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HETA 98-0026-2745 Ronald McDonald House of Durham Durham, North Carolina

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PREFACE

The Hazard Evaluations and Technical Assistance Branch 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 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 employer 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.

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ACKNOWLEDGMENTS AND AVAILABILITY OF REPORT

This report was prepared by Angela Weber and Elena Page of the Hazard Evaluations and Technical Assistance Branch, Division of Surveillance, Hazard Evaluations and Field Studies (DSHEFS). Field assistance was provided by Kenneth Martinez and Gregory Burr. Analytical support was provided by P & K Microbiology Services, Inc. Desktop publishing was performed by Pat Lovell. Review and preparation for printing was performed by Penny Arthur.

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Health Hazard Evaluation Report 98-0026-2745 Ronald McDonald House of Durham Durham, North Carolina July 1999

Angela M. Weber, M.S. Elena H. Page, M.D., M.P.H.

SUMMARY

In November 1997, the National Institute for Occupational Safety and Health (NIOSH) received a management request from the Steven Brouillard Construction Company (SBCC) for a health hazard evaluation (HHE) at the Ronald McDonald House (RMDH) in Durham, North Carolina. Employees were concerned about adverse health effects (diarrhea, stomach cramps, vomiting, dizziness, weakness, and fatigue), which they attributed to exposure to fungi and toxins encountered during the renovation of contaminated building materials at the RMDH. The house, which is used by outpatient pediatric cancer patients from Duke University Hospital, had experienced moisture problems since 1986 from water incursion through the exterior walls, from roof leaks, and from improperly draining thru-wall heat pump units. In 1993, a flood caused major water damage on both floors of the house, including rooms used as suites for transplant patients.

NIOSH investigators conducted an initial site visit at the RMDH on November 12-13, 1997, and returned to conduct microbial sampling on November 24-25, 1997. Microbial sampling was performed to evaluate whether the numbers and kinds of fungi in the RMDH were atypical of those fungi found in non-contaminated buildings. There was also concern that prior remediation work (performed without isolation barriers in place) may have contaminated surrounding areas as well as the ventilation system. The environmental evaluation included: (1) physical inspection of heat pump systems and contaminated building materials, (2) collection of air samples for culturable fungi and total spores, (3) collection of air samples for thermotolerant fungi including *Aspergillus fumigatus*, (4) real-time measurements of temperature and relative humidity, and (5) collection of bulk materials and settled dust for culturable fungi. The medical evaluation consisted of confidential medical interviews with seven RMDH employees and five SBCC employees. Medical records for four of the five SBCC employees were reviewed.

Results of airborne and bulk sampling indicated extensive contamination of the building with a variety of molds that are capable of producing mycotoxins under certain conditions, including *Aspergillus versicolor*, *Stachybotrys chartarum (atra)*, *Memnoniella echinata*, and *Penicillium aurantiogriseum*. Aspergillus versicolor was present in all 17 indoor sample locations. In comparison, only two colonies of *Aspergillus versicolor* were detected in the outdoor air on the dates of sampling. Airborne concentrations of *Stachybotrys chartarum*, while not found in any of the outdoor samples, were detected in six of the indoor locations (all areas had yet to be remediated). Analysis of bulk samples of contaminated building materials (e.g., sheetrock, baseboard molding, and thru-wall heat pump filters) revealed that these areas were serving as amplification sites for microbiological growth. *Aspergillus fumigatus* (which can cause aspergillosis in immuno-compromised patients) was the predominant thermotolerant organism found in dust collected from the thru-wall heat pump unit filters in two of the transplant suites. Spores

in settled dust collected from the return air plenum above the ceiling tiles indicated that previous remediation efforts, conducted without isolation barriers in place, may have resulted in the dissemination of spores throughout the ventilation system. Based on these findings, NIOSH recommended immediate microbial remediation of the house.

In the medical interviews, the SBCC employees reported diarrhea, vomiting, and fatigue in the absence of respiratory and/or dermatological symptoms. Employees of the RMDH did not report any significant symptoms. Medical records review did not reveal any evidence of fungal infections or mycotoxicoses in the employees of the SBCC. There was evidence of an infectious gastroenteritis (bacterial) in one SBCC employee. The illnesses experienced by the 5 SBCC employees were not consistent with fungal allergy, infection, or mycotoxicosis. A specific cause for their illnesses was not identified.

It is not likely that the workers' illnesses were related to a workplace exposure. The materials the workers were tearing out were contaminated with fungus, however, the workers' illnesses were not consistent with allergy, fungal infection, or mycotoxicosis. Although exposures to employees at the time the symptoms were reported were not determined, a health hazard potentially existed for employees of both the SBCC and the RMDH, as well as to the clients of the RMDH. Also, past flooding, moisture incursion through exterior walls, roof leaks, and improperly draining thru-wall heat pump units resulted in providing chronically moist building materials ideal for fungal growth. The potential for fungal growth to re-occur on newly replaced materials is likely, since the leaks in the foundation of the building had not been corrected.

Keywords: SIC 8361 (Residential Care), indoor environmental quality, bioaerosols, microorganisms, fungi, spores, mycotoxins, microbial remediation, IEQ, IAQ.

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INTRODUCTION

In November 1997, the National Institute for Occupational Safety and Health (NIOSH) received a management request from the Steven Brouillard Construction Company (SBCC) for a health hazard evaluation (HHE) at the Ronald McDonald House (RMDH) in Durham. North Carolina. The HHE requesters expressed concern over employees' reports of adverse health effects (diarrhea, stomach cramps, vomiting, dizziness, weakness, and fatigue), which the SBCC employees attributed to exposures to fungi and their toxins while performing renovations on contaminated building materials at the RMDH. In response, NIOSH investigators conducted an initial site visit at the RMDH on November 12-13, 1997, and returned to conduct microbial sampling on November 24-25, 1997. Preliminary sampling results indicated that immediate microbial remediation actions were These findings, as well as initial necessary. recommendations, were sent to the RMDH in an interim letter on December 29, 1997. The information in this report reflects conditions at the facility at the time of these evaluations.

BACKGROUND

Facility Description

The RMDH is a facility for outpatient pediatric cancer patients at Duke University Hospital. The house is a residential facility for children and their parents who live more than 50 miles away and would have to travel or stay in a hotel to receive outpatient care. Families must receive permission from Duke University to stay at the RMDH. There are six employees who work between 20 and 40 hours weekly, and a maintenance employee who works about ten hours per week. The resident manager lives in the building with her family. There are also eight employees who rotate weekend coverage and thus are in the building an average of one weekend every two months.

The 5,000-square foot building consists of four transplant suites for children who have received bone marrow transplants, 18 guest rooms, a resident manager's apartment, kitchen, laundry facilities,

offices, and common areas such as a play room and lobby. The evaluated areas of the building can be found in Appendix A of this report and are shown in Figures 1 - ground floor (page 20) and Figure 2 main floor (page 21). The maximum facility capacity is 22 families, and the maximum guest room capacity is four persons per room. The original two-story facility was built in 1961 with additions made in 1985 (a resident manager's apartment, transplant suites A-9 and B-10, and guest rooms A-8, A-10, and B-9 were added to the south side of the building), in 1991 (a smoking lounge was added to the northwest corner of the building), and in 1993 (a dining room and transplant suites B-4 and B-5 were added to the east side of the building). Office space is located in the loft of the facility.

All transplant suites and guest rooms (except rooms A-6 and A-7) are ventilated by thru-wall heat pump systems. All other areas of the building are served by one of five central heat pump units. Air is returned to the central units from the occupied spaces via a common return air ceiling plenum.

History of Water Damage

According to RMDH management representatives, the building has experienced moisture problems since 1986 from water incursion through the exterior walls, from roof leaks, and from improperly draining thru-wall heat pump units. RMDH management representatives reported that musty odors have been present in the building since at least 1993. In May 1993, a flood caused major water damage on both floors of the building, including the resident manager's apartment, several guest rooms, and the smoking lounge. Transplant suites B-4 and B-5 were flooded from water entering through the thru-wall heat pump units into the rooms. As part of the flood remediation activities performed by a professional contractor, contaminated (moldy) baseboard molding was cleaned and/or removed. Most of the porous materials (e.g., sheetrock, ceiling tile) which had become wet during the flood were not replaced.

Duke University Occupational and Environmental Safety Office representatives indicated they had previously been involved in the evaluation of microbial contamination problems at the RMDH. In 1995, they found elevated concentrations of *Penicillium* in the lobby of the building; the presence of fungi was thought to be associated with condensation and moisture incursion around the chimney located in that room. In addition, they evaluated moisture problems related to the thru-wall heat pumps and found elevated concentrations of Gram-negative bacteria (specifically, *Pseudomonas*) in the drain pans of the units. A strong musty odor was associated with the contamination.

Remodeling Activities

Remodeling work at the RMDH included the installation of new flooring throughout the building, as well as re-installing the cleaned thru-wall heat pump units to prevent further water damage from improper draining of condensation into the wall cavity. Remodeling work also included repairing the water-damaged sheetrock under the heat pump units. In June 1997, a general contractor was hired; however, employees of the SBCC reported that the original contractor became ill while performing the remodeling work. They were unaware of the details of his illness. However, this individual reported to the NIOSH physician that he was not ill from the workplace, but had an unrelated problem, and left the job for personal reasons. The SBCC was then hired and began work on August 1, 1997. Employees were general contractors with no training or experience in handling microbial-contaminated building materials. Personal protective equipment (PPE) was not worn by the construction workers, and no isolation precautions were taken to prevent contamination of other areas of the building. The building was occupied by patients, guests, and RMDH employees during the remodeling work.

During the process of remodeling the rooms, SBCC employees noted large amounts of black and golden fungal growth on the back of the water-damaged sheetrock, located under the thru-wall heat pump units where the water had been draining, and in the heat pump units themselves. Water was also seeping through the foundation of the building through holes in the block wall and through leaks in the expansion joints due to an inadequate drainage system. According to the construction company's project notes, most wall cavities were not sealed behind the wall sleeves, leaving open the space between the interior block wall and the exterior brick. Since the wall cavity was not sealed, damp air was allowed to enter the building. Vapor barriers were absent throughout the building, and electrical boxes were rusty due to water incursion.

Of the 16 carpeted areas which were scheduled for replacement with PergoTM flooring, all but two (A-6 and A-7) were wet below the thru-wall heat pump units. Three different types of flooring were used throughout the building. Five rooms had vinyl flooring (A-9, B-4, B-5, B-6, B-10), four rooms had congolium flooring (A-3, B-2, B-7, B-8), while all other rooms were carpeted.

Two to three weeks after starting work at the RMDH, SBCC employees began experiencing health problems and expressed their concerns to RMDH management regarding their exposures to contaminated building materials. The Duke University Occupational and Environmental Safety Office was then asked by the RMDH to evaluate the renovation activities. On September 4, 1997, Duke University collected one air sample for viable fungi in the construction area of the basement (the room location was not identified). No comparison samples were collected from the outdoor air or from control locations (areas not under construction). The sampling was limited in scope and did not include assessing the presence of thermotolerant species of Aspergillus. According to a memo sent from Duke University to the RMDH, a concentration greater than 2,628 colony forming units per cubic meter (cfm/m³) was recovered (location is not mentioned), consisting predominantly of Cladosporium, Penicillium, and other fungi not speciated. An exact concentration was not determined because the culture plate was overgrown with fungi. Analysis of tape preparations collected from the back of the sheetrock demonstrated the presence of Stachybotrys chartarum.

The Infection Control Department of Duke University Hospital provided the construction crew with recommendations on September 17, 1997. Although recommendations included using PPE (i.e., particulate respirators, coveralls, and gloves), restricting access to the work area, and using plastic barriers between the work areas and resident rooms, they were not consistent with the current recommended guidelines for the remediation of fungi.¹

Approximately two weeks prior to the initial site visit (when NIOSH first contacted the requester), NIOSH recommended that all remediation work be stopped until the appropriate environmental controls were put into place and appropriate PPE was available. The SBCC was sent published guidelines concerning remediation of microbial-contaminated materials.¹

METHODS

Medical Evaluation

During the initial site visit on November 12-13, 1998, NIOSH representatives met at the RMDH with local representatives from the SBCC, the RMDH, and Duke University. Interviews were conducted with all five employees of the SBCC, the original contractor who began the job, as well as all seven full-time employees of the RMDH. Volunteers who work for the RMDH, as well as the eight persons who rotate weekends at the RMDH, were not interviewed because of the limited time they spent at the facility. Medical records were reviewed for four of the SBCC employees; one SBCC employee did not seek medical care. The interviews covered occupational, environmental, and medical histories, a review of systems, work practices, and detailed questioning about any symptoms reported, including their relationship to work. The goal was to identify any potential work-related illness or symptoms.

