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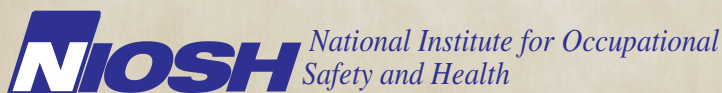


Evaluation of Mold Contamination in a Hotel Before and After Remediation

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HIGHLIGHTS OF THE NIOSH HEALTH HAZARD EVALUATION

On October 22, 1998, the National Institute for Occupational Safety and Health (NIOSH) received a request from the Health Commissioner of Springdale, Ohio, for technical assistance in evaluating hotel employee health and employee exposures to mold at the Springdale Best Western Hotel in Springdale, Ohio. Guests and hotel employees had complained about water leakage and odors, and hotel managers had discovered mold in the walls of guest rooms. NIOSH investigators conducted evaluations in November 1998, February and April 1999, and July 2000.

What NIOSH Did

- We analyzed wallpaper, gypsum board, ventilator filters, and wall surfaces for mold and mycotoxins (toxins produced by mold).
- We analyzed air samples for mold.
- We asked employees about health symptoms.
- We used a research test to see if we could determine if employees were exposed to mycotoxins. This test involved checking employee blood samples for antibodies (substances produced by the immune system) that might indicate exposure to a mycotoxin.

What NIOSH Found

- We found high levels of mold contamination prior to remediation work. Concentrations of mold in the air returned to background levels after remediation.
- One employee had a serious lung illness thought by his doctor to be due to exposure to mold at the hotel. Other employees had symptoms that improved when they left work.
- On the whole, the blood test results for mycotoxin antibodies for employees who had probably been exposed to mold were not different than the results for unexposed employees.

What Springdale Best Western Hotel Managers Can Do

- Close off any areas contaminated with mold.
- Make sure any remediation work is done by qualified workers who use environmental controls and work practices that do not result in contamination of other areas of the hotel.

What Springdale Best Western Hotel Employees Can Do

- Inform management of unusual odors, visible mold, or signs of water leakage.
- Unless trained to do so, do not attempt any inspection for mold that involves removal of wallpaper, drilling holes, or other invasive procedures.

The NIOSH investigation documented the presence of active fungal reservoirs behind vinyl wallpaper of exterior hotel walls, and air sampling indicated the dissemination of fungi into the rooms of the hotel. Follow-up surveys after tear-out, build-out, and re-occupancy indicated that the fungal contamination had been satisfactorily remediated. Inhalation exposures to various fungal structures, and consequently their mycotoxins, were possible at the time of the NIOSH investigation, so it is reasonable to assume that similar exposure conditions were present prior to remediation activities when wallpaper was removed. Although one employee developed an illness that seemed unexplained other than by exposures to fungi at the hotel, the extent to which other employees had symptoms related to these exposures was not determined. The antibody test for the mycotoxin roridin was not useful for assessing exposure.

On October 22, 1998, NIOSH received a request from the Springdale, Ohio, Health Department for technical assistance in evaluating worker exposures to mold at the Springdale Best Western Hotel. Since May 1998, guests and hotel employees had complained to hotel managers about water leakage and odors in guest rooms. Extensive fungal (mold) growth was found and, in October, the hotel was closed for renovation. A manager who had inspected contaminated areas and removed pieces of wallpaper was diagnosed with a lung disease consistent with hypersensitivity pneumonitis and thought to be caused by exposure to mold at the hotel.

NIOSH investigators conducted several site visits in November 1998 to inspect the hotel and collect bulk samples of gypsum board, unit ventilator filters, and wallpaper; tape samples of wall surfaces; and air samples for culturable fungi and nonculturable fungal structures. The rooms sampled included those on the second, third, and fifth floors, the latter two floors appearing to be more contaminated than the second floor. NIOSH personnel returned to collect air samples in February 1999 (after tear-out), in April 1999 (after build-out), and in July 2000 (after re-occupancy). The NIOSH medical investigator surveyed exposed (those who worked in the mold-contaminated areas) and unexposed (those who worked only in the bar and restaurant area) employees, and obtained blood samples for a research test for antibodies to roridin, a mycotoxin produced by various fungi.

