory tests identified *Stachybotrys chartarum* (*S. chartarum*) on bulk samples collected in Room 18 and the women's restroom. Following this, NIOSH investigators pursued a research protocol concerning validation of Stachylysin™ as a biomarker of exposure to this fungus.

Three groups of employees (a total of 33 people) were asked to participate in this serum survey conducted during the second site visit: massage therapists, maintenance workers, and management employees who had no known contact with the Spa. Massage therapists were chosen because they had reported odors and symptoms related to those odors, in Room 18 and in other locations. Other Spa employees were unlikely to have worked in Room 18 because it was a massage room. Spa records were used to identify which massage therapists had worked in Room 18 in the 2 weeks before it was closed on October 17, 2003. NIOSH investigators did not attempt to identify employees who used the restroom as time spent in a restroom would be minimal. In addition, the maintenance supervisor identified which maintenance employees had been involved in attempting to identify the source of moldy odors in Room 18 and the women's restroom, which included activities such as cutting access holes in the ceiling. These activities occurred after the rooms were closed, and likely represented the most significant exposure to fungi among employees. A group of resort management employees who had not been in the Spa were selected as a comparison group because they had

no known occupational exposure to *S. chartarum*. Informed consent was obtained. A serum specimen was obtained from all participants and tested for Stachylysin™. In addition, a questionnaire concerning the participants' workplace, job duties, medical history, and current health symptoms was administered.

EVALUATION CRITERIA

Microbial Contamination

Exposure to microbes is not unique to the indoor environment. No environment, indoors or out, is completely free from microbes, not even a surgical operating room. Nevertheless, media reports and some scientific studies have suggested an association between building occupant symptoms and indoor fungi (mold), bacteria, or endotoxin concentrations. Remediation of microbial contamination may improve indoor environmental quality (IEQ) conditions even though a specific cause-effect relationship is not determined. NIOSH investigators routinely recommend the remediation of observed microbial contamination and the correction of situations favorable for microbial growth and bioaerosol dissemination.

Mold

The types and severity of symptoms related to exposure to mold in the indoor environment depend in part on the extent of the mold present, the extent of the individual's exposure, and the susceptibility of individuals (for example, whether they have pre-existing allergies or asthma). In general, excessive exposure to fungi may produce health problems by several primary mechanisms, including: (1) allergy or hypersensitivity, (2) infection, and (3) toxic effects. Additionally, molds produce a variety of volatile organic compounds, the most common of which is ethanol.

Allergic responses are the most common type of health problem associated with exposure to molds. These health problems may include sneezing; itching of the nose, eyes, mouth, or throat; nasal stuffiness and runny nose; and red, itchy eyes. Repeated or single exposure to mold or mold spores may cause previously non-sensitized individuals to become sensitized. Molds can trigger asthma symptoms (shortness of breath, wheezing, cough) in persons who are allergic to mold. A recent review of the scientific literature concluded that exposure to molds in the indoor environment may make pre-existing asthma worse, but also concluded that there was not enough evidence to determine whether exposure to mold in the indoor environment could cause asthma.[2] Hypersensitivity pneumonitis is another allergic response that has developed in people following extensive short-term (acute) or long-term (chronic) exposure to molds. It is a very rare illness, which may resemble bacterial pneumonia, and typically involves respiratory symptoms (such as cough, wheezing, or shortness of breath) as well as other symptoms (such as extreme fatigue and low-grade fever).

People with weakened immune systems (immune-compromised or immune-suppressed individuals) may be more vulnerable to infections by molds. For example, *Aspergillus fumigatus*, a mold that has been found on almost every substrate, has been known to infect the lungs of immune-compromised individuals after inhalation of the airborne spores.[3] Healthy individuals are usually not vulnerable to infections from airborne mold exposure.

Recently, there has been increased concern related to exposure to specific molds that produce toxic substances called mycotoxins. Illness associated with exposures (from inhalation and/or skin contact) to mycotoxins in agricultural or industrial environments has been reported. However, there is currently no conclusive evidence of a link between mycotoxin exposure in the indoor environment and human illness.[4,5,6] It is important to note that many molds potentially produce toxins given the right conditions.

No exposure guidelines for mold in air have been established, because it is not possible to distinguish between "safe" and "unsafe" levels of exposure. Nevertheless, the potential for health problems is an important reason to prevent indoor mold growth and to remediate

any indoor mold contamination. Moisture intrusion along with nutrient sources such as building materials or furnishings allows mold to grow indoors, so it is important to keep the building interior and furnishings dry. NIOSH investigators concur with the EPA's recommendations to remedy mold contamination in indoor environments (www.epa.gov/iaq/molds/mold_remediation.htmL).[7]

Heating, Ventilating, and Air Conditioning

One of the most common deficiencies in the indoor environment is the improper operation and maintenance of ventilation systems and other building components.[8] NIOSH investigators have found that correcting HVAC problems often reduces reported symptoms. The majority of studies of ventilation rates and building occupant symptoms have shown that rates below 10 liters per second per person (Ls^{-1}/person) (which equates to 20 cubic feet per minute per person [cfm/person]), are associated with one or more health symptoms.[9] Moreover, higher ventilation rates, from 10 Ls^{-1}/person up to 20 Ls^{-1}/person, have been associated with further significant decreases in the prevalence of symptoms.[9] Thus, improved HVAC operation and maintenance, higher ventilation rates, and comfortable temperature and RH can all potentially serve to improve symptoms without ever identifying any specific cause-effect relationships. When conducting an IEQ survey, NIOSH investigators often measure ventilation and comfort indicators, such as CO_2, temperature, and RH to provide information relative to the functioning and control of HVAC systems.

Carbon Dioxide

CO_2 is a normal constituent of exhaled breath and is not considered a building air pollutant. It is an indicator of whether sufficient quantities of outdoor air are being introduced into an occupied space. However, CO_2 is not an effective indicator of ventilation adequacy if the ventilated area is not occupied at its usual level at the time the CO_2 is measured. The American Society for Heating, Refrigerating, and Air-Conditioning Engineers, Inc. recommends an indoor CO_2 concentration within 700 ppm of the outdoor concentration for comfort (odor) reasons.[10] Elevated CO_2 concentrations suggest that other indoor contaminants may also be increased. If CO_2 concentrations are elevated, the amount of outdoor air introduced into the ventilated space needs to be increased. ASHRAE's most recently published ventilation standard, *ANSI/ASHRAE 62.1-2004: Ventilation for Acceptable Indoor Air Quality*, recommends outdoor air supply rates of 17 cfm/person for office spaces and libraries, 7 cfm/person for reception areas, and 5 cfm/person for lobbies.[10]

Temperature and Relative Humidity

Temperature and RH measurements are often collected as part of an IEQ investigation because these parameters affect the perception of comfort in an indoor environment. The perception of thermal comfort is related to one's metabolic heat production, the transfer of heat to the environment, physiological adjustments, and body temperature.[11] Heat transfer from the body to the environment is influenced by factors such as temperature, humidity, air movement, personal activities, and clothing. The *ANSI/ASHRAE Standard 55-2004: Thermal Environmental Conditions for Human Occupancy*, specifies conditions in which 80% or more of the occupants would be expected to find the environment thermally acceptable.[12] Assuming slow air movement and 50% RH, the operative temperatures recommended by ASHRAE range from 68.5°F to 76°F in the winter, and from 75°F to 80.5°F in the summer. The difference between the two is largely due to seasonal clothing selection. ASHRAE also recommends maintaining RH at or below 65%.[10] Increased humidity can promote the excessive growth of microorganisms and dust mites.