Environmental Evaluation

During the initial site visit, NIOSH conducted a visual inspection of the entire facility, which included areas that had already been remediated and

those which were still scheduled to be remediated. Based on the visual assessment, NIOSH determined that a follow-up visit was warranted to evaluate whether the concentrations and the species of fungi in the RMDH were atypical of those fungi found in non-contaminated buildings. There was also concern that prior remediation work (performed without isolation barriers in place) may have contaminated surrounding areas as well as the heating, ventilating, and air conditioning (HVAC) system.

The follow-up site visit was made on November 24 and 25, 1997, and consisted of sampling for airborne culturable fungi, total airborne spore concentrations (both viable and nonviable), culturable fungi in settled dust and bulk materials, and cello-tape sampling of surfaces. Remediation work was not being performed during the evaluation. The goal of the NIOSH sampling strategy was threefold: (1) to determine whether dissemination of spores had occurred from areas previously renovated, (2) to evaluate whether fungi had been adequately removed from the renovated areas, and (3) to assess the background concentrations and species of fungi in rooms which were scheduled for remediation. At the time of the evaluation, the following rooms had already been remediated: transplant suite B-10, the laundry room, the play room (south wall only), and guest rooms B-1, B-2, B-3, B-7, B-8, B-9, A-1, and A-11.

Microbial Air Sampling

To determine the concentrations of culturable airborne fungi, Anderson 2-stage viable cascade impactors were used at a calibrated flow rate of 28.3 liters per minute (lpm) over a sampling period of 10 minutes. Only one petri dish was loaded in the lower stage of the impactors thus using it as a one-stage impactor. Air samples were collected over the course of the day from each location on two types of media: malt extract agar (MEA) for hydrophilic fungi and corn meal agar (CMA) for *Stachybotrys chartarum*. An additional sample was collected at each site on MEA to evaluate the presence of thermotolerant fungi (incubated at 35[°] C) including *Aspergillus fumigatus*. Thermotolerant fungi, such

as *Aspergillus fumigatus* and *Aspergillus flavus*, are of primary concern in facilities such as the RMDH since they can cause infection (i.e., aspergillosis) in at-risk patients even when exposure occurs at very low concentrations.² The taxa and rank of the collected microorganisms were determined by morphological characteristics.

Air samples for culturable fungi were collected from 17 interior locations and 3 outdoor locations over a two-day period. Interior locations included both floors of the resident manager's apartment, transplant suites B-10, B-5, B-4, and A-9, the office located in the loft, the smoking lounge, the play room, the dining room, and guest rooms A-2, A-4, A-6, A-12, B-2, B-7, and B-9. Four replicate samples were collected in the transplant suites, three replicate samples were collected at all other interior locations, and two replicate samples were collected at each of the three outdoor sample locations. Temperature and relative humidity (RH) were recorded at each location.

Airborne concentrations of total spores (both viable and nonviable) were measured in the same 17 interior locations and 3 outdoor locations that were sampled for culturable fungi. Spores were collected with polycarbonate filters with a pore size of 0.2 micrometers (µm) and a diameter of 37 millimeters (mm). The filters were placed on cellulose support pads and sealed in plastic filter cassettes. The filter holders were connected via TygonTM tubing to Gillian Hi Flow SamplerTM battery-operated personal sampling pumps operating at a flow rate of three lpm over a seven hour time period. Calibration of the flow rates was performed immediately prior to, and after, sampling. For subsequent calculation of sample volumes, the mean of the pre- and post-sampling flow rates was used. Samples were analyzed for fungal spore counts by optical microscopy. Filters were cleared with acetone vapor, mounted in cotton blue/lactic acid, and scanned at 400X magnification with bright field or phase contrast illumination. Two hundred fields were counted for each sample. Only particles greater than $2 \mu m$ in diameter were considered to be possible fungal spores.

Bulk Material Sampling

Settled dust samples were collected from four areas of the building: two samples consisted of dust collected on return air filters located in the thru-wall heat pump units in transplant suites B-4 and B-5; the other two dust samples were collected from the upper surfaces of ceiling tile located in guest room B-2 and transplant suite B-10 (dust was collected from the ceiling tiles in these remediated rooms due to concerns regarding potential dissemination of remediation dust into the return air system). Dust amples were collected on 37 mm polycarbonate filters which had been placed on cellulose support pads and sealed in plastic filter cassettes. The filter holders were connected via TygonTM tubing to a high flow electrical-powered sampling pump. The opening of the cassette was used for dust collection.

Nine bulk samples were collected of visiblycontaminated building materials such as moldy sheetrock, filters from the thru-wall heat pump units, paper-backing behind baseboards, and materials from the central heat pump units. Both types of samples (dust and bulk) were mixed with sterile water and serially diluted on MEA (one plate incubated at 25[°]C and another plate incubated at 35[°]C) and CMA. Fungi were enumerated and identified (results are expressed as colony forming units per gram of material [CFU/g]).

Four cello-tape samples were collected from surfaces in the heat pump serving the resident manager's apartment and were analyzed by direct microscopic examination. This is a semi-quantitative (reported by the laboratory as few, many, massive amounts of spores present) and a qualitative (type of fungal structure) evaluation of visible mold growth. This method was used to determine whether fungi were actively growing (at some time) on the sampled surface.

Temperature and RH measurements were made using a Vaisala, Model HM 34, battery-operated meter. This meter is capable of providing direct readings for dry-bulb temperature and RH, ranging from -4 to 140[°] F and 0 to 100%, respectively.

EVALUATION CRITERIA

Health Effects of Fungi

Fungi comprise 25% of the biomass of earth;³ therefore, human exposure to fungi is ubiquitous. Although there are thousands of fungal species, reports of human and animal diseases have involved fewer than 100 species.³ Saprophytic fungi (i.e., those utilizing non-living organic matter as a food source) inhabit soil, vegetation, water, or any reservoir that can provide an ample supply of nutrients. Fungi can produce adverse health effects by three known mechanisms: (1) immunologic hypersensitivity to the fungus (allergy), (2) fungal infection (i.e., mycosis), and (3) mycotoxicosis, a reaction to toxins produced by the fungus.⁴

Health effects related to allergic responses are based, partly, on a genetic predisposition.⁵ Allergic diseases typically associated with exposures in indoor environments include allergic rhinitis (nasal allergy), allergic asthma, allergic bronchopulmonary aspergillosis (ABPA), and extrinsic allergic alveolitis (hypersensitivity pneumonitis).⁶ Allergic respiratory diseases resulting from exposures to microbial agents have been documented in agricultural, biotechnology, office. and home environments.7,8,9,10,11,12,13 Allergic rhinitis is characterized by sneezing; itching of the nose, eyes, palate, or pharynx; nasal stuffiness with partial or total airflow obstruction; and rhinorrhea (runny nose) with postnasal drainage. Allergic asthma is characterized by episodic or prolonged wheezing and shortness of breath in response to bronchial (airways) ABPA is characterized by cough, narrowing. exhaustion, low-grade fever, and wheezing.^{5,14} Heavy exposures to airborne microorganisms can cause an acute form of extrinsic allergic alveolitis, which is characterized by chills, fever, malaise, cough, and dyspnea (shortness of breath) appearing four to eight hours after exposure. In the chronic form, thought to be induced by continuous low-level exposure, onset occurs without chills, fever, or malaise and is characterized by progressive shortness of breath with weight loss.¹⁵

Mycotoxins

Many, if not all, molds can produce mycotoxins,³ which are non-volatile fungal metabolites with the potential to cause toxic reactions.^{16,17} There are more than 300 known mycotoxins.¹⁸ Mycotoxin production is affected by a variety of conditions such as fungal strain, genetic susceptibility of the host plant or commodity, moisture content, temperature, aeration, microbial population, and stress factors.¹⁹ Human disease thought to be caused by the tricothecenes, a commonly occurring category of mycotoxins, was first reported in Russia. Between 1942 and 1947, there were epidemics of alimentary toxic aleukia (ATA), which was often fatal and characterized by vomiting, skin inflammation, hemorrhaging of the gastrointestinal tract and mucous membranes, immunosuppression, and pancytopenia (a simultaneous decrease in the numbers of red cells, white cells, and platelets in the blood).^{20,21,22,23,24,25} ATA was attributed to eating overwintered grain contaminated by Fusarium species, and T-2 toxin (a specific trichothecene) has been implicated as the causative agent.^{22,24,25} The symptoms of ATA usually occurred after eating at least two kilograms of grain, and poor nutritional status appeared to be a risk factor.²⁴

In 1987 an outbreak of gastrointestinal illness related to the consumption of bread made from wheat contaminated with *Aspergillus* and *Fusarium* occurred in India.²⁶ Ninety-seven people reported abdominal pain or fullness occurring within 15-60 minutes after eating the bread. Sixty-three percent also had sore throat, 39% had diarrhea, and 7% vomited. The symptoms were reported to have lasted as long as the food was being consumed, and resolved upon cessation of consumption. Several tricothecenes, including vomitoxin (the predominant isolate), nivalenol, and acetyldeoxynivalenol were isolated from wheat samples. A similar illness was reported after consumption of moldy rice in China in the early 1990's.²⁷ T-2 toxin was reported to be the etiologic agent in that outbreak.

Stachybotrys

Fungi of the genus Stachybotrys are found worldwide²⁸ and have been isolated from soil and a wide variety of substances rich in cellulose, such as hay, wood pulp, cotton, grains, various dead plant components, paper, and glue in book bindings.²⁹ Buildings where Stachybotrys growth problems are reported have typically experienced chronic water damage and were kept at a temperature conducive to the growth of Stachybotrys (72-82 degrees Fahrenheit). Stachybotrys chartarum (atra) is one of many fungi capable of producing tricothecene mycotoxins (including roridan, verrucarin, and the satratoxins) under certain environmental conditions. Tricothecenes have been found in the aerosolized spores of this fungus, indicating the potential for inhalation exposure to these compounds.³⁰

Mycotoxicosis produced by Stachybotrys species is termed stachybotryotoxicosis, and is well known to veterinarians. Stachybotryotoxicosis has been reported to affect both large and small animals, especially during the early 1900's in Russia and Europe, when ingestion of mold-contaminated feeds (hay, grains, etc.) was thought to be responsible for disease manifested in animals by stomatitis, hemorrhage and necrosis of the gastrointestinal tract, leukopenia, and immunosuppression.^{16,25,28,31,32} Characteristic dermal manifestations are ulcerations, hyperemia (redness), edema, and tissue necrosis of varying severity. An atypical form can occur with loss of reflexes, hyperirritability, loss of vision, and inability to move about.²⁴ Laboratory studies revealed that the severity of the illness was dosedependent and that the tricothecene mycotoxins elaborated by the fungi were the responsible agents.³¹

There are several potential routes of human exposure to the mycotoxins produced, including inhalation, ingestion, and absorption through the skin. Local skin irritation due to handling of material contaminated by this fungus has been reported, but whether or not systemic effects occur due to skin absorption is unknown.²⁰ An experimental study of T-2 toxin showed low penetration through excised human abdominal skin,³³ but it is not known if these results would apply to the other tricothecenes.