Fungal concentrations in the bulk material samples ranged from 8.8×10^4 to 5.2×10^7 colony forming units per gram of material. The predominant fungi were *Acremonium* sp., *Alternaria* sp., *Aspergillus niger*, *Aspergillus sydowii*, *Mucor* sp., *Penicillium* sp., *Phoma* sp., *Stachybotrys chartarum* (previously known as *Stachybotrys atra*), *Ulocladium chartarum*, and yeasts. The predominant fungi in the tape samples were *Acremonium* sp., *Alternaria* sp., *Aspergillus*/*Penicillium*-like, *Chaetomium*-like, *Penicillium* sp., *Stachybotrys chartarum*, *Stachybotrys*-like, *Ulocladium* sp., and *Ulocladium*-like. Mycotoxins were identified in 8 of 18 samples.

In November 1998, indoor geometric mean culturable fungal concentrations ranged from 294 to 2,690 colony-forming units per cubic meter (CFU/m³); most were above the outdoor concentration of 380 CFU/m³. Fungal concentrations on the third and fifth floors were generally higher than those on the second floor. Outdoors and on the second floor, the predominant

SUMMARY (CONTINUED)

genus was *Cladosporium*, comprising approximately 70% of the total. On the third and fifth floors, however, *Penicillium* sp. were predominant. *Stachybotrys chartarum* was identified in five rooms, representing all three floors. With the exception of two fifth-floor rooms, the indoor geometric mean fungal structure (spores and hyphal fragments) concentrations (1,772 to 296,513 fungal structures per cubic meter [FS/m³], median 16,201 FS/m³) were above the outdoor concentration (5,269 FS/m³). Concentrations on the third floor (median 63,400 FS/m³) and fifth floor (38,500 FS/m³) were higher than in the one sampled location on the second floor (5,575 FS/m³). *Stachybotrys* was identified in all but one of the sampled rooms (0% to 35% of the sample, median 4%). It was not found outdoors.

In February and April 1998 and July 2000, culturable and nonculturable fungal concentrations indoors were generally lower than those outdoors. The percentages of *Cladosporium* and *Aspergillus/Penicillium* in the samples remained relatively stable over time, although they were somewhat lower in July 2000. Both the proportion of sampling locations at which *Stachybotrys* was found and its percentage in those samples declined over time. The air samples collected indoors in July 2000 had no *Stachybotrys*.

Eight employees participated in the medical evaluation; six had housekeeping, maintenance, or supervisory jobs that involved work in mold-contaminated areas. The six mold-exposed employees reported a variety of symptoms, some of which improved away from work, but none of the symptoms was suggestive of hypersensitivity pneumonitis, the illness diagnosed in the manager. Two employees, one exposed and one unexposed, had an elevated immunoglobulin G (IgG) titer to roridin. No employee had specific immunoglobulin M (IgM) antibodies. (IgG indicates exposure at some time; IgM indicates recent exposure.)

Keywords: NAICS Code 721110 (Hotels [except Casino Hotels] and Motels), fungi, mold, mycotoxin, roridin, hypersensitivity pneumonitis

INTRODUCTION

On October 22, 1998, NIOSH received a request from the Springdale, Ohio, Health Department for technical assistance in evaluating worker exposures to mold at the Springdale Best Western Hotel. Since May 1998, guests and hotel employees had complained to hotel managers about water leakage and odors in guest rooms. Extensive fungal (mold) growth was found, and in October the hotel was closed for renovation.

NIOSH industrial hygiene and medical investigators made site visits on November 2, November 19–20, and November 24, 1998; February 8 and April 12–14, 1999; and July 25–26, 2000. The November environmental surveys took place prior to tear-out, and the April 1999 survey took place after build-out but prior to re-occupancy. The July 2000 survey took place after the hotel re-opened.