Stachylysin™

Within the scientific community and the general public, there has been considerable attention and concern regarding fungi and mycotoxins, especially *S. chartarum*, in the indoor environment. *S. chartarum* is a saprophytic fungus (those utilizing non-living organic matter

as a food source) commonly found on cellulosic materials (wallpaper, drywall) in office buildings with wet environments or in those with high humidity. Although anecdotal reports have attributed a wide variety of health effects to exposure to certain fungi (specifically, *S. chartarum*) in the indoor environment, no clear relationship has been documented. The paucity of good scientific data about the specific effects in humans of exposure to fungi is due, in part, to the lack of a valid, measurable indicator of human exposure.

S. chartarum, like other microorganisms, produces proteinaceous substances called hemolysins. The hemolysin produced by *S. chartarum* has been termed Stachylysin™. Recently, an enzyme-linked immunosorbent assay (ELISA) measurement of Stachylysin™ in serum has been developed that may allow quantification of human exposure.[13] Animal studies indicate that the presence of Stachylysin™ in the serum is a fairly specific indicator of exposure to *S. chartarum* (i.e., there were no false positives); however, the sensitivity is not as high (i.e., animals with known exposure did not have detectable Stachylysin™ in the serum). It appears that Stachylysin™ usually disappears from the serum of exposed animals about 4 weeks after cessation of exposure, although in some cases it did not disappear until about 8 weeks later. [14] We are unsure whether Stachylysin™ acts similarly in humans.

Endotoxin

Endotoxin, a lipopolysaccharide compound from the outer cell wall of GNB, is released from the bacteria when the GNB die or undergo growth.[15,16] GNB are ubiquitous in the environment. In experimental studies, human volunteers exposed via inhalation to high levels of endotoxin experience airway and alveolar inflammation as well as chest tightness, fever, and malaise and have an acute reduction in lung function, as measured by the forced expiratory volume in one second.[17,18] Airborne endotoxin exposures between 45 and 400 endotoxin units per cubic meter (EU/m^3) have been associated with acute airflow obstruction, mucous membrane irritation, chest tightness, cough, shortness of breath, fever, and wheezing.[18,19,20,21] Chronic health effects that have been associated with airborne endotoxin exposures include chronic bronchitis, bronchial hyperreactivity, chronic airway obstruction, hypersensitivity pneumonitis, and emphysema.[18] A permanent decrease in pulmonary function, along with respiratory symptoms, has been reported in several cross-sectional epidemiological studies.[17]

Mycobacteria

Mycobacteria are rod-shaped bacteria that have cell walls with a high lipid (fat) content. Mycobacteria are found in a great variety of natural and human-influenced aquatic environments, including in and around swimming pools and spas, treated drinking water, and aerosols. They are readily aerosolized from aqueous suspension. Aerosolization is caused by the generation of airborne droplets from bubbles bursting at the water surface. Recently reports have linked exposure to various species of mycobacteria in pools and natural waters to the development of various respiratory illnesses. These include bronchitis, hypersensitivity pneumonitis, granulomatous pneumonitis, and allergic alveolitis. [22] For example, *Mycobacterium avium* in spa water has been linked to hypersensitivity pneumonitis and possibly pneumonia.[23] Symptoms were flu-like and included cough, fever, chills, malaise, and headaches. The illnesses followed the inhalation of heavily contaminated aerosols generated by the spa.

Due to the high lipid content of their cell wall, mycobacteria are very resistant to the disinfectants used in water treatment, including chlorine and ozone.[24,25] Therefore, it is essential to maintain recommended disinfection residuals in spas and pools at all times in order to reduce the risks of acquiring swimming pool granuloma or respiratory illness caused by mycobacteria. Thorough cleaning of surfaces and materials around pools and Spas where the organism may persist is also necessary.[26]

Water

Proper water chemistry is essential to maintaining safe and consistent swimming pool and spa operation. Chemicals used in swimming pools and spas include disinfectants, alkalinity and pH adjusters, and filter aids. The North

Carolina Department of Environment and Natural Resources has established standards for water quality of public swimming pools (Title 15A Subchapter 18A of the North Carolina Administrative Code Section 2500).[27] These parameters include pH (a scale representing relative acidity or alkalinity) ranging from 7.2 to 7.8, total alkalinity ranging from 80 to 150 parts per million (ppm), calcium hardness of approximately 250 ppm, and free chlorine ranging from 1 to 3 ppm. ANSI, along with the National Spa and Pool Institute has also published similar standards for public swimming pools and spas (ANSI/NSPI-2 1999) with ideal conditions of pH ranging from 7.4 to 7.6 (7.8 maximum), total alkalinity ranging from 80 to 100 ppm, calcium hardness ranging from 200 to 400 ppm (1000+ maximum), and free chlorine ranging from 1.0 to 3.0 ppm.[28]

RESULTS

Environmental Evaluation

Moisture Assessment

Visual inspection rarely showed surface water damage in the occupied spaces. However, visual inspection did show water damage and mold growth in the static space between the finished ceiling and the concrete deck above the ceiling, and the qualitative assessment revealed significant moisture problems in two rooms (18 and the women's restroom). Low to moderate moisture levels were also found in several other rooms (1, 2, 3, 5, 14, 16, 17). The relative moisture content of the floors, walls, and ceilings is shown in Table 1.

As described above, visual inspection did not reveal any surface water damage in the occupied space of Room 18, but the walls in this room had elevated moisture levels. With Spa management cooperation, NIOSH investigators bored a hole in one wall for further inspection. Pieces of the two layers of wallboard material removed showed visual evidence of mold growth.