Stachybotryotoxicosis in humans has been described in case reports from eastern Europe and Russia. Russian investigators reported stachybotryotoxicosis in humans who had contact with straw or hay in areas where stachybotryotoxicosis was enzootic in horses.³¹ The affected individuals developed severe dermatitis, chest pain, sore throat, bloody rhinitis, cough, and (in some) leukopenia.25,28,31 In experimental human studies, mold placed on the skin reproduced the clinical syndrome described above.^{29,31} An outbreak of stachybotryotoxicosis in 1977 involved 23 workers who loaded and supplied moldy hay.³⁴ Symptoms began within 24 hours of exposure and consisted of dyspnea, sore throat, bloody nose or bloody nasal discharge, and burning and watering of the eyes. The affected workers had hyperemic, swollen, crusted skin on the face, and dermatitis in the groin and buttocks. Recovery occurred within one week after cessation of exposure. Skin scrapings and nasal and throat swabs grew Stachybotrys chartarum. Specimens from the straw also grew Stachybotrys chartarum. Other cases of stachybotryotoxicosis related to occupational exposure have been reported among workers at farms, cottonseed oil mills, grain elevators, and facilities used for reprocessing moldy grain, malt grain processing facilities, textile mills using plant fibers, and bindertwine factories.²⁴ The persons affected were reported to have recovered rapidly after cessation of workplace exposure, and re-exposure resulted in more serious sequellae.²⁴

In summary, several case reports and studies have suggested *Stachybotrys* species and its mycotoxins as causes of human illness in the non-agricultural environment.^{35,36,37,38,39,40} Because of methodologic limitations, however, these studies cannot be regarded as conclusive evidence of a link between mycotoxin exposure in the home or office environment and human illness.^{41, 42,43}

Aspergillus

Aspergillus is a ubiquitous mold; there are over 600 species in the genus *Aspergillus*. Most *Aspergillus* species are found in soil, although many species can be found on a wide variety of substrates, including forage and food products, cotton, and other organic debris. *Aspergillus fumigatus*, the most common species, accounts for most disease attributable to *Aspergillus*, both allergic and infectious. Groups at risk of high exposure to this fungus and resultant hypersensitivity pneumonitis, include farmers; bird hobbyists; workers in sawmills, greenhouses, cane mills, or breweries; and people who work around mushrooms, tobacco, grain, compost piles, or decomposing haystacks.^{444,546,47,48,49}

Aspergillus versicolor has the potential to produce sterigmatocystin, a mycotoxin closely related in structure and biological activity to the aflatoxins. Although, aflatoxins are potent liver carcinogens and represent a risk to those exposed to high concentrations, there are no reported cases of liver cancer or other toxic effects associated with exposures to Aspergillus in office buildings.

Exposure to Aspergillus species has been reported to cause a variety of health problems. These include asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis, allergic sinusitis, The clinical manifestations of and infection. Aspergillus-related asthma are no different than other forms of asthma. Symptoms include cough, wheezing, chest tightness, and dyspnea, and obstructive changes on pulmonary function testing are present during acute attacks. Hypersensitivity pneumonitis can occur in individuals with repeated exposure to organic dusts containing Aspergillus species. Acute symptoms may occur 6-12 hours after exposure. They include myalgias (muscle aches), weight loss, fatigue, chest tightness, cough, and shortness of breath on exertion. Acute episodes are self-limiting, but upon repeated exposure, the condition can become chronic. ABPA is an inflammatory disease caused by an immunologic response to Aspergillus fumigatus and other Aspergillus species growing in the bronchi of patients with asthma.⁵⁰ Allergic fungal sinusitis

(AFS) due to *Aspergillus* species typically occurs in allergic immunocompromised patients. Most patients have asthma, and 85% have nasal polyps. Invasive aspergillosis is a very serious infectious disease which can be fatal, and typically occurs in immunocompromised patients, most notably those with leukemia or lymphoma.

Penicillium

The blue-green molds of *Penicillium* are common contaminants of indoor environments. Exposure to *Penicillium* can occur as a result of contaminated humidifier water or moldy HVAC systems. Inhalation of airborne spores is the major route of entry. These molds are common contaminants of agricultural commodities and some of the mycotoxins produced by these species are also produced by fungi common in house dust.⁵¹ *Penicillium* infections of clinical importance are very rare, although this mold has been associated with asthma and hypersensitivity pneumonitis.

RESULTS

Medical Evaluation

Medical interviews were conducted on-site with each of the seven full-time employees of the RMDH, the five employees of SBCC, and by telephone with the general contractor who was initially hired to complete the renovations at the RMDH. This contractor reported that he had an illness unrelated to work and that he quit the job for personal reasons. One employee of the RMDH had afternoon headaches that resolved after leaving work. The headaches had been present for several years. None of the other employees of the RMDH had any health complaints related to work. Two children of an employee who lives in the RMDH have allergic rhinitis, and both (according to the parent) reported experiencing brief exacerbations of their rhinitis at the beginning of the remediation project.

Each of the five SBCC employees reported health effects possibly related to work at the RMDH. Three of them worked 40 hours per week, every week, while one worked full weeks intermittently. These four employees were involved in the tearout of moldy sheetrock. The fifth was on site 20-30 hours per week and involved in administrative duties offsite the rest of the time. The most common symptoms reported were diarrhea or loose stools and vomiting while on the job. Three of the employees had brief episodes of diarrhea or loose stools. Two of five reported isolated incidents of vomiting on separate occasions. One of the five employees was very ill with a prolonged diarrheal illness, occasional vomiting, and pronounced weight loss. The fifth denied having either diarrhea or vomiting. The employee who first reported diarrhea told his physician he was ill from fungal exposure, was treated with fluconazole (an oral antifungal agent), and reported resolution of diarrhea in two to three days. Three other employees subsequently visited a physician, and all told their physicians that they were exposed to fungi, and that their co-worker had been treated with fluconazole. They, too, received fluconazole. All employees denied any respiratory or dermatologic symptoms. Other symptoms included fatigue unrelated to the workplace (four employees), occasional lightheadedness (three employees), and headache (two employees).

The first episode of diarrheal illness among the SBCC employees occurred after two to three weeks on the job at the RMDH. In the other three affected employees, symptoms occurred 3-10 weeks later. Two employees missed work due to illness, one for four days, and the other for several weeks. Neither reported a recurrence of symptoms when they returned to work at the RMDH. The other two employees with gastrointestinal illness continued working at the RMDH and their illnesses resolved. In the first week of September, some of the employees began using filter masks and gloves at the recommendation of one employee's physician.

Recommendations for use of respirators, goggles, gloves, and coveralls were made by Duke University Hospital Infection Control on September 17, 1997. Of the employees who missed work, one returned to work before these measures were implemented and remained asymptomatic except for an isolated episode of vomiting that occurred in early November 1997. The other employee's onset of illness was September 5, 1997, and he worked only intermittently due to very severe symptoms from September 22 to October 1, 1997 (mostly doing paperwork and running errands), indicating continued illness despite minimal exposure to the workplace.

The five SBCC employees worked closely together. They brought lunch from home, stored it in their work area and ate in the kitchen of the RMDH or in their trailer. Although none reported eating or smoking in the work area, three reported they did not wash their hands before they ate, while two did wash their hands before eating. Several of the employees shared off-duty activities, none of the workers had traveled recently, and no family members were ill. Two of the employees lived alone.

Medical records review did not reveal any evidence that fungal infections or mycotoxicoses in the employees of the SBCC was considered. Nor were there any medical findings to suggest that such an uncommon cause of gastrointestinal illness (in multiple, otherwise healthy persons) should have been considered. There was evidence of an infectious (bacterial) gastroenteritis in one SBCC employee.

Environmental Evaluation

Microbial Air Sampling

Outdoor culturable air sampling results are presented in Appendix B (Tables B-1 and B-3). Nineteen air samples (ten on MEA and nine on CMA) were collected at outdoor sites. Results revealed total fungal concentrations ranging from 92 to 1,004 colony forming units per cubic meter (CFU/m³), with an overall average concentration of 312 CFU/m³. Speciation of fungal plates showed that 18 of the 19 samples were dominated by *Cladosporium*(74% of the total number of colonies); 1 sample (A-4015) was dominated by Basidiomycetes. *Penicillium* accounted for only 5% of the isolates collected outdoors. During the sampling period, temperatures ranged from 46[°] F to 60[°] F, while RH measurements ranged from 27% to 35%.

Indoor culturable air sampling results are presented in Appendix B (Tables B-2 and B-4). A total of 100 culturable samples (54 on MEA and 46 on CMA) were collected indoors with an overall average concentration of 147 CFU/m³ (concentrations ranged from 25 to too numerous to count [>400 colonies]). Average concentrations in the remediated areas versus non-remediated areas were 196 and 126 CFU/m³, respectively. Cladosporium dominated 45 out of 100 (or 45%) indoor samples compared to 95% of the outdoor samples, while Basidiomycetes dominated 40% of the indoor samples (compared to 5% of the outdoor samples). Basidiomycetes have been associated with wood rot. While Penicillium accounted for only 5% of the isolates collected outdoors, 15% of the indoor samples were dominated by *Penicillium*. Memnoniella echinata, while not detected in the outdoor samples, dominated samples collected in the play room, dining room, and guest room A-2. Memnoniella echinata was also present in guest rooms A-4, A-6, and B-7. Aspergillus versicolor was present in all sample locations throughout the building. In comparison, only 2 colonies of Aspergillus versicolor were detected in the outdoor air on the dates of sampling. Seven additional species of Aspergillus were detected in the indoor samples that were not present on the outdoor samples. Temperatures ranged from 64" F to 78" F, while RH measurements ranged from 18% to 51% during the sampling period.

The results of sampling for thermotolerant (incubated at 35[°]C) fungi are presented in Tables B-5 and B-6 located in Appendix B. Concentrations of thermotolerant fungi outdoors ranged from none

detected (<4 CFU/m³) to 35 CFU/m³ with an average concentration of 13 CFU/m³ over the two-day sampling period. *Aspergillus fumigatus* was present in seven of the ten outdoor samples with colony counts ranging from one to nine in those seven samples. *Aspergillus fumigatus* was detected in low concentrations at three indoor locations including the play room and transplant suites B-4 and B-5. The average indoor concentration of airborne thermotolerant fungi was 18 CFU/m³. Other thermotolerant organisms found indoors included *Aspergillus niger, Aspergillus nidulans, Paecilmyces variotii, Mucor, Penicillium*, and sterile fungi.

The results of the air sampling for total spores are shown in Appendix B (Tables B-7 and B-8). Spore concentrations throughout the building ranged from none detected (<877 spores per cubic meter [spores/m³]) to 160,825 spores/m³ (collected in guest room A-12). Average spore concentrations inside the building $(13,333 \text{ spores/m}^3)$ were approximately the same as outdoor concentrations $(10,519 \text{ spores/m}^3)$. Areas inside the building which had higher average concentrations than those found outdoors included guest rooms B-7 (26,989 spores/m³) and A-12 (160,825 spores/m³). Spores in room B-7 were dominated by Cladosporium, while spores in room A-12 were dominated by Aspergillus, Penicillium, and Stachybotrys. Guest room A-12 had not been remediated at the time of the NIOSH sampling evaluation.

Bulk Material Sampling

The analyses for bulk samples are presented in Table C-1 (Appendix C). Samples showed a range of microbiological colonization from non-detectable (thermotolerant organisms only) to 14,122,220 CFU/g in transplant suite B-4. The wet paper-backing behind the baseboard in the bathroom of transplant suite B-4 was dominated by *Acremonium* and *Stachybotrys chartarum*. Bulk sampling of water damaged, rotted or visually contaminated materials, especially related to the leaking thru-wall heat pumps, confirmed the presence of amplification (growth) sites for such

fungi as *Stachybotrys chartarum*, *Memnoniella echinata*, *Paecilomyces variotii*, *Acremonium*, and *Aspergillus ustus*. *Rhodatorula* dominated the wet cardboard filter frame in guest room A-5. *Cladosporium* dominated the wet insulation materials collected from the heat pump serving the kitchen, entry, bath, and laundry area. *Penicillium* and *Aspergillus versicolor* were also actively growing on these materials.

Dust samples from thru-wall heat pump filters in transplant suites B-4 and B-5 and ceiling tile surfaces in guest room B-2 and transplant suite B-10 (both remediated) were collected and analyzed for culturable fungi (Table C-2; Appendix C). The total concentration of culturable fungi from the filters cultured at 25"C ranged from 2,031,858 to 5,959,084 CFU/g. The samples were dominated by Cladosporium and Penicillium. The presence of Rhodotorula, Trichoderma koningii, and Paecilomyces variotii indicate that the filters were chronically moist. Aspergillus fumigatus was the predominant thermotolerant organism found in the dust from the through-the-wall unit ventilation filters in transplant suites B-4(77%) and B-5(62%). These samples were collected from the return air side of the filters indicating that these organisms were airborne in the transplant suite at some time.

Spores in settled dust collected from the return air plenum above the ceiling tiles in guest room B-2 and transplant suite B-10 indicate that previous remediation efforts conducted without isolation barriers in place may have resulted in the dissemination of spores throughout the ventilation system. Samples from these areas were dominated by *Aspergillus versicolor*, *Aspergillus sydowii*, and *Memnoniella echinata*. However, only one colony each of *Aspergillus versicolor* and *Momnoniella echinata* were present on the airborne culturable samples collected in these rooms. *Chaetomium*, *Stachybotrys chartarum*, and *Penicillium* were also present in the settled dust samples.

A total of five tape slide samples were collected from visible fungal contamination on material surfaces (including insulation materials, condensate pipe wrap, and exterior components of the ductwork) within the heat pump mechanical closet serving the resident manager's apartment. The presence of "many" fungal spores was reported for all five samples. Hyphae and conidiophores of *Cladosporium herbarum* and *Cladosporium cladosporioides* were present on each sample suggesting active fungal growth.

DISCUSSION

Environmental

Air sampling was performed by NIOSH to evaluate whether fungi had been adequately removed from the renovated areas and to assess background concentrations and species of fungi in rooms which were going to be renovated. As a general principal, the mix of airborne fungal species indoors should be similar to that found in the outdoor air. The predominance of one or more species of fungi indoors that is not present in the outdoor air or in "control" locations suggests the presence of an amplifier (growth site) for that species of fungus in the building. A mixture of fungal species that is dominated by toxigenic fungi such as *Aspergillus versicolor, Aspergillus funigatus,* or *Stachybotrys chartarum* is an indicator of a moisture problem.⁵²

Although average air concentrations of culturable fungi indoors were considerably lower than those found outdoors, the types of fungi indoors were different than those outdoors. For example, species of *Penicillium* accounted for 15% of the isolates collected indoors, compared to 5% outdoors. Penicillium spores dominated samples collected in the smoking room, both floors of the resident manager's apartment, the dining room, and guest rooms A-2 and A-6 (all non-remediated areas); five species of *Penicillium* were present indoors that were not present outdoors. In addition, while Stachybotrys chartarum was not found in any of the outdoor samples, it was detected in six of the indoor sample locations (second floor office, play room, dining room, and guest rooms A-2, A-4, and A-6). These six sample locations had not been remediated at the time of sampling. Contaminated sheetrock, thru-wall heat pumps, and wet carpeting were the most likely amplification sites for these and other organisms.

The reason(s) remediated areas still had a higher average concentration of culturable airborne fungi (196 CFU/m³) than non-remediated areas (126 CFU/m^3) could not be determined. One contributing factor may have been the larger amounts of visible mold growth reportedly present on the ground floor where most of the remediation work in the building had been completed. Since background levels in these areas were not measured prior to NIOSH's involvement in the project, we were unable to determine if the levels had increased or decreased as a result of removing contaminated building materials. The presence of the previously mentioned fungi indicates that the ground floor has provided a chronically moist environment for these fungi to proliferate. The fact that these fungi were still present in the remediated areas may indicate that contaminated building materials had not been appropriately remediated and/or growth on newly replaced materials was occurring since the moisture incursion problems had yet to be corrected.

The presence of aspergillosis-causing fungi, such as Aspergillus fumigatus, are of particular concern in this facility due to the presence of immunocompromised children. Airborne concentrations of Aspergillus species at or below 0.1 CFU/m³ have been recommended for the prevention of nosocomial aspergillosis.² The average concentrations of Aspergillus fumigatus in transplant suites B-4 and B-5 were approximately 4 CFU/m³ and 1 CFU/m³, respectively (see Table B-5). As discussed in the Results section, Aspergillus fumigatus was the predominant thermotolerant organism found in the dust from the thru-wall heat pump return air filters. Aspergillus fumigatus may have been actively growing and disseminating spores from the wet filter, or the outdoor air was not adequately filtered before being supplied to this room. The play room had the highest average airborne concentration of Aspergillus fumigatus (5 CFU/m³); potential growth sites in the play room for this organism were not assessed. It is also important to note that almost any

fungus can become an opportunistic pathogen in a severely immuno-compromised patient.⁵³

On December 24, 1997, NIOSH sent a letter containing recommendations for remediation to the RMDH based on the initial microbiological sampling results which indicated extensive contamination of the building with a variety of molds that are capable of producing mycotoxins. At the time, NIOSH investigators planned to follow-up with further environmental sampling during the on-going remediation to evaluate the effectiveness of isolation barriers and to characterize the types of exposures the remediation workers may experience. However, NIOSH investigators were notified approximately two months later that a consultant had been hired by the RMDH on December 29, 1997, to remediate the contaminated building materials. Since the work had already been completed, NIOSH was unable to conduct further sampling during the remediation process.

Medical

Although this work environment was very heavily contaminated with mold/fungus, the health problems experienced by the SBCC employees do not appear to be related to the contamination. In spite of one employee exhibiting a syndrome clinically similar to an infectious gastroenteritis, a fungal cause is extremely uncommon. Fungal causes of diarrhea are not even listed in the differential diagnosis of acute diarrhea in medical texts.⁵⁴ The illnesses occurred at different times and resolved despite continued presence at the worksite in all but one worker. Diarrheal illnesses due to viruses, bacteria, and protozoa are very common, and are transmitted through the fecal-oral route, via food sources, person-to-person, and via fomites.

In this environment, the primary route of exposure to fungi or mycotoxins, including *Stachybotrys*, would be inhalation or skin contact. Symptoms and signs of stachybotryotoxicosis in humans are dermatitis, chest pain, sore throat, bloody rhinitis, cough, and in some, leukopenia.^{24,28,31} Skin irritation or sores have been a consistent feature in previous reports of human exposure to tricothecenes, and have been the

basis for using a skin test to determine the presence of tricothecenes.^{16,17,32} These employees did not report any respiratory or dermatologic symptoms.

There are no reports in the medical literature of isolated gastrointestinal illness associated with exposure to fungi via the inhalational or dermal routes, only by ingestion of contaminated foodstuffs. Symptoms, including sore throat, diarrhea, and vomiting, occurred within 5-60 minutes after ingestion, and resolved within 1-2 days after cessation of ingestion.^{26,27} In contrast, the SBCC employees did not begin to have diarrhea or loose stools until 2-6 weeks after beginning work, and (except for one) their diarrhea resolved despite continuing work or returning to work. Though one could argue that exposures only occurred during a few-week period, the illnesses were not suggestive of mycotoxicosis.

Finally, fluconazole is a fungistatic agent used to treat infection with fungi. It would not be effective in the treatment of mycotoxicosis because there is not replication of the fungi. Toxins are secondary metabolites of the fungi and often are present even though the fungi are no longer viable. The diagnosis of fungal infection in the four workers was apparently based solely on the first worker's selfdiagnosis. There were no supporting medical findings found in the medical records.

CONCLUSIONS

It is not likely that the workers' illnesses were related to a workplace exposure to fungi or mycotoxins, or to other identified workplace exposures. The materials the workers were tearing out were contaminated with fungus; however, the workers' illnesses were not consistent with allergy, fungal infection, or mycotoxicosis. There is a continuing debate in the scientific community over the type and extent of health effects, if any, associated with exposures to mycotoxins in the non-industrial indoor environment. However, the issue of mycotoxins aside, a potential health hazard existed for the remediation employees from a basic absence of

appropriate PPE and lack of guidance on work practices. In addition to the SBCC employees, immunocompromised children housed in transplant suites B-4 and B-5 may have been exposed to hazardous concentrations of Aspergillus fumigatus (one of the causative agents for apergillosis). Also, past flooding, moisture incursion through exterior walls, roof leaks and improperly draining thru-wall heat pump units resulted in providing chronically moist building materials ideal for fungal growth. Bulk samples revealed potential fungal reservoirs in areas throughout the building. Air samples as well as settled dust samples indicated dissemination from these reservoirs. Remediation of the contaminated building materials also appears to have contributed to the spread of fungal spores in the return air ceiling plenum. The potential for fungal growth to re-occur on newly replaced materials is likely, since the leaks in the foundation of the building have not been corrected.

RECOMMENDATIONS

The following recommendations are offered to prevent further microbial contamination in the RMDH, to assess previous remediation efforts, and to ensure that any future microbial remediation of the building is appropriately performed.

1. All sources of moisture incursion should have been identified and repaired prior to the installation of new building materials. This includes fixing roof leaks, envelope leaks, drainage away from the foundation, and leakage from the thru-wall heat pump units. A firm specializing in building design and construction should be consulted to ensure that the architectural recommendations sent to the RMDH by W.W. Kingsbury on July 10, 1995, have been addressed. This should also include the installation of an appropriate vapor barrier in the exterior walls of the building. Failure to prevent further water incursion will result in conditions conducive to microbial growth.

2. Any future remediation of contaminated building materials should be performed using

appropriate risk management procedures. Sources of guidelines are the Control of Moisture Problems Affecting Biological Indoor Air Quality,² Guidelines on Assessment and Remediation of Stachybotrys atra in Indoor Environments,⁵⁵ and Fungal Contamination in Public Buildings: A Guide to Recognition and Management.⁵⁶ Removal techniques should be chosen based on the amount of surface area contaminated and should include the use of isolation barriers (including sealing off ductwork and the ceiling plenum), PPE, negative pressurization, cleaning with a high efficiency particulate air (HEPA) vacuum, and damp-wiping non-porous surfaces. Ceiling tiles in rooms which were remediated without critical barriers in place should be removed. The guidelines state that such precautions are especially warranted when a "stressed" population, e.g. those who are immunocompromised or have asthma or other respiratory conditions, occupy or will reoccupy the area where work is currently being undertaken. Killing of mold spores with biocides without physical removal of porous materials is of limited value because allergenicity and toxicity are unaffected by viability.

3. Future efforts should ensure that remediation personnel are appropriately equipped with PPE (i.e., HEPA-filtered respirators, clothing, gloves, etc.). For respirator use, the Occupational Safety and Health Administration (OSHA) requires a respiratory protection program that includes the following components: written standard operating procedures, user instruction and training, cleaning and disinfection, storage, inspection, surveillance of work area conditions, evaluation of respirator protection program, medical review, and use of certified respirators.⁵⁷

4. The microbially-contaminated fiberglass sound liner downstream of the cooling coils in the central heat pump which serves the kitchen, etc. should be discarded and replaced, preferably with a smoothsurfaced insulation to prevent the collection of microbial contaminants. Subsequent to the removal of the insulation, all surfaces (nonporous and porous) should be dried and cleaned with a HEPA-filtered vacuum to remove dirt, debris, and microorganisms before removal. The surface of the insulation should not be damaged by vacuuming. All remedial activities should be performed when the building is unoccupied and when the heat pump is not operating. The interior of all other central heat pumps remaining in the building (i.e., those units that were not inspected during the NIOSH investigation) should be inspected for microbial contamination.

5. After all the debris is removed, the components of the heat pump should be cleaned with a dilute aqueous household bleach solution (10%) while the ventilation system is not operating. A water rinse should follow cleaning. Hypochlorites have a relatively low order of toxicity and skin irritation potential. During the cleaning, workers should wear the protective equipment mentioned previously.

6. The condensate drip pans in the thru-wall heat pump units should be kept free of standing water and visible microbial growth. At the time of the NIOSH evaluation, many of the filters in the operating thruwall heat pumps (including transplant suites B-4 and B-5) were wet from the over-spray of standing water in the drain pans. Throughout the year, coils, condensate pans, and drains should be inspected monthly and, if necessary, cleaned. Pill packs should not be used to keep the drip pans free of debris or biological growth. These tablets are not effective unless a sufficient pool of water in the pan enables the tablet to dissolve evenly throughout the pan.

7. Serious consideration should be given to increasing the filter efficiency of the thru-wall and central heat pump systems due to the presence of immuno-compromised patients in the facility. The most efficient filter should be used in order to remove microbial particulates from the airstream whose pressure drop does not seriously affect the airflow rate of the fans. The manufacturer of the heat pumps should be consulted to determine the impact of increasing the filter efficiency upon the operation of the system. In addition, all the filter systems should be checked for filter by-pass.

8. A formal written preventive maintenance schedule for all of the heat pumps should be written

and implemented in consultation with the manufacturers of the equipment. Preventive maintenance on the equipment should be documented. Central files should be developed for all of the heat pump units according to type. These files should contain specifications, design drawings, product literature, operational parameters, theory of operation, and other important information needed to assure continuity between mechanical personnel.