Microbial Assessment

The results of the sticky tape sample analyses are summarized in Table 2. Tape samples were collected from discolored areas suspected of mold growth in Rooms 14 and 17. The Room 14 samples revealed the presence of *Stachybotrys, Chaetomium,* and *Alterneria*-type fungal genera, and the Room 17 samples identified *Chaetomium* as the predominant fungal genus. Other tape samples were collected of dust, wallboard, and discolored grout in Room 18. Direct optical microscopy examination of the Room 18 samples identified *Aspergillus/ Penicillium*, *Stachybotrys*, *Cladosporium*, and *Dicyma* mold species. The women's restroom samples revealed the presence of predominantly *Aspergillus/Penicillium*, *Stachybotrys*, and *Cladosporium* mold species by direct microscopy examination.

Bulk samples of wallboard, wallboard paperback, and pool tile were collected in Room 18 and the women's restroom. Optical examination of sticky tape samples from the bulk materials showed that Room 18 had *Aspergillus/Penicillium* and *Stachybotrys* genera contamination and the women's restroom had *Aspergillus/Penicillium* and *Cladosporium* genera contamination. Cultures of the Room 18 bulk wallboard samples showed fungal concentrations ranging from 6.0×10^5 to 9.0×10^6 colony forming units per gram (CFU/g) with *Aspergillus*, *Penicillium* and *Acremonium* as the predominant genera and concentrations of mixed bacteria ranging from 2.9×10^7 to 8.3×10^7 CFU/g. Cultures of the bulk samples collected in the women's restroom showed fungal concentrations ranging from 2.0×10^4 to 2.0×10^7 CFU/g with *Aspergillus*, *Penicillium,* *Cladosporium,* and *Acremonium* as the predominant genera and concentrations of mixed bacteria ranging from 4.8×10^6 to 7.1×10^6 CFU/g.

After draining the decorative wall fountain near Room 18, two swab samples were taken of scum found on the bottom. The results are given in Table 4. Under optical microscopic examination, these samples showed fungal structures, protozoans, and bacteria. Cultures revealed fungal concentrations ranging from 60 to

$2.0x10^5$ CFU/swab and concentrations of mixed bacteria ranging from $1.6x10^6$ and $1.1x10^7$ CFU/swab.

A summary of the bioaerosol sampling results collected on December 2, 2003, is presented in Table 5. For the viable samples collected using the Andersen N-6 sampler, *Aspergillus* was the predominant genus. In Room 18, the below-ceiling culturable samples showed a higher count than the above-ceiling samples. In terms of total spore counts, *Aspergillus*/*Penicillium* was the predominant genera. For both Room 18 and the women's restroom, the above-ceiling concentrations of spores were higher than those found below the ceiling. The QPCR results showed similar concentrations of spore equivalents above and below the ceiling in Room 18; above-ceiling concentration of spore equivalents was higher in the women's restroom than in the restroom area itself.

Ventilation Assessment

Inspection of the air handling units revealed that they were clean and well maintained. It was noted that all supply and return air was completely ducted to and from each room of the Spa. In lieu of air flow measurements, CO_2, air temperature, and RH were monitored for 18 hours to determine the adequacy of the ventilation in treatment rooms 2, 5, 14, and 16 while the doors were closed during treatments and after Spa business hours. The CO_2 levels ranged from 320 to 760 ppm; temperature ranged from 71.5 to 75.1°F; and RH was between 40% and 70%. The highest RH was measured in treatment room 14. These results indicate that the ventilation was adequate in supplying air and controlling CO_2 concentrations, air temperature, and RH to within acceptable ranges as specified by ANSI/ASHRAE guidelines. Similarly, results of smoke tube observations showed adequate air movement in the treatment rooms.

Water Assessment

Bacteria concentrations in the bulk water samples ranged from non-detectable (ND) in several water systems to 1.3×10^6 CFU/mL of water in the lap pool water system. The data are summarized in Table 6. The predominant bacterial species identified were *Pseudomonas aeruginosa* and *Pseudomonas flourescens*. In general, higher concentrations of bacteria were seen in the samples taken from the water line for each water system than samples taken from other sample locations within the water system.

Mycobacteria concentrations in the bulk water samples ranged from ND in most water systems to 7 CFU/mL in the men's waterfall whirlpool water system. The data are summarized in Table 6. The predominant mycobacterial species identified were *Mycobacterium avium* and *Mycobacterium fortuitum*. Fungal concentrations in the bulk water samples ranged from ND in most water systems to 1600 CFU/mL in the lap pool water system. The data are summarized in Table 6. The predominant fungal species identified were *Exophiala* and *Aureobasidium*.

All bulk water samples from the pool systems were also analyzed by direct microscopy as well. Generally, the microbiological species characterized were similar to those found on the culture. However, in samples from the men's and women's hot tub systems taken at each respective Accutrol™ monitoring point, *Acanthamoeba* protozoan species was identified by the direct exam but was not cultured. The results of the direct exam for all water samples are included in Table 4.

Bulk water samples were also collected from the decorative water fountains in the hallway near treatment Rooms 3, 8, 12, and 18. Bacterial concentrations in these water samples ranged from 2×10^2 CFU/mL (fountain near Room 18) to 3×10^4 CFU/mL (fountain near Room 8); the predominant bacterial species was *Pseudomonas florescens*. Mycobacterial concentrations ranged from ND in most fountains to 2 CFU/mL in the fountain near Room 18. *Mycobacterium fortuitum* and *Mycobacterium gordonae* were identified. Fungal concentrations ranged from 4 CFU/mL in the fountain near Room 12 to 100 CFU/mL in the fountain near Room 18. The fungal species identified included *Scolecobasidium*, *Aureobasidium*, *Phialphora*, and *Exophiala*. Optical microscopic examination identified protozoans (including *Naegleria* species, flagellates, and unidentified trophozoites) in a sample taken from the

fountain near Room 3. The results of these water samples are summarized in Table 7.

Concentrations of endotoxin ranged from 0.29 endotoxin units per milliliter of water (EU/mL) in the lap water system to 93 EU/mL in the water collected from the women's waterfall whirlpool system. These results are summarized in Table 8. The lowest concentrations of endotoxin were seen in the waters with the highest reported bacteria levels. The endotoxin levels indicate the presence of GNB.

Average concentrations of chloride in the samples determined by laboratory analysis (collected on the third site visit) ranged from 51 ppm in the double water fall fountain water to 1833 ppm in the Spa mineral pool water. The result of the Spa mineral pool is high due to the sodium chloride added to the pool system. This water chemistry information from the December 2, 2003, visit is summarized in Table 9. It is recommended that the combined chlorine residual should be kept to a minimum, preferably below 0.2 ppm.[27] Chlorine was not detected in the air on any sample taken (concentrations were less than 0.2 ppm, the limit of detection of the detector tube).