9. Air intakes should not be located near areas where accumulation of decaying leaf litter, soil and other organic debris (which serves as resevoirs for bacteria and fungi) occurs, especially intakes serving transplant areas. Most of the thru-wall heat pump systems on the bottom floor of the facility were only a foot above the ground. On one occasion, water actually entered transplant suites B-4 and B-5 through the air intakes of the heat pumps since they were located so close to the ground. Ideally, the location of the air intake should be moved to an area at least 25 feet away from external bioaerosol amplification sites.⁵⁸

10. Consideration should be given to re-locating all transplant suites to the main floor, where moisture incursion has been less of a problem. Due to poor construction of the exterior walls and inadequate drainage away from the foundation, moisture problems are likely to occur in the future.

11. Post-remediation culturable air and surface sampling (including settled dust) should be conducted in order to determine the effectiveness of clean-up activities and moisture reduction. The media used during the NIOSH investigation is recommended, as well as evaluating the presence of thermotolerant fungi. Information on species is crucial for determining whether such organisms as *Stachybotrys chartarum*, *Aspergillus versicolor*, and *Aspergillus fumigatus* are present. The taxa and concentrations of fungi should be compared with outdoor air and indoor control locations. Replicate samples must be taken at each location to address sampling variability.⁵⁹

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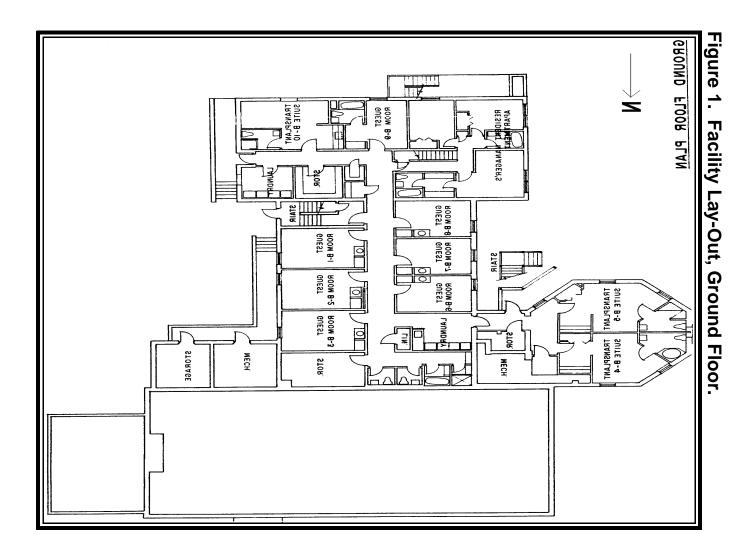
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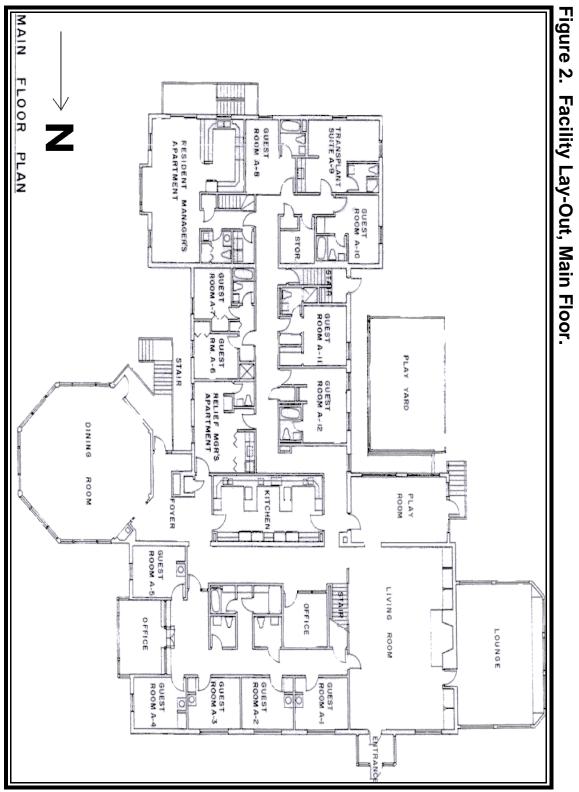
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Appendix A Facility Lay-out of Evaluated Areas





Appendix B Microbial Air Sampling Results

Table B-1 Air Sampling for Culturable Fungi Outdoors Ronald McDonald House Durham, North Carolina Sampling Date: November 24, 1997

Sample		Malt	Extract Agar @ 25°C	Cor	n Meal Agar @ 25°C
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)
A-1009 (14:22) C-1009 (14:35)	Outdoor Air - East Side of Building	466	Cladosporium (98) Penicillium (14) Epicoccum nigrum (6) Alternaria (5) Aureobasidium pullulans (2) Pithomyces chartarum (2) yeasts (2) Aspergillus versicolor (1) Fusarium (1) Rhodotorula (1)	523	Cladosporium (118) Alternaria (7) Basidiomycetes (6) Penicillium (5) Epicoccum nigrum (4) yeasts (4) Curvularia (3) Aureobasidium pullulans (1)
A-1010 (14:49) C-1010 (14:49)	Outdoor Air - East Side of Building	357	Cladosporium (77) Penicillium (7) yeasts (6) Alternaria (5) Epicoccum nigrum (4) Aureobasidium pullulans (1) sterile fungi (1)	353	Cladosporium (79) Basidiomycetes (7) Alternaria (6) Penicillium (4) Aureobasidium pullulans (3) Arthrinium (1)
A-2008 (13:04) C-2008 (13:17)	Outdoor Air - West Side of Building	297	Cladosporium (58) Epicoccum nigrum (9) Penicillium (6) Alternaria (5) sterile fungi (3) yeasts (2) Aureobasidium pullulans (1)	297	Cladosporium (56) yeasts (9) Basidiomycetes (6) Alternaria (4) Aureobasidium pullulans (4) Epicoccum nigrum (2) Penicillium (2) Curvularia (1)
A-2009 (13:31)	Outdoor Air - West Side of Building	382	Cladosporium (92) Alternaria (8) Epicoccum nigrum (3) Basidiomycetes (2) Penicillium (2) Trichoderma koningii (1)	NA	No sample collected.

^a Sample numbers starting with "A" were collected on malt extract agar; sample numbers starting with "C" were collected on corn meal agar.

^b CFU/ m^3 = Colony forming units per cubic meter of air.

Table B-2 Air Sampling for Culturable Fungi Indoors Ronald McDonald House Durham, North Carolina Sampling Date: November 24, 1997

Sample		Malt Extract Agar @ 25°C		Corr	n Meal Agar @ 25°C
Numbers ^a Location (Start Time)	Total Fungi (CFU/m³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	
A-1000 (9:45) C-1000 (10:10)	Transplant Suite B-10	191	Basidiomycetes (28) <i>Cladosporium</i> (17) <i>Penicillium</i> (3) <i>Rhodotorula</i> (2) yeasts (2) <i>Alternaria</i> (1) <i>Oidiodendron</i> (1)	131	Cladosporium (16) Basidiomycetes (12) yeasts (4) Penicillium (3) Alternaria (1) Aspergillus niger (1)
A-1001 (9:58) C-1001 (10:22)	Transplant Suite B-10	102	Basidiomycetes (13) <i>Cladosporium</i> (9) <i>Penicillium</i> (2) <i>Tritirachium</i> (2) yeasts (2) <i>Chaetomium</i> (1)	170	Cladosporium (29) Basidiomycetes (5) yeasts (5) Penicillium (4) Alternaria (2) Phoma (1) Aspergillus ochraceus (1) Aspergillus versicolor (1)
A-2010 (14:23) C-2010 (14:37)	Transplant Suite B-10	247	Cladosporium (38) Penicillium spp. (8) Basidiomycetes (7) yeasts (5) Penicillium aurantiogriseum (4) Aspergillus versicolor (3) Penicillium brevicompactum (2) Tritirachium (2) Alternaria (1)	NA	Not analyzed by laboratory.
A-2011 (14:50) C-2011 (14:23)	Transplant Suite B-10	205	Cladosporium (34) Basidiomycetes (5) Penicillium spp. (5) Alternaria (2) Penicillium corylophilum (2) Penicillium variabile (2) yeasts (2) Arthrinium (1) Aspergillus versicolor (1) Aureobasidium pullulans (1) Penicillium aurantiogriseum (1) Penicillium brevicompactum (1) Oidiodendron (1)	NA	Not analyzed by laboratory.

Sample		Ma	lt Extract Agar @ 25°C	Corr	n Meal Agar @ 25°C
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)
A-1002 (10:52) C-1002 (11:20)	Transplant Suite B-5	28	Cladosporium (5) Penicillium (2) Trichoderma koningii (1)	49	Basidiomycetes (5) <i>Cladosporium</i> (4) <i>Penicillium</i> (3) yeasts (1) <i>Rhodotorula</i> (1)
A-1003 (11:05) C-1003 (11:20)	Transplant Suite B-5	28	Cladosporium (2) Penicillium (2) Tritirachium (2) sterile fungi (1) yeasts (1)	42	Cladosporium (5) Basidiomycetes (3) Aspergillus versicolor (2) Penicillium (1) Aspergillus ochraceus (1)
A-2014 (15:56) C-2015 (15:56)	Transplant Suite B-5	49	Cladosporium (8) Aureobasidium pullulans (4) Penicillium (1) Aspergillus penicillioides (1)	NA	Not analyzed by laboratory.
A-2015 (16:20) C-2014 (16:08)	Transplant Suite B-5	39	<i>Cladosporium</i> (6) Basidiomycetes (4) <i>Rhodotorula</i> (1)	NA	Not analyzed by laboratory.
A-1004 (12:08) C-1004 (12:08)	Transplant Suite B-4	148	Cladosporium (24) Basidiomycetes (8) Penicillium (6) Aspergillus versicolor (2) Botrytis (1) Epicoccum nigrum (1)	110	Cladosporium (20) Aspergillus versicolor (3) Basidiomycetes (2) Penicillium (2) yeasts (2) Beauveria (1) Pithomyces chartarum (1)
A-1005 (11:55) C-1005 (12:24)	Transplant Suite B-4	177	Cladosporium (23) Basidiomycetes (15) Penicillium (9) Alternaria (2) yeasts (1)	177	Cladosporium (26) Penicillium (8) Basidiomycetes (7) Aspergillus versicolor (4) yeasts (2) Tritirachium (2) Alternaria (1)
A-2012 (15:11) C-2013 (15:11)	Transplant Suite B-4	346	Cladosporium (81) Penicillium spp. (5) Penicillium minioluteum (3) Alternaria (2) Penicillium aurantiogriseum (2) yeasts (2) Epicoccum nigrum (1) Penicillium brevicompactum (1) Rhodotorula (1)	NA	Not analyzed by laboratory.