Medical Evaluation

Interviews

Of the 29 persons interviewed, 11 reported they had no symptoms related to the work environment. Of the 18 who did report symptoms they related to the work environment, 7 reported nasal symptoms (runny nose, itching, or sneezing), 6 reported headache, 5 reported eye irritation, 4 reported cough, 3 reported rash and 2 reported each of the following: fatigue, nausea, joint pain, shortness of breath or wheezing, pneumonia, and dizziness. One reported poor concentration.

Biological Monitoring and Questionnaire

Thirty-three employees participated in this evaluation: 8 massage therapists, 7 maintenance workers, and 18 management workers. One eligible Spa employee did not participate. Demographic comparisons of these three groups are described in Table 8. There was a significant difference in tenure and hours worked per week among the three groups. A higher percentage of management employees were current smokers, but this was not statistically significant. There was no significant difference in the prevalence of atopy (hereditary predisposition to allergies) between groups. Three management employees had been diagnosed with mold allergy by their physicians, but no massage therapists or maintenance employees had. There were very few physician-diagnosed respiratory illnesses among employees in the last two years (approximately the time frame the Spa had been open). One massage therapist and one management employee each reported physician-diagnosed bronchitis during that time, one employee from each of the three groups reported a physician-diagnosed sinus infection during that time, and one management and one maintenance employee reported an asthma attack in the last 12 months.

Participants were asked about the occurrence of a variety of symptoms at work in the previous 4 weeks, which is about the period of time the treatment room and women's restroom had been closed. Symptoms were considered work-related if they sometimes or usually occurred at work and improved on days off work. Massage therapists reported significantly more cough, achiness, sinus problems, dry or sore throat, sneezing, and fatigue than did management, which served as the referent group. Maintenance employees did not have a significantly higher prevalence of any work-related symptom than management employees. These results are presented in Table 9.

Four persons had detectable amounts of Stachylysin™ in their serum. Three were management employees with no known exposure to the Spa area or to treatment Room 18. One was a maintenance employee who had been working to identify the source of moldy odors in the Spa. No massage therapists had Stachlysin™ detected in their serum.

DISCUSSION & CONCLUSIONS

Environmental Evaluation

The environmental evaluation identified problems with mold and moisture in the facility. Isolated areas of mold were found behind walls and above the ceilings of the women's restroom and Room 18. The source of the water was not definitely determined, but was suspected to be moisture migrating through the walls of the steam room, which shares common walls with both the women's restroom and Room 18. Other areas of water damage and mold growth were repaired by wrapping exposed pipes. Moisture intrusion along with nutrient sources such as building materials or furnishings allows mold to grow indoors. It is extremely important, therefore, to keep the building interior and furnishings dry to prevent mold growth.

Because concentrations of microbes varied between sampling locations in the respective pool systems and between dates of sampling, it is difficult to determine the nature and extent of the microbial contamination in the pools and fountains. However, the presence of Mycobacterium species, GNB, and protozoa should be addressed.

Medical Evaluation

Despite lacking an obvious pathway for exposure to fungi and evidence of exposure to *S. chartarum* based upon Stachylysin™ results, massage therapists were more likely to report work-related symptoms than either management employees, who had no known exposure to occupational fungal contamination, or maintenance employees, who likely did have exposure to fungi in the course of their work. NIOSH investigators were unable to identify any exposure in the Spa to account for their reported symptoms. However, odors may have played a role in the reporting of subjective symptoms by this group of employees. Odors figure prominently in IEQ complaints, have historically guided ventilation practice, and are often used to make judgments on the healthfulness of indoor spaces.[29] Even though it may be difficult to associate an unpleasant odor with an illness, objectionable odors connote an unhealthful environment. For example, one study found that persons exposed to unpleasant odors may feel these odors adversely affect their health, mood, and performance.[30] Although the sense of smell should not be relied on to evaluate workplace hazards, odor can be a helpful guide in a building investigation. Odors in the environment may be unwanted, repulsive to some people, and difficult to tolerate. Resolution of odor problems is an important aspect of maintaining good IEQ.

There are several potential explanations for the failure of the Stachylysin™ test to detect Stachylysin™ in the samples from the massage therapists. The massage therapists utilized the room only for brief periods of time, from 50-80 minutes per session, with individual therapists giving from one to six sessions in the 2 weeks before the room was closed. The room was closed about 4 weeks prior to the serum being drawn. In addition, there was no obvious route of exposure to the fungi, because the ceiling was drywall and the ventilation system ducted, while the fungal growth was found on the back of the drywall. Volatile organic compounds responsible for moldy odors could have emanated through outlets, but it is unlikely any significant fungal exposure took place. Therefore, the failure of the Stachylysin™ test to detect Stachylysin™ in the samples from the massage therapists could reflect an absence of exposure, or too much time may have elapsed since exposure, and the Stachylysin™ may have cleared from the serum. It could also reflect poor test sensitivity.

Maintenance personnel were in the rooms at various times after they were closed looking specifically for the source of the moldy odors reported. They cut access panels in the drywall, which would likely have released fungi into their breathing zone. These employees likely had the most significant and most recent exposure to fungi in the Spa. The duration of their exposure is unclear, however. The positive findings in three of the management workers who had no known occupational exposure to fungi may reflect an unidentified exposure. It could also reflect cross-reactivity with other antigens, such as common environmental fungi. Finally, it may

be that this test is not a good biomarker for exposure to *S. chartarum.*

RECOMMENDATIONS

The following recommendations are based on the observations of NIOSH investigators. Most of these recommendations were discussed in the interim letter.

1. Remediate mold in Room 18 and the women's restroom. These rooms should remain closed until remediation is complete. Information on mold remediation is available in the EPA's document, "Mold Remediation in Schools and Commercial Buildings."[7] Information on consultants is available from the American Industrial Hygiene Association's "Guidelines for Selecting an Indoor Air Quality Consultant".[31]
2. Install vapor barriers between the steam room and the surrounding rooms to prevent water vapor from entering the interior wall cavities.[7]
3. Monitor moisture levels in the walls of treatment rooms that remain open, especially those adjacent to the men's steam room.
4. Identify and promptly eliminate sources of excess moisture or leaks that may cause water damage and lead to microbial growth in the facility.
5. Contact the North Carolina Department of Environment and Natural Resources or the National Spa and Pool Institute to determine the most appropriate method to reduce levels of microbiological agents in pools and fountains. Continue to monitor concentrations of microbes in the pool and fountain systems to ensure the adequacy of any disinfecting efforts.
6. Increase communication between employees and management to facilitate the discussion of concerns about environmental conditions at the Spa.
7. Implement an IEQ Management Plan for the Spa to address the IEQ issues that have evolved over the past several years. An IEQ manager or administrator with clearly defined responsibilities, authority, and resources should be selected. This individual should have a good understanding of the building's structure and function, and should be able to effectively communicate with occupants. An employee representative should be included in the program. The NIOSH/EPA document, "Building Air Quality: A Guide for Building Owners and Facility Managers" [http://www.cdc.gov/niosh/pdfs/iaq.pdf] may be helpful for developing and implementing the IEQ management plan.[32] A companion NIOSH/EPA guide: "Building Air Quality Action Plan" can serve as a checklist for developing and assessing an IEQ management program [http://www.epa.gov/iaq/largebldgs/graphics/baqactionplan.pdf].[33] The EPA has also established an IEQ information clearinghouse that can provide information on a number of IEQ-related topics and has a website specifically for IEQ issues [http://www.epa.gov/iaq/index.html].
8. Encourage/refer employees who continue to experience health problems to see a physician trained in occupational safety and health.

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Table 1
Results of Moisture Meter Assessment
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3034

	Moisture Content			
Location	**Walls**	**Ceiling**	**Floor**	**Location of Highest Moisture Levels**
Room 1 *	5%-50%	5%-15%	10%-20%	50% on right corner of back wall
Room 2	0%-100%	5%-30%	5%-10%	100% under light on back wall
Room 3	5%-100%	5%-20%	10%-40%	100% on offset corner on left wall
Room 5	NA (tile)	5%-20%	10%-20%	None observed
Room 14	0%-20%	5%-80%	10%-40%	80% on wrinkled portion ceiling near diffuser
Room 16 *	0%-100%	5%-30%	30%-40%	100% on offset corner on right wall
Room 17 *	0%-15%	5%-40%	5%-20%	None observed
Room 18 †,‡	0%-100%	5%-40%	15%-20%	100% on all but right wall
Women's Restroom †	0%-100%	5%-15%	10%-50%	100% on back and right walls

***** Clean and dry above ceiling
† Apparent fungal growth above ceiling
‡ Apparent fungal growth between gypsum board layers on back wall

Table 2
Results of Sticky Tape Sample Analysis
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Room	Sample Location	Results
Room 14	Taken from exhaust vent in ceiling of shower	Few conidia/spores suggestive of *Stachybotrys* species, rare conidia/spores suggestive of *Alternaria*/*Pithomyces*/*Ulocladium* group, rare conidia/spores suggestive of *Chaetomium* species, rare dematiaceous hyphae
Room 14	Taken from exhaust vent in ceiling of shower	Rare *Cladosporium* species, rare dematiaceous hyphae, rare *Stachybotrys* species, rare unidentified hyaline conidia/spores
Room 17	Discolored grout near floor	Rare ascospores, rare conidia/spores suggestive of *Chaetomium* species
Room 18	Wall along fountain, taken from wall grout near floor	Rare ascospores, rare *Cladosporium* species
Room 18	Taken from wood shelf- wall along fountain	Few conidia/spores suggestive of *Aspergillus*/*Penicillium* group, rare *Cladosporium* species, rare dematiaceous hyphae
Room 18	Lifted from paper back of wallboard	Many ascospores, many dematiaceous hyphae, moderate ascocarps-most closely resembles *Ascotricha* species, moderate dematiaceous conidia/ spores-most closely resembles dicyma species, few conidia/spores suggestive of *Aspergillus*/*Penicillium* group
Women's Restroom	From discolored area on metal beam (behind wall)	Rare dematiaceous hyphae, rare conidia/spores suggestive of *Stachybotrys* species
Women's Restroom	From visible mold found under face plate of vent over door	Moderate conidia/spores-most closely resembles *Pyrenochaeta* species, moderate dematiaceous hyphae, rare *Alternaria* species
Women's Restroom	From visible mold found under face plate of vent over door	Many *Chaetomium* species, many dematiaceous hyphae, moderate conidia/spores suggestive of *Aspergillus*/*Penicillium* group, moderate *Cladosporium* species, moderate hyaline hyphae

Table 3
Results of Bulk Sample Analyses
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Room	Sample Location	Analysis	Results
Room 18	Paperback of wallboard behind wall shared with steam room	Direct Exam	Moderate conidia/spores suggestive of *Aspergillus*/*Penicillium* group, moderate dematiaceous hyphae, moderate *Stachybotrys* species, moderate dematiaceous conidia/spores- most closely resembles *Dicyma* species, few ascospores, rare conidia/spores suggestive of *Chaetomium* species, moderate bacterial rods/cocci
		Fungal Culture	$3.6x10^6$ CFU/g* of *Penicillium* species Morphotype 1, $1.2 x10^6$ CFU/g of *Acremonium* species, $1.2 x10^6$ CFU/g of *Aspergillus versicolor*, $1.2 x10^6$ CFU/g of *Scopulariopsis* species, $6.0 x10^5$ CFU/g of sterile dematiaceous mold-unable to identify further due to overgrowth of other mould *Aspergillus* species- Subgenus *Nidulantes* (*Aspergillus nidulans*/*ustus*)
		Bacterial Culture	$3.6 x10^7$ CFU/g of mixed bacteria including $9.8 x10^4$ CFU/g of *Pseudomonas aeruginosa*, 2 morphotypes
Room 18	Piece of wallboard	Direct Exam	Moderate ascospores- most closely resembles *Ascotricha* species, moderate conidia/spores suggestive of *Aspergillus*/*Penicillium* group, moderate dematiaceous hyphae, moderate dematiaceous conidia/spores-most closely resembles *Dicyma* species, few conidia/spores suggestive of *Stachybotrys* species, moderate bacterial rods/cocci
		Fungal Culture	$9.0 x10^6$ CFU/g of *Acremonium* species, $3.6 x10^6$ CFU/g of *Aspergillus versicolor*, $1.8 x10^6$ CFU/g of *Aureobasidium* species, $9.0 x10^5$ CFU/g of *Cladosporium* species, *Aspergillus* species subgenus *Nidulantes* (*Aspergillus nidulans*/*ustus*)
		Bacterial Culture	$8.3 x10^7$ CFU/g of mixed bacteria, including $1.8 x10^4$ CFU/g of *Pseudomonas aeruginosa*, two morphotypes
Room 18	Piece of wallboard	Direct Exam	Many ascospores, moderate ascocarps-most closely resembles *Ascotricha* species, both sexual and asexual (*Dicyma* species) forms, moderate conidia/spores suggestive of *Aspergillus*/*Penicillium* group, moderate dematiaceous hyphae, moderate *Stachybotrys* species, moderate dematiaceous conidia/spores, most closely resembles *Dicyma* species, moderate bacterial rods/cocci
		Fungal Culture	$4.8 x10^6$ CFU/g of *Aspergillus versicolor*, $1.6 x10^6$ CFU/g of *Dicyma* species- asexual form of *Ascotricha* species, $8.0 x10^5$ CFU/g of *Aspergillus flavus*, *Acremonium* species
		Bacterial Culture	$2.9 x10^7$ CFU/g of mixed bacteria