Sample		Mal	lt Extract Agar @ 25°C	Corr	n Meal Agar @ 25°C
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)
A-2013 (15:37) C-2012 (15:25)	Transplant Suite B-4	258	Cladosporium (45) Penicillium spp. (10) Basidiomycetes (7) yeasts (4) Penicillium aurantiogriseum (2) Acremonium (1) Alternaria (1) Aspergillus (1) Epicoccum nigrum (1) Rhodotorula (1)	NA	Not analyzed by laboratory.
A-1015 (17:03) C-1015 (17:17)	Transplant Suite A-9	95	Cladosporium (12) Basidiomycetes (11) Penicillium (2) Aspergillus versicolor (1) Curvularia (1)	117	<i>Cladosporium</i> (21) Basidiomycetes (8) <i>Penicillium</i> (3) <i>Alternaria</i> (1)
A-1016 (17:30) C-1016 (17:30)	Transplant Suite A-9	155	Basidiomycetes (25) Cladosporium (15) Penicillium (2) Aureobasidium pullulans (1) yeasts (1)	159	Basidiomycetes (26) <i>Cladosporium</i> (10) <i>Penicillium</i> (5) yeasts (2) <i>Tritirachium</i> (1) <i>Rhodotorula</i> (1)
A-2000 (9:41) C-2001 (9:41)	Transplant Suite A-9	92	Basidiomycetes (12) Cladosporium (4) Penicillium (3) Aspergillus versicolor (3) yeasts (3) Tritirachium (1)	110	Basidiomycetes (17) <i>Cladosporium</i> (6) <i>Penicillium</i> (3) <i>Aspergillus versicolor</i> (2) yeasts (2) <i>Alternaria</i> (1)
A-2001 (10:08) C-2000 (9:55)	Transplant Suite A-9	99	Basidiomycetes (12) Cladosporium (6) Penicillium (1) Aspergillus (1) Aspergillus ustus (1) Aureobasidium pullulans (1) Beauveria (1)	117	Basidiomycetes (20) <i>Cladosporium</i> (4) <i>Penicillium</i> (4) yeasts (3) <i>Aspergillus versicolor</i> (1) <i>Aureobasidium pullulans</i> (1)
A-1017 (17:17) C-1017 (17:17)	Office on Second Floor	64	Basidiomycetes (6) Cladosporium (5) Penicillium (4) Alternaria (1) Aspergillus sydowii (1) Aspergillus versicolor (1)	67	<i>Cladosporium</i> (8) Basidiomycetes (5) <i>Penicillium</i> (4) <i>Pithomyces chartarum</i> (1) yeasts (1)

Sample		Ma	lt Extract Agar @ 25°C	© 25°C Corn Meal Agar @ 25°C		
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	
A-2006 (12:22) C-2007 (12:22)	Office on Second Floor	28	<i>Cladosporium</i> (3) Basidiomycetes (2) <i>Penicillium</i> (2) yeasts (1)	35	<i>Cladosporium</i> (4) Basidiomycetes (3) yeasts (2) <i>Penicillium</i> (1)	
A-2007 (12:35) C-2006 (12:35)	Office on Second Floor	32	Cladosporium (3) Basidiomycetes (3) Penicillium (1) Phialophora (1) Stachybotrys chartarum (1)	25	Basidiomycetes (3) <i>Penicillium</i> (2) <i>Cladosporium</i> (1) yeasts (1)	
A-1012 (15:46) C-1012 (15:46)	Smoking Room	141	Cladosporium (12) Penicillium aurantiogriseum (9) Basidiomycetes (8) Penicillium spp. (6) yeasts (4) Aspergillus sydowii (1)	145	Penicillium (19) Cladosporium (14) yeasts (5) Basidiomycetes (2) Aspergillus versicolor (1)	
A-2004 (11:30) C-2005 (11:30)	Smoking Room	155	Cladosporium (26) Penicillium (8) Basidiomycetes (5) Alternaria (3) Aureobasidium pullulans (1) yeasts (1)	194	Cladosporium (39) Penicillium (5) yeasts (5) Basidiomycetes (2) Alternaria (2) Aureobasidium pullulans (1) Arthrinium (1)	
A-2005 (11:55) C-2004 (11:43)	Smoking Room	145	Cladosporium (21) Basidiomycetes (7) Penicillium spp. (6) Penicillium aurantiogriseum (2) Paecilomyces variotii (2) Alternaria (1) Penicillium brevicompactum (1) Tritirachium (1)	131	<i>Cladosporium</i> (18) Basidiomycetes (6) <i>Penicillium</i> (5) yeasts (5) <i>Tritirachium</i> (2) <i>Phialophora</i> (1)	
A-1008 (13:02) C-1008 (13:17)	Resident Manager's Apartment - Ground Floor	113	Penicillium (8) Basidiomycetes (8) Cladosporium (7) Aspergillus versicolor (6) yeasts (2) Aspergillus ochraceus (1)	110	Basidiomycetes (11) Cladosporium (7) Penicillium (5) Aspergillus versicolor (2) yeasts (2) Tritirachium (1) Aspergillus penicillioides (1) Aureobasidium pullulans (1) Phialocephala (1)	

Sample		Mal	lt Extract Agar @ 25°C	Corn Meal Agar @ 25°C		
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	
A-2016 (16:38) C-2017 (16:38)	Resident Manager's Apartment - Ground Floor	163	Cladosporium (10) Penicillium spp. (10) Penicillium corylophilum (9) Aureobasidium pullulans (5) Aspergillus versicolor (4) Penicillium aurantiogriseum (2) Alternaria (1) Penicillium minioluteum (1) Tritirachium (1) Aspergillus fumigatus (1) Pithomyces chartarum (1) yeasts (1)	NA	Not analyzed by laboratory.	
A-2017 (16:50) C-2016 (16:50)	Resident Manager's Apartment - Ground Floor	201	Penicillium spp. (14) Cladosporium (13) Penicillium corylophilum (7) Tritirachium (6) Basidiomycetes (5) Aspergillus versicolor (4) Penicillium aurantiogriseum (3) Alternaria (2) Pithomyces chartarum (2) Penicillium minioluteum (1)	NA	Not analyzed by laboratory.	
A-1014 (16:36) C-1014 (16:44)	Resident Manager's Apartment - Main Floor	216	Penicillium corylophilum (26) Cladosporium (19) Tritirachium (8) Penicillium spp. (6) yeasts (2)	286	Penicillium (39) Tritirachium (23) Cladosporium (9) yeasts (4) Alternaria (2) Aureobasidium pullulans (1) Aspergillus fumigatus (1) Arthrinium (1) Botrytis (1)	
A-2002 (10:34) C-2003 (10:34)	Resident Manager's Apartment - Main Floor	74	Basidiomycetes (7) <i>Cladosporium</i> (7) <i>Penicillium</i> (4) yeasts (1) <i>Aureobasidium pullulans</i> (1) <i>Chrysosporium</i> (1)	92	Basidiomycetes (10) <i>Cladosporium</i> (9) yeasts (3) <i>Penicillium</i> (2) <i>Alternaria</i> (1) <i>Aspergillus versicolor</i> (1)	
A-2003 (10:57) C-2002 (10:44)	Resident Manager's Apartment - Main Floor	110	Basidiomycetes (10) Cladosporium (8) Penicillium spp. (4) Penicillium aurantiogriseum (3) Aspergillus versicolor (3) Penicillium corylophilum (2) Tritirachium (1)	110	Basidiomycetes (9) Cladosporium (8) Penicillium (4) Aspergillus versicolor (3) yeasts (3) Tritirachium (2) Aureobasidium pullulans (1) Rhodotorula (1)	

^aSample numbers starting with "A" were collected on malt extract agar; sample numbers starting with "C" were collected on corn meal agar.

Table B-3 Air Sampling for Culturable Fungi Outdoors Ronald McDonald House Durham, North Carolina Sampling Date: November 25, 1997

Sample		Malt Extract Agar @ 25°C		Corn	Meal Agar @ 25°C
NumbersaLocation(Start Time)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	
A-3005 (10:54) C-3005 (11:08)	Outdoor Air - East Side of Building	1,004	Cladosporium (236) Penicillium (22) Alternaria (16) Epicoccum nigrum (5) Pithomyces chartarum (4) sterile fungi (1)	551	<i>Cladosporium</i> (138) <i>Alternaria</i> (6) Basidiomycetes (6) yeasts (3) <i>Epicoccum nigrum</i> (2) sterile fungi (1)
A-3006 (11:22) C-3006 (11:22)	Outdoor Air - East Side of Building	117	Cladosporium (26) Alternaria (2) Penicillium (2) Fusarium (1) sterile fungi (1) Trichoderma koningii (1)	92	Cladosporium (15) Basidiomycetes (6) Alternaria (1) Penicillium (1) Chaetomium globosum (1) Drechslera (1) sterile fungi (1)
A-4014 (13:32) C-4015 (13:03)	Outdoor Air - West Side of Building	208	Cladosporium (35) Basidiomycetes (12) Penicillium (4) Alternaria (3) Epicoccum nigrum (2) Aspergillus fumigatus (1) Botrytis (1) sterile fungi (1)	194	Cladosporium (32) Basidiomycetes (12) yeasts (4) Penicillium (2) Pithomyces chartarum (2) sterile fungi (1) Beauveria (1) Curvularia (1)
A-4015 (13:16) C-4014 (13:16)	Outdoor Air - West Side of Building	307	Basidiomycetes (38) Cladosporium (37) Penicillium (4) Epicoccum nigrum (3) Alternaria (2) Phoma (1) Botrytis (1) sterile fungi (1)	184	<i>Cladosporium</i> (39) Basidiomycetes (6) <i>Alternaria</i> (4) yeasts (2) sterile fungi (1)
A-4012 (12:17) C-4012 (12:17)	Outdoor Air - North Side of Building	141	Cladosporium (18) Basidiomycetes (8) Aspergillus fumigatus (5) Alternaria (3) Penicillium (3) Beauveria (1) Chrysosporium (1) sterile fungi (1)	127	Cladosporium (22) Basidiomycetes (8) Aspergillus fumigatus (2) Alternaria (1) Aspergillus versicolor (1) Botrytis (1) Penicillium (1)

Sample		Malt Extract Agar @ 25°C		Corn Meal Agar @ 25°C	
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)
A-4013 (12:43)	Outdoor Air - North Side of Building	194	<i>Cladosporium</i> (28) Basidiomycetes (14) <i>Penicillium</i> (7)	131	<i>Cladosporium</i> (23) Basidiomycetes (4) <i>Aspergillus fumigatus</i> (4)
C-4013 (12:30)	C		sterile fungi (2) Aspergillus fumigatus (2) Alternaria (1)		Penicillium (2) yeasts (1) sterile fungi (1)
			Pithomyces chartarum (1)		Epicoccum nigrum (1) Aspergillus niger (1)

^aSample numbers starting with "A" were collected on malt extract agar; sample numbers starting with "C" were collected on corn meal agar. ^bCFU/m³ = Colony forming units per cubic meter of air.

Table B-4 Air Sampling for Culturable Fungi Indoors Ronald McDonald House Durham, North Carolina Sampling Date: November 25, 1997

Sample		Malt Extract Agar @ 25°C		Corn Meal Agar @ 25°C		
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	
A-3000 (8:10) C-3000 (8:22)	Play Room	159	Basidiomycetes (38) <i>Penicillium</i> (4) <i>Cladosporium</i> (3)	127	Basidiomycetes (22) <i>Cladosporium</i> (8) <i>Penicillium</i> (3) sterile fungi (2) <i>Aureobasidium pullulans</i> (1)	
A-3007 (11:54) C-3007 (12:06)	Play Room	57	Cladosporium (6) Penicillium (4) Alternaria (3) sterile fungi (1) Rhizopus stolonifer (1) Rhodotorula (1)	88	Cladosporium (9) Penicillium (6) yeasts (5) Basidiomycetes (2) Alternaria (1) Aspergillus versicolor (1) sterile fungi (1)	
A-3015 (14:49) C-3015 (14:49)	Play Room	NA	OVERLOADED (>400) Memnoniella echinata (dominant) Cladosporium (NA) Penicillium (NA) yeasts (NA) Aspergillus versicolor (NA) Aspergillus versicolor (NA) Aspergillus ustus (NA) Paecilomycese variotii (NA) Stachybotrys chartarum (NA)	1,113	Memnoniella echinata (186) Stachybotrys chartarum (43) Paecilomycese variotii (28) Aspergillus ustus (19) Cladosporium (15) Aspergillus sydowii (14) Penicillium (10)	
A-4002 (8:15) C-4001 (8:29)	Dining Room	237	Basidiomycetes (38) Penicillium corylophilum (10) Penicillium spp. (8) Cladosporium (7) Aspergillus versicolor (4)	134	Penicillium (17) Basidiomycetes (16) Cladosporium (2) sterile fungi (2) Aspergillus flavus (1)	
A-4010 (11:22) C-4009 (11:22)	Dining Room	85	Basidiomycetes (10) <i>Cladosporium</i> (9) <i>Penicillium</i> (5)	71	Basidiomycetes (8) <i>Cladosporium</i> (5) <i>Penicillium</i> (4) <i>Aspergillus versicolor</i> (2) <i>Aspergillus sydowii</i> (1)	