*CFU/g- colony forming unit per gram

Table 3 (Con't)
Results of Bulk Sample Analyses
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Room	Sample Location	Analysis	Results
Women's Restroom	From wall above toilet (opposite rock wall/planter)	Direct Exam	Moderate conidia/spores suggestive of *Aspergillus*/*Penicillium* group, moderate *Cladosporium* species, few dematiaceous hyphae, few unidentified dematiaceous conidia/spores, rare hyaline hyphae, moderate bacterial rods/cocci
		Fungal Culture	2.0 x10^6 CFU/g *of *Aspergillus versicolor*, 1.5 x10^6 CFU/g of *Aspergillus flavus*, 5.0 x10^5 CFU/g of sterile dematiaceous mold- unable to identify further due to over growth of other mold, 1.1 x10^5 CFU/g of *Cladosporium* species, *Acremonium* species
		Bacterial Culture	7.1 x10^6 CFU/g of mixed bacteria
Women's Restroom	From wall above toilet (opposite rock wall/planter)	Direct Exam	Moderate conidia/spores suggestive of *Aspergillus*/*Penicillium* group, moderate *Cladosporium* species, moderate yeast w/o pseudohyphae, few dematiaceous hyphae, few unidentified hyaline conidia/spores, moderate bacterial rods/cocci
		Fungal Culture	2.0 x10^7 CFU/g of *Cladosporium* species., 1.1 x10^7 CFU/g of sterile hyaline mold- 4.0 x10^6 CFU/g of black yeast-unable to identify further due to non-viability on subculture, 8.0 x10^4 of cream yeast- unable to identify further due to non-viability of subculture, 2.0 x10^4 CFU/g of *Aspergillus versicolor*, *Fusarium* species
		Bacterial Culture	4.8 x10^6 CFU/g of mixed bacteria
N/A	Sample of pool tile	Direct Exam	Rare dematiaceous hyphae, rare unidentified dematiaceous conidia/spores, few bacterial rods/cocci
		Fungal Culture	60 CFU/swab of *Sporothrix* species, 30 CFU/swab of *Penicillium* species- morphotype 2, 30 CFU/swab of *Pseudoallescheria boydii*, 30 CFU/swab of sterile dematiaceous mold
		Bacterial Culture	60 CFU/swab of mixed bacteria
		Fungal Culture	2.0 x10^5 CFU/swab of *Cladosporium* species, 2.0 x10^3 CFU/swab of A*cremonium* species, 240 CFU/swab of cream yeast; *Pithomyces* species
		Bacterial Culture	1.6 x10^6 CFU/swab of mixed bacteria

*CFU/g- colony forming unit per gram

Table 4
Results of Swab Sample Analysis
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Room	Sample Location	Analysis	Results
Fountain 4 (near Rm 18)	Scum around sump area on bottom of fountain after water drained	Direct Exam	Rare yeast w/o pseudohyphae, few bacterial rods/cocci, few flagellates, rare Rotifers
		Fungal Culture	6.0×10^3 CFU/swab* of black yeast,, 6.0×10^3 CFU/swab of sterile dematiaceous mould- morphotype 1; 2.0×10^3 CFU/swab of sterile dematiaceous mould- morphotype 2, 180 CFU/swab of *Cladosporium* species, 60 CFU/swab of *Aspergillus versicolor*
		Bacterial Culture	1.1×10^7 CFU/swab of mixed bacteria
Fountain 4 (near Rm 18)	Scum around sump area on bottom of fountain after water drained	Direct Exam	Few yeast w/ pseudohyphae, rare dematiaceous hyphae, few bacterial rods/cocci, few flagellates
		Fungal Culture	2.0×10^5 CFU/swab of *Cladosporium* species, 2.0×10^3 CFU/swab of A*cremonium* species, 240 CFU/swab of cream yeast, *Pithomyces* species
		Bacterial Culture	1.6×10^6 CFU/swab of mixed bacteria

*CFU/swab- colony forming unit per swab

Table 5
Summary of Air Sampling Results (Research) as Arithmetic Averages
December 2, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Location	PCR* (SE/m^3)**	Total Spores (S/m^3)†	Viable Fungi (CFU/m^3)‡
Room18 – Below Ceiling	1517	413	241
Room 18 – Above Ceiling	1579	614	157
Women's Restroom – Below Ceiling	479	282	154
Women's Restroom – Above Ceiling	2728	1450	436

*PCR – polymerase chain reaction analyses
**SE/m^3 – Spore equivalents per cubic meter (cassette)
†S/m^3 – Spores per cubic meter (spore trap)
‡CFU/m^3 – Colony forming units per cubic meter (Andersen N-6)

Table 6
Results of Pool Water Sample Analyses
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Pool	Sample Location	Analysis	Results
Double Waterfall (DWF)	Monitor	Direct Exam	No fungal elements seen, few bacterial rods/cocci
		Fungal Culture	No growth, no fungus isolated
		Bacterial Culture	$4.5x10^3$ CFU/mL* *Pseudomonas aeruginosa*
		Mycobacterial Culture	No mycobacterium species isolated
	Water Line	Direct Exam	No fungal elements seen, rare bacterial rods/cocci
		Fungal Culture	2 CFU/mL of sterile dematiaceous mold
		Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated
Lap pool (LAP)	Monitor	Direct Exam	No fungal elements seen, moderate bacteria rods/cocci
		Fungal Culture	No fungus isolated
		Bacterial Culture	$5.5x10^5$ CFU/mL of mixed bacteria including $5.0x10^5$ CFU/mL of *Pseudomonas aeruginosa*
		Mycobacterial Culture	No mycobacterium species isolated
	Water Line	Direct Exam	Few yeast w/o pseudohyphae, few bacteria rods/cocci
		Fungal Culture	1600 CFU/mL of *Exophiala* species,1 CFU/mL of *Acremonium* species
		Bacterial Culture	$1.3x10^6$ CFU/mL of mixed bacteria
		Mycobacterial Culture	No mycobacterium species isolated
	Pool	Direct Exam	No fungal elements seen, no bacteria seen
		Fungal Culture	No growth
		Bacterial Culture	1.0 CFU/mL of a catalase-positive, Gram-positive rod
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated
Men's Cold Plunge (MCP)	Monitor	Direct Exam	No fungal elements seen, moderate bacteria rods/cocci
		Fungal Culture	No fungus isolated
		Bacterial Culture	$3.2x10^5$ CFU/mL of mixed bacteria including $1.1x10^4$ CFU/mL *Pseudomonas* species-including $9.0x10^4$ CFU/mL of *Pseudomonas fluorescens* group (not *Pseudomonas aeruginosa*) and $2.0x10^4$ CFU/mL of *Pseudomonas aeruginosa*
		Mycobacterial Culture	No mycobacterium species isolated
	Water Line	Direct Exam	No fungal elements seen, moderate bacteria rods/cocci
		Fungal Culture	2 CFU/mL of *Exophiala* species
		Bacterial Culture	$2.0x10^5$ CFU/mL of *Pseudomonas fluorescens* group (not *Pseudomonas aeruginosa*)
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated

*CFU/mL = colony forming units per milliliter

Table 6 (con't)
Results of Pool Water Sample Analyses
November 3-4, 2003-3024
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005

Pool	Sample Location	Analysis	Results
Men's Hot Tub (MHT)	Monitor	Direct Exam	No fungal elements seen, moderate bacteria rods/cocci, few *Acanthamoeba* species
		Fungal Culture	No fungus isolated
		Bacterial Culture	$3.7x10^5$ CFU/mL* of mixed bacteria including $6.0x10^4$ CFU/mL of *Pseudomonas aeruginosa*
		Mycobacterial Culture	No mycobacterium species isolated
	Water Line	Direct Exam	No fungal elements seen, moderate bacteria rods/cocci
		Fungal Culture	No fungus isolated
		Bacterial Culture	$7.8x10^5$ CFU/mL of mixed bacteria
		Mycobacterial Culture	*Mycobacterium abscessus* isolated from broth only
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated
Men's Waterfall Whirlpool (MWF)	Monitor	Direct Exam	No fungal elements seen, moderate bacteria rods/cocci
		Fungal Culture	No fungus isolated
		Bacterial Culture	$5.0x10^5$ CFU/mL of mixed bacteria including $5.0x10^4$ CFU/mL of *Pseudomonas aeruginosa*
		Mycobacterial Culture	1 CFU/mL of *Mycobacterium avium* complex (two possible morphotypes)
	Water Line	Direct Exam	Rare unidentified hyaline conidia/spores, moderate bacteria rods/cocci
		Fungal Culture	2 CFU/mL of sterile dematiaceous mold
		Bacterial Culture	$9.4x10^5$ CFU/mL of mixed bacteria
		Mycobacterial Culture	6.6 CFU/mL of *Mycobacterium avium* complex (three possible morphotypes)
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated

*CFU/mL = colony forming units per milliliter

Table 6 (con't)
Results of Pool Water Sample Analyses
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Pool	Sample Location	Analysis	Results
Spa Mineral Pool (SPA)	Monitor	Direct Exam	No fungal elements seen, moderate bacterial rods/cocci
		Fungal Culture	No fungus isolated
		Bacterial Culture	$4.0x10^5$ CFU/mL* of mixed bacteria including $2.0x10^4$ CFU/mL of *Pseudomonas aeruginosa*
		Mycobacterial Culture	No mycobacterium species isolated
	Water Line	Direct Exam	rare dematiaceous hyphae, rare yeast w/o pseudohyphae, moderate bacterial rods/cocci
		Fungal Culture	3 CFU/mL of *Scedosporium apiospermum*
		Bacterial Culture	$6.6x10^5$ CFU/mL of mixed bacteria
		Mycobacterial Culture	No mycobacterium species isolated
	Pool	Direct Exam	No fungal elements seen, no bacteria seen
		Fungal Culture	No growth
		Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated
Women's Cold Plunge (WCP)	Monitor	Direct Exam	No fungal elements seen, no bacteria seen,
		Fungal Culture	No growth, no fungus isolated
		Bacterial Culture	2.0 CFU/mL of mixed bacteria
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated
Women's Hot Tub (WHT)	Monitor	Direct Exam	No fungal elements seen, moderate bacterial rods/cocci, few *Acanthamoeba* species, trophozoites
		Fungal Culture	No fungus isolated
		Bacterial Culture	$1.0x10^6$ CFU/mL *Pseudomonas aeruginosa*
		Mycobacterial Culture	No mycobacterium species isolated
	Water Line	Direct Exam	No fungal elements seen, rare bacterial rods/cocci
		Fungal Culture	2 CFU/mL of *Fusarium* species
		Bacterial Culture	$2.0x10^2$ CFU/mL bacteria
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	$8.0x10^2$ CFU/mL of an oxidase-positive, nonfermentative, gram-negative rod
		Mycobacterial Culture	No mycobacterium species isolated
		Bacterial Culture	1.0 CFU/mL of a catalase-positive, Gram-positive rod,1.0 CFU/mL of a catalase-positive, Gram-positive cocci
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated

*CFU/mL = colony forming units per milliliter

Table 6 (con't)
Results of Pool Water Sample Analyses
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Pool	Sample Location	Analysis	Results
Women's Waterfall Whirpool (WWF)	Monitor	Direct Exam	No fungal elements seen, moderate bacterial rods/cocci
		Fungal Culture	No fungus isolated
		Bacterial Culture	$5.1x10^5$ CFU/mL* of *Pseudomonas aeruginosa*, 2 morphotypes
		Mycobacterial Culture	No mycobacterium species isolated
	Water Line	Direct Exam	Rare hyaline hyphae, rare unidentified hyaline conidia/spores, moderate bacteria rods/cocci
		Fungal Culture	100 CFU/mL of Aureobasidium species
		Bacterial Culture	$1.0x10^6$ CFU/mL mixed bacteria
		Mycobacterial Culture	1CFU/mL *Mycobacterium avium* complex
	Pool	Direct Exam	No fungal elements seen, no bacteria seen
		Fungal Culture	No fungus isolated
		Bacterial Culture	1.0 CFU/mL of a catalase-positive, gram-positive rod,1.0 CFU/mL of a catalase-positive, gram-positive cocci
		Mycobacterial Culture	No mycobacterium species isolated
	Filter	Bacterial Culture	No growth
		Mycobacterial Culture	No mycobacterium species isolated