Sample		Ma	lt Extract Agar @ 25°C	Cor	rn Meal Agar @ 25°C
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)
A-4016 (13:53) C-4016 (13:53)	Dining Room	180	Memnoniella echinata (16) Cladosporium (13) Basidiomycetes (8) Penicillium (7) Stachybotrys chartarum (5) Paecilomycese lilacinus (1) Alternaria (1)	155	Memnoniella echinata (19) Cladosporium (9) Penicillium (5) Stachybotrys chartarum (5) Aspergillus versicolor (2) Alternaria (1) sterile fungi (1) yeasts (1) Ulocladium chartarum (1)
A-3004 (10:12) C-3004 (10:12)	Guest Room A-2	88	<i>Cladosporium</i> (11) <i>Penicillium</i> (6) Basidiomycetes (4) <i>Aspergillus versicolor</i> (2) <i>Exophiala</i> (1) sterile fungi (1)	49	<i>Cladosporium</i> (4) <i>Penicillium</i> (3) Basidiomycetes (2) <i>Aspergillus ustus</i> (2) <i>Exophiala</i> (1) sterile fungi (1) yeasts (1)
A-3008 (12:23) C-3008 (12:22)	Guest Room A-2	78	Cladosporium (8) Basidiomycetes (4) Penicillium (3) Aspergillus versicolor (2) sterile fungi (1) yeasts (1) Curvularia (1) Chrysosporium (1) Alternaria (1)	95	Cladosporium (11) Aspergillus fumigatus (5) Penicillium (5) Basidiomycetes (2) Phoma (2) Myrothecium (1) sterile fungi (1)
A-3012 (13:56) C-3012 (13:56)	Guest Room A-2	286	Penicillium (23) Cladosporium (22) Memnoniella echinata (20) Basidiomycetes (6) Aspergillus versicolor (5) Stachybotrys chartarum (3) Paecilomycese lilacinus (2)	360	Memnoniella echinata (35) Cladosporium (31) Penicillium (15) Stachybotrys chartarum (11) Aspergillus versicolor (2) Tritirachium (2) yeasts (1) Aspergillus ustus (1) Paecilomycese lilacinus (1) Aureobasidium pullulans (1) Beauveria (1) sterile fungi (1)

Sample		Ma	lt Extract Agar @ 25°C	Со	rn Meal Agar @ 25°C
Numbers ^a (Start Time)	Total Rungi Predominant Tava		Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	
A-3001 (8:40) C-3001 (8:53)	Guest Room A-4	205	Basidiomycetes (32) <i>Cladosporium</i> (18) <i>Penicillium</i> (5) <i>Aspergillus niger</i> (1) sterile fungi (1) yeasts (1)	159	Cladosporium (22) Basidiomycetes (14) Penicillium (4) yeasts (2) Chaetomium globosum (1) Drechslera (1) Exophiala (1)
A-3003 (9:41) C-3003 (9:41)	Guest Room A-4	92	<i>Cladosporium</i> (14) Basidiomycetes (6) <i>Penicillium</i> (3) sterile fungi (2) <i>Alternaria</i> (1)	asidiomycetes (6) enicillium (3) erile fungi (2)	
A-3011 (13:38) C-3011 (13:38)	Guest Room A-4	307	Cladosporium (47) Memnoniella echinata (14) Penicillium (8) Stachybotrys chartarum (8) Chaetomium (4) Aspergillus versicolor (3) Basidiomycetes (2) sterile fungi (1)	336	Cladosporium (55) Memnoniella echinata (15) Penicillium (8) Stachybotrys chartarum (7) Paecilomycese lilacinus (3) Basidiomycetes (2) Alternaria (2) yeasts (1) sterile fungi (1) Curvularia (1)
A-3002 (9:10) C-3002 (9:10)	Guest Room A-6	53	Basidiomycetes (8) Penicillium (4) Cladosporium (1) Alternaria (1) Aspergillus versicolor (1)	67 Basidiomycetes (6) <i>Cladosporium</i> (4) <i>Penicillium</i> (4) yeasts (2) <i>Aspergillus versicolor</i> (1) <i>Alternaria</i> (1) <i>Acrodontium</i> (1)	
A-3009 (12:44) C-3009 (12:44)	Guest Room A-6	67	Cladosporium (10) yeasts (4) Basidiomycetes (2) Alternaria (1) sterile fungi (1) Pythomyces chartarum (1)	49	Cladosporium (8) Basidiomycetes (2) yeasts (1) Alternaria (1) Penicillium (1) Fusarium (1)

Sample		Ma	lt Extract Agar @ 25°C	Cor	rn Meal Agar @ 25°C
Numbers ^a (Start Time)	Location	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)
A-3013 (14:20) C-3013 (14:20)	Guest Room A-6	113	<i>Penicillium</i> (13) Basidiomycetes (8) <i>Memnoniella echinata</i> (6) <i>Cladosporium</i> (3) yeasts (1) sterile fungi (1)	74	Aspergillus versicolor (8) Aspergillus ustus (3) Cladosporium (2) Penicillium (2) Memnoniella echinata (1) Stachybotrys chartarum (1) Paecilomycese variotti (1) Alternaria (1) Beauveria (1) Epicoccum nigrum (1)
A-3010 (12:59) C-3010 (13:11)	Guest Room A- 12	46	Basidiomycetes (10) <i>Penicillium</i> (1) yeasts (1) <i>Epicoccum nigrum</i> (1)	60	Basidiomycetes (8) <i>Cladosporium</i> (4) <i>Penicillium</i> (4) sterile fungi (1)
A-4003 (9:26) C-4004 (9:26)	Guest Room A- 12	81	Basidiomycetes (16) <i>Cladosporium</i> (4) <i>Penicillium</i> (1) <i>Pythomyces chartarum</i> (1) <i>Aspergillus sydowii</i> (1)	57	Basidiomycetes (8) Penicillium (3) Cladosporium (2) yeasts (1) sterile fungi (1) Aspergillus fumigatus (1)
A-4011 (11:57) C-4011 (11:57)	Guest Room A- 12	57	Basidiomycetes (8) Penicillium (3) Cladosporium (1) yeasts (1) Aspergillus fumigatus (1) sterile fungi (1) Epicoccum nigrum (1)	53	Basidiomycetes (8) Penicillium (3) Cladosporium (2) Aspergillus versicolor (1) Aspergillus fumigatus (1)
A-4004 (9:57) C-4003 (9:57)	Guest Room B-2	113	Basidiomycetes (26) <i>Penicillium</i> (4) <i>Cladosporium</i> (1) sterile fungi (1)	95	Basidiomycetes (20) <i>Cladosporium</i> (4) <i>Aspergillus versicolor</i> (1) yeasts (1) sterile fungi (1)
A-4008 (10:43) C-4007 (10:43)	Guest Room B-2	53	Basidiomycetes (10) <i>Cladosporium</i> (4) <i>Aspergillus versicolor</i> (1)	88	Basidiomycetes (16) <i>Cladosporium</i> (5) <i>Penicillium</i> (4)

Sample				Cor	rn Meal Agar @ 25°C
Numbers ^a (Start Time)			Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)	
A-4006 (10:12) C-4005 (10:12)	Guest Room B-7	81	Basidiomycetes (16) <i>Cladosporium</i> (4) <i>Penicillium</i> (2) <i>Epicoccum nigrum</i> (1)	95	Basidiomycetes (16) <i>Cladosporium</i> (4) <i>Penicillium</i> (3) yeasts (1) sterile fungi (1) <i>Alternaria</i> (1) <i>Aspergillus glaucus</i> (1)
A-4009 (10:59) C-4008 (10:59)	Guest Room B-7	110	<i>Cladosporium</i> (18) Basidiomycetes (8) <i>Penicillium</i> (4) yeasts (1)	99	Cladosporium (15) Basidiomycetes (6) Penicillium (5) Aphanocladium (1) Oidiodendron (1)
A-4018 (14:40) C-4018 (14:40)	Guest Room B-7	322	yeasts (53) <i>Cladosporium</i> (14) Basidiomycetes (10) <i>Penicillium</i> (4) <i>Chrysosporium</i> (4) <i>Aureobasidium pullulans</i> (2) <i>Acremonium</i> (2) <i>Aspergillus versicolor</i> (1) <i>Memnoniella echinata</i> (1)	166	Cladosporium (17) yeasts (15) Basidiomycetes (6) Penicillium (3) sterile fungi (2) Rhodotorula (1) Aspergillus versicolor (1) Aspergillus niger (1) Alternaria (1)
A-4001 (8:56) C-4002 (8:56)	Guest Room B-9	208	Basidiomycetes (30) <i>Cladosporium</i> (21) <i>Penicillium</i> (6) <i>Alternaria</i> (1) <i>Aspergillus versicolor</i> (1)	177	Basidiomycetes (22) <i>Cladosporium</i> (9) <i>Aspergillus versicolor</i> (6) <i>Penicillium</i> (4) <i>Paecilomyces variotii</i> (3) sterile fungi (2) yeasts (1) <i>Alternaria</i> (1) <i>Aspergillus fumigatus</i> (1) <i>Aspergillus ustus</i> (1)

Sample		Ma	lt Extract Agar @ 25°C	Cor	rn Meal Agar @ 25°C
Numbers ^a (Start Time)	Total Rungi Predominant Lava			Total Fungi (CFU/m ³) ^b	Predominant Taxa (number of colonies)
A-4007 (10:27) C-4006 (10:27)	Guest Room B-9	85	Basidiomycetes (14) <i>Cladosporium</i> (3) <i>Penicillium</i> (3) <i>Aspergillus versicolor</i> (2) <i>Aspergillus nidulans</i> (1) <i>Chrysosporium</i> (1)	110	Basidiomycetes (16) Cladosporium (4) Penicillium (2) Aspergillus versicolor (2) Aspergillus niger (1) Aspergillus ochraceus (1) Aspergillus sclerotiorum (1) yeasts (1) sterile fungi (1) Pythomyces chartarum (1) Oidiodendron (1)
A-4017 (14:22) C-4017 (14:22)	Guest Room B-9	85	<i>Cladosporium</i> (10) Basidiomycetes (8) <i>Penicillium</i> (3) yeasts (1) <i>Alternaria</i> (1) <i>Phoma</i> (1)	81	Cladosporium (8) Basidiomycetes (6) Penicillium (2) Aureobasidium pullulans (2) Alternaria (2) yeasts (1) Acrodontium (1) sterile fungi (1)

^aSample numbers starting with "A" were collected on malt extract agar; sample numbers starting with "C" were collected on corn meal agar. ^bCFU/m³ = Colony forming units per cubic meter of air.

Table B-5 Air Sampling for Thermotolerant Fungi Ronald McDonald House Durham, North Carolina Sampling Date: November 24, 1997

Sample			Malt Extract Agar @ 35°C
Number (Start Time)	Location	Total Fungi (CFU/m ³) ^a	Predominant Taxa (number of colonies)
B-1009 (14:22)	Outdoor Air - East Side of Building	4	Aspergillus fumigatus (1)
B-1010 (14:35)	Outdoor Air - East Side of Building	25	Penicillium (3) yeasts (2) sterile fungi (1) Aspergillus fumigatus (1)
B-2008 (13:31)	Outdoor Air - West Side of Building	<4	No Growth
B-2009 (13:17)	Outdoor Air - West Side of Building	<4	No Growth
B-1000 (9:45)	Transplant Suite B-10	<4	No Growth
B-1001 (9:58)	Transplant Suite B-10	<4	No Growth
B-2010 (14:50)	Transplant Suite B-10	<4	No Growth
B-2011 (14:37)	Transplant Suite B-10	<4	No Growth
B-1002 (10:52)	Transplant Suite B-5	<4	No Growth
B-1003 (11:05)	Transplant Suite B-5	<4	No Growth
B-2014 (16:20)	Transplant Suite B-5	<4	No Growth
B-2015 (16:08)	Transplant Suite B-5	4	Aspergillus fumigatus (1)

Sample		Malt Extract Agar @ 35°C				
Number (Start Time)	Location	Total Fungi (CFU/m ³) ^a	Predominant Taxa (number of colonies)			
B-1004 (11:40)	Transplant Suite B-4	<4	No Growth			
B-1005 (11:55)	Transplant Suite B-4	<4	No Growth			
B-2012 (15:37)	Transplant Suite B-4	11	Aspergillus fumigatus (3)			
B-2013 (15:25)	Transplant Suite B-4	4	Mucor (1)			
B-1015 (17:03)	Transplant Suite A-9	<4	No Growth			
B-1016 (17:17)	Transplant Suite A-9	<4	No Growth			
B-2000 (10:09)	Transplant Suite A-9	<4	No Growth			
B-2001 (9:55)	Transplant Suite A-9	<4	No Growth			
B-2004 (11:55)	Smoking Room	<4	No Growth			
B-2005 (11:43)	Smoking Room	<4	No Growth			
B-2002 (10:57)	Resident Manager's Apartment - Main Floor	<4	No Growth			
B-2003 (10:44)	Resident Manager's Apartment - Main Floor	<4	No Growth			

 ${}^{a}CFU/m^{3} = Colony$ forming units per cubic meter of air.

Table B-6 Air Sampling for Thermotolerant Fungi Ronald McDonald House Durham, North Carolina Sampling Date: November 25, 1997

Commle Number		Malt Extract Agar @ 35°C			
Sample Number (Start Time)	Location	Total Fungi (CFU/m ³) ^a	Predominant Taxa (number of colonies)		
B-3001 (10:54)	Outdoor Air - East Side of Building	18	Aspergillus fumigatus (3) Aspergillus niger (1) Emericella (Aspergillus) nidulans (1)		
B-3002 (11:08)	Outdoor Air - East Side of Building	<4	No Growth		
B-4006 (13:03)	Outdoor Air - West Side of Building	14	Aspergillus fumigatus (3) Paecilomyces variotii (1)		
B-4007 (13:32)	Outdoor Air - West Side of Building	11	Aspergillus fumigatus (2) Penicillium (1)		
B-4004 (12:30)	Outdoor Air - North Side of Building	35	Aspergillus fumigatus (9) Penicillium (1)		
B-4005 (12:43)	Outdoor Air - North Side of Building	25	Aspergillus fumigatus (5) Aspergillus niger (1) Penicillium (1)		
B-3000 (8:10)	Play Room	11	Aspergillus fumigatus (3)		
B-3003 (11:54)	Play Room	4	Aspergillus fumigatus (1)		
3005 (15:06)	Play Room	431	Paecilomyces variotii (121) Aspergillus niger (1)		
B-4001 (8:15)	Dining Room	4	sterile fungi (1)		
B-4002 (8:29)	Dining Room	4	Penicillium (1)		
B-4003 (11:35)	Dining Room	4	sterile fungi (1)		
B-3004 (14:35)	Guest Room A-6	<4	No Growth		

 ${}^{a}CFU/m^{3} = Colony$ forming units per cubic meter of air.

Table B-7 Air Sampling for Total Fungal Spores Ronald McDonald House Durham, North Carolina Sampling Date: November 24, 1997

Sample Number (Start Time)	Location	Sample Collection Volume (liters)	Concentration (Total Fungal Structures/m ³) ^a	Predominant Taxa (number of fungal structures)
S-9 (10:42)	Outdoor Air - East Side of Building	1,254	3,691	Aspergillus/Penicillium-like (2) Cladosporium (2)
S-8 (10:37)	Outdoor Air - West Side of Building	1,268	17,333	unknown (14) <i>Cladosporium</i> (3) <i>Epicoccum</i> (1) hyphal fragments (1)
S-4 (10:18)	Transplant Suite B-10	1,355	4,269	Aspergillus/Penicillium-like (4) basidiospores (1)
S-10 (10:55)	Transplant Suite B-5	1,194	3,875	unknown (2) Aspergillus/Penicillium-like (1) hyphal fragments (1)
S-5 (10:23)	Transplant Suite B-4	1,299	1,780	<i>Cladosporium</i> (1) basidiospores (1)
S-1 (9:21)	Transplant Suite A-9	1,470	2,360	Aspergillus/Penicillium-like (2) Cladosporium (1)
S-3 (10:11)	Office on Second Floor	1,299	5,343	Cladosporium (6)
S-2 (10:03)	Smoking Room	1,362	4,246	Aspergillus/Penicillium-like (2) Cladosporium (2) unknown (1)
S-6 (10:26)	Resident Manager's Apartment - Ground Floor	1,269	912	Aspergillus/Penicillium-like (1)
S-7 (10:32)	Resident Manager's Apartment - Main Floor	1,248	5,563	Cladosporium (6)

^aTotal fungal structures/ m^3 = Total fungal structures per cubic meter of air.

Table B-8 Air Sampling for Total Fungal Spores Ronald McDonald House Durham, North Carolina Sampling Date: November 25, 1997

Sample Number (Start Time)	Location	Sample Collection Volume (liters)	Concentration (Total Fungal Structures/m ³) ^a	Predominant Taxa (number of fungal structures)
S-21 (8:27)	Outdoor Air - North Side of Building	1,538	10,532	<i>Cladosporium</i> (7) basidiospores (6) unknown (1)
S-16 (8:12)	Play Room	1,509	2,300	Aspergillus/Penicillium-like (2) basidiospores (1)
S-15 (8:16)	Dining Room	1,456	1,589	basidiospores (2)
S-19 (10:11)	Guest Room A-2	1,208	1,915	basidiospores (2)
S-18 (8:21)	Guest Room A-4	1,497	2,318	Cladosporium (3)
S-20 (9:17)	Guest Room A-6	1,320	<877	No Spores Detected
S-17 (9:14)	Guest Room A-12	1,322	160,825	Aspergillus/Penicillium-like (129) Stachybotrys (26) Cladosporium (5) basidiospores (1)
S-12 (10:16)	Guest Room B-2	1,232	<939	No Spores Detected
S-13 (10:17)	Guest Room B-7	1,200	26,989	Cladosporium (28)
S-14 (8:47)	Guest Room B-9	1,464	2,370	Cladosporium (3)

^aTotal fungal structures/ m^3 = Total fungal structures per cubic meter of air.

Appendix C Microbial Bulk Sampling Results

Table C-1 Results of Culture Analysis for Fungi in Bulk Materials Ronald McDonald House Durham, North Carolina Sampling Date: November 25, 1997

	Malt Extract Agar @ 25°C		Malt Extract Agar @ 35°C		Corn Meal Agar @ 25°C	
Sample Location	Total Fungi (CFU/g)ª	Predominant Taxa (number of colonies)	Total Fungi (CFU/g)ª	Predominant Taxa (number of colonies)	Total Fungi (CFU/g) ^a	Predominant Taxa (number of colonies)
Guest Room A-5 (paper-backing on sheetrock; bottom right corner of AC unit)	5,776,101	Cladosporium (153) Stachybotrys chartarum (69) Penicillium (2)	<629	No Growth	7,735,850	Cladosporium (222) Stachybotrys chartarum (69)
Guest Room A-5 (paper-backing on sheetrock; bottom right corner of AC unit)	6,842,208	Memnoniella echinata (135) Stachybotrys chartarum (106) Penicillium (10) Cladosporium (4) Aspergillus ustus (2)	49,351	Aspergillus ustus (76)	7,640,909	Stachybotrys chartarum (142) Memnoniella echinata (126) Penicillium (12) Aspergillus ustus (4) Cladosporium (3)
Guest Room A-5 (wet cardboard frame for AC filter)	7,466,888	Rhodatorula (268) yeasts (6) Cladosporium (1)	2,649	yeasts (4)	5,810,507	Rhodatorula (210) Cladosporium (2) yeasts (1) Acremonium (1)
Guest Room A-12 (paper-backing behind baseboard)	7,175,000	Memnoniella echinata (224) Stachybotrys chartarum (22) Aspergillus sydowii (18) Penicillium (6) Aspergillus ustus (3)	22,436	Paecilomyces variotii (25) Aspergillus ustus (10)	9,461,538	Memnoniella echinata (386) Aspergillus sydowii (37) Stachybotrys chartarum (33) Aspergillus ustus (4)

	Malt I	Extract Agar @ 25°C	Malt Ex	tract Agar @ 35°C	Cor	n Meal Agar @ 25°C
Sample Location	Total Fungi (CFU/g) ^a	Predominant Taxa (number of colonies)	Total Fungi (CFU/g) ^a	Predominant Taxa (number of colonies)	Total Fungi (CFU/g) ^a	Predominant Taxa (number of colonies)
Transplant Suite B-4 (wet paper-backing on baseboard behind tile)	10,370,590	Acremonium (368) Stachybotrys chartarum (10) Penicillium (3) Cladosporium (3) Aspergillus versicolor (2) Fusarium (1)	3,939,216	Acremonium (147)	14,122,220	Acremonium (386) Stachybotrys chartarum (138) Penicillium (2) Cladosporium (1)
Resident Manager's Apartment (fiberglass insulation in AHU)	4,545	Penicillium (3) Cladosporium (1) sterile fungi (1) Curvularia (1) Acremonium (1)	<649	No Growth	8,442	Cladosporium (4) Penicillium (4) Aspergillus versicolor (2) Memnoniella echinata (1) sterile fungi (1) yeasts (1)
Mechanical room adjacent to B-4 (under AHU)	298,676	Cladosporium (5) Gilomastix murorum (3) Penicillium (1) sterile fungi (1) yeasts (1)	662	Penicillium (1)	434,437	Acremonium (9) Phoma (2) Cladosporium (2) Gilomastix murorum (1)
Mechanical room under play room (paper from blower in AHU)	6,431,373	Cladosporium (182) Aspergillus versicolor (47) Penicillium (9) Aspergillus restrictus (2)	<654	No Growth	6,029,412	Cladosporium (176) Aspergillus versicolor (42) Penicillium (7)
Mechanical room under play room (insulation liner of AHU downstream of cooling coil)	2,905,298	Cladosporium (65) Penicillium (29) Acremonium (11) Aspergillus versicolor (2)	<662	No Growth	3,258,278	Cladosporium (76) Penicillium (28) Acremonium (3) Aspergillus versicolor (2)

 a CFU/ g = Colony forming units gram of material.

Table C-2 Results of Culture Analysis for Fungi in Settled Dusts Ronald McDonald House Durham, North Carolina Sampling Date: November 25, 1997

	Malt Extract Agar @ 25°C		Malt E	xtract Agar @ 35°C	Corn Meal Agar @ 25°C	
Sample Location	Total Fungi (CFU/g)ª	Predominant Taxa (number of colonies)	Total Fungi (CFU/g)ª	Predominant Taxa (number of colonies)	Total Fungi (CFU/g)ª	Predominant Taxa (number of colonies)
Transplant Suite B-4 (dust collected on return air filter)	5,771,298	Cladosporium (388) Penicillium (56) Rhodotorula (5) Aspergillus ustus (4) Acremoniella (2) sterile fungi (2) Epicoccum nigrum (2) Alternaria (1) Trichoderma koningii (1)	16,794	Aspergillus fumigatus (17) Aspergillus niger (4) Rhizopus stolonifer (1)	5,959,084	Cladosporium (424) Penicillium (45) Aspergillus ustus (3) Rhodotorula (1) Epicoccum nigrum (1) Trichoderma koningii (1) Mucor (1)
Transplant Suite B-5 (dust collected on return air filter)	2,031,859	Cladosporium (126) Paecilomyces variotii (9) sterile fungi (2) Rhodotorula (1) Aspergillus versicolor (1) Sporobolomyces (1)	11,504	Aspergillus fumigatus (8) Aspergillus niger (2) Paecilomyces variotii (2) Aspergillus terreus (1)	2,655,929	Cladosporium (173) Penicillium (8) Aspergillus ustus (1) Alternaria (1)

Sample Location	Malt Extract Agar @ 25°C		Malt Extract Agar @ 35°C		Corn Meal Agar @ 25°C	
	Total Fungi (CFU/g)ª	Predominant Taxa (number of colonies)	Total Fungi (CFU/g) ^a	Predominant Taxa (number of colonies)	Total Fungi (CFU/g) ^a	Predominant Taxa (number of colonies)
Guest Room B-2 (dust on top of ceiling tile)	757,983	Aspergillus sydowii (20) Memnoniella echinata (17) Aspergillus ustus (10) Penicillium (5) Cladosporium (1) Chaetomium (1) Rhizopus stolonifer (1)	5,882	Paecilomyces variotii (4) Aspergillus fumigatus (1) Aspergillus niger (1) Trichoderma koningii (1)	895,798	Memnoniella echinata (29) Aspergillus ustus (27) Aspergillus sydowii (6) Stachybotrys chartarum (2) Chaetomium (1)
Transplant Suite B-10 (dust on top of ceiling tile)	67,290	Aspergillus versicolor (35) Penicillium (24) Chaetomium (9) Paecilomyces variotii (2) Alternaria (1) Aspergillus niger (1)	3,738	Paecilomyces variotii (2) Aspergillus ustus (1) Aspergillus terreus (1)	104,673	Aspergillus versicolor (71) Penicillium (14) Stachybotrys chartarum (9) Cladosporium (8) Aspergillus sydowii (3) Aureobasidium pullulans (2) Memnoniella echinata (2) Drechslera (1) Paecilomyces variotii (1) Trichoderma Koningii (1)

^aCFU/g = Colony forming units gram of material.

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