*CFU/mL = colony forming units per milliliter

Table 7
Results of Fountain Water Sample Analyses
November 3-4, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Fountain	Analysis	Results
Fountain 1 (Near Room 3)	Direct Exam	Few unidentified hyaline conidia/spores, rare *Cladosporium* species, rare dematiaceous hyphae, few bacterial rods/cocci, few flagellates, rare *Naegleria* species, trophozoites
	Fungal Culture	12 CFU/mL* of *Exophiala* species
	Bacterial Culture	$1.3x10^3$ CFU/mL of mixed bacteria including 6.0 CFU/mL *Pseudomonas fluorescens* group
	Mycobacterial Culture	No mycobacterium species
Fountain 2 (Near Room 8)	Direct Exam	Rare dematiaceous hyphae, rare yeast w/o pseudohyphae, few bacterial rods/cocci, few flagellates, few debris
	Fungal Culture	14 CFU/mL of *Phialophora* species, 2 CFU/mL of a sterile hyaline mold
	Bacterial Culture	$3.0x10^4$ CFU/mL of mixed bacteria including 31 CFU/mL *Pseudomonas fluorescens* group
	Mycobacterial Culture	No mycobacterium species isolated
Fountain 3 (Near Room 12)	Direct Exam	Rare yeast w/o pseudohyphae, moderate bacterial rods/cocci, few flagellates
	Fungal Culture	4 CFU/mL of a sterile dematiaceous mold
	Bacterial Culture	$3.0 x10^3$ CFU/mL mixed bacteria
	Mycobacterial Culture	No mycobacterium species isolated
Fountain 4 (Near Room 18) Sample 1	Direct Exam	Rare dematiaceous hyphae, rare yeast w/o pseudohyphae, rare bacterial rods/cocci, rare debris
	Fungal Culture	100 CFU/mL of *Scolecobasidium* species, 3 CFU/mL of a sterile hyaline mold, 2 CFU/mL of *Aureobasidium* species
	Bacterial Culture	200 CFU/mL mixed bacteria
	Mycobacterial Culture	1 CFU/mL of an organism closely resembling *Mycobacterium fortuitum*, 1CFU/mL *Mycobacterium gordonae*
Fountain 4 (Near Room 18) Sample 2	Direct Exam	Rare dematiaceous hyphae, rare yeast w/o pseudohyphae, few bacterial rods/cocci, few flagellates
	Fungal Culture	12 CFU/mL of a sterile dematiaceous mold (unable to identify further due to non-viability on subculture), 6 CFU/mL of *Aureobasidium* species, 5 CFU/mL of a sterile hyaline mold, morphotype1, 1 CFU/mL of a sterile hyaline mold, morphotype2
	Bacterial Culture	100 CFU/mL mixed bacteria including 3 CFU/mL of *Pseudomonas fluorescens* group
	Mycobacterial Culture	No mycobacterium species isolated

*CFU/mL = colony forming units per milliliter

Table 8
Results of Pool Water Sample Analysis for Endotoxin
December 2, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

Location	Result (EU/mL)*		
	Monitor	Water line	Pool
Double Waterfall Pool (DWF)	1.5	1.3	N/A
Lap Pool	0.44	0.38	0.29
Men's Cold Plunge (MCP)	17	15	N/A
Men's Hot Tub (MHT)	4.8	7.7	N/A
Men's Waterfall Whirlpool (MWF)	24	26	N/A
Spa Pool	4	1.7	3.2
Women's Cold Plunge (WCP)	4.7	2.8	N/A
Women's Hot Tub (WHT)	17	20	N/A
Women's Waterfall Whirlpool (WWF)	61	8.6	93

Limit of detection = 0.005
Limit of quantification = 0.05
*EU/mL = endotoxin units per milliliter

Table 9
Pool Water Chemistry – December 2, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

	Pool									
	DWF	**LAP**	**MCP**	**MHT**	**MWF**	**OHT**	**SPA**	**WCP**	**WHT**	**WWF**
Volume (gal.)*	3372	19382	377	1387	2515	9067	23075	377	1387	2515
Temperature	78	83	65	102	102	103	87	67	101	102
ORP†	688	774	733	732	759	720	2.5	781	729	732
Salt (ppm)‡	1	2	3-3.5	5	5	2.5	2.5	5	2.5-3	3
pH	7.6	7.5	7.4	7.6	7.6	7.5	7.5	7.5	7.6	7.6
Total alkalinity (ppm)	60	100	80	110	100	100	100	90	120	90
Calcium hardness (ppm)	60	280	150	240	160	230	250	200	300	170
Free chlorine (ppm)	0.94	2.19	3.05	4.11	4.06	2.67	2.79	6.95	2.84	4.3
Total chlorine (ppm)	1.04	2.38	4.58	5.72	6.92	2.94	3.73	8.93	4.4	5.65
Chloride (ppm)	51	527	215	640	330	NA	1833	135	755	347

*gal. – gallons
†ORP – oxidation reduction potential
‡ppm – parts per million

DWF – Double Waterfall Pool
LAP – Lap Pool
MCP – Men's Cold Plunge
MHT – Men's Hot Tub
MWF – Men's Waterfall Whirlpool
OHT – Outdoor Hot Tub
SPA – Spa Pool
WCP – Women's Cold Plunge
WHT – Women's Hot Tub
WWF – Women's Waterfall Whirlpool

Table 10
Demographics of Employees Interviewed
November 10-14, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

		Massage Therapists (n=8)	Maintenance (n=7)	Management (n=18)
Average Age		41	39 46	
Average Tenure (years)*		2.5	3.6	10.3
Average Weekly hours*		37	48 55	
Smoking history	Current	1 (13%)	1 (14%)	5 (28%)
	Former	2 (25%)	2 (29%)	6 (33%)
	Never	5 (63%)	4 (57%)	7 (39%)
Male		4 (50%)	6 (86%)	13 (72%)
Female		4 (50%)	1 (14%)	5 (28%)
Atopy†		3 (43%)	2 (29%)	7 (39%)
Physician diagnosed allergy to mold		0	0	3 (17%)

* Significant difference between groups ($p < 0.05$)
†Atopy is a history of hay fever, eczema, or asthma, and indicates a genetic predisposition toward allergic disorders

Table 11
Prevalence of Work-related* Symptoms in the 4 Weeks
Prior to the Survey of Employees Interviewed
November 10-13, 2003
Grove Park Inn Resort and Spa, Asheville, North Carolina
HETA 2004-0005-3024

	Massage Therapists (n=8)	Maintenance (n=7)	Management (n=18)
Wheezing	1/7 (14%)	0/7	0/18
Cough	5/7 (71%)†	1/7 (14%)	1/18 (6%)
Shortness of breath	3/7 (43%)	0/7	1/18 (6%)
Fever	2/7 (29%)	1/7 (14%)	0/18
Achiness	5/7 (71%)†	1/7 (14%)	1/18 (6%)
Sinus problem	4/8 (50%)†	0/7	0/17
Rash, dermatitis, or eczema	2/7 (29%)	0/7	0/18
Dry or irritated eyes	3/8 (38%)	0/7	1/18 (6%)
Headache	4/8 (50%)	0/7	4/18 (22%)
Sore or dry throat	4/7 (57%)†	0/7	1/18 (6%)
Sneezing	5/8 (63%)†	0/7	2/18 (11%)
Fatigue	4/8 (50%)†	2/7 (29%)	1/18 (6%)

*Work-related is defined as sometimes or usually present at work and improving on days off work.
† Significant difference when compared to referent group (management)

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