BACKGROUND

In May 1998, the hotel manager and facilities manager began assessing water damage and fungal growth in hotel rooms following complaints of water leakage and odors. These inspections involved stripping wallpaper and making holes in walls. The managers did not wear respirators. Grossly visible mold was found under the wallpaper and on interior surfaces of exterior walls. Fungal growth was surmised to have been facilitated by water incursion at the seams of the building insulation and protective facing panels. The hotel manager continued work at the hotel supervising the remediation. In 1998, the hotel manager developed an illness that was subsequently diagnosed as restrictive lung disease consistent with hypersensitivity pneumonitis; exposure to fungi at the hotel seemed to be the most likely etiology.¹

NIOSH investigators observed substantial fungal contamination on the inside surface of gypsum board (beneath vinyl wallpaper) in various rooms of the hotel during the walk-through inspection on November 2, 1998. The contaminated surfaces were primarily located on exterior building walls. The rooms at the ends of each wing had the largest surface areas of contamination. Additionally, contamination was most pronounced on the odd-numbered floors. Small, localized patches of fungal growth were observed on bathroom walls at water pipe penetrations and on the walls adjacent to the soft drink vending machines.

BACKGROUND

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The results of the November 1998 environmental evaluations were reported by letter dated August 13, 1999. On February 17, 1999, and March 1, 2000, individual employee participants were notified of their medical test results. On the latter date, the Health Department was provided a summary of the medical test results.

METHODS

Industrial Hygiene Evaluation^{2,3}

Bulk Samples

Bulk samples of material for fungal analysis were placed into sealable plastic storage bags and shipped to the analytical laboratory, where representative portions of each sample were weighed and then vortexed in a recorded volume of 0.2% Tween® 20. Serial dilutions of the prepared samples were then plated to the appropriate nutrient media. The nutrient media used for fungi included cornmeal agar (CMA) and dichloran glycerol (DG18). CMA is a selective nutrient medium that promotes the growth of *Stachybotrys* species; DG18 is a selective nutrient medium that promotes the growth of low-moisture tolerating fungi (xerophiles). Plated samples were incubated at 25°C.

Samples of material to be analyzed for mycotoxins were shipped to the laboratory in the same manner. In the laboratory, they were placed into an Erlenmeyer flask, and the contents extracted with methanol:chloroform (1:1, 20 milliliters) using ultrasound sonication for 1 hour. The flask and contents were stored overnight at 4°C prior to filtration and concentration of the organic extract. The concentrated organic extract was dissolved in a minimum amount of methylene chloride and applied to a polyethylene amine column.

Cellophane tape samples (using the adhesive side of the tape to pull spore structures and hyphae from the growth surface) were mounted in the field sticky side up to a glass slide using a solution of lactophenol and cotton blue stain. Samples were shipped to the analytical laboratory and microscopically analyzed semiquantitatively.

Fourteen bulk samples of materials that appeared to be contaminated with fungi were collected on November 2, 1998, in locations from the third, fourth, and fifth floors. Bulk samples of

METHODS

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materials were also collected from locations with an environment conducive to collection and/or growth of microorganisms (for example, unit ventilator filters). Twelve bulk samples of gypsum board were collected by cutting a sizeable section (approximately 30 square inches [in²]) from the wall. Similarly, two 4-in² samples were cut from the unit ventilator filters. Fifteen sticky cellophane tape samples were collected in rooms on the third, fourth, and fifth floors.

On November 20, 1998, materials contaminated with fungi were collected from rooms on the second, third, and fifth floors for mycotoxin analysis, specifically for toxins produced by *Stachybotrys chartarum*.

Air Samples

To determine the concentrations of culturable airborne fungi, an Andersen single stage viable cascade impactor was used at a calibrated flow rate of 28.3 liters per minute (Lpm). All culturable samples were collected over 5 minutes (with the exception of the outdoor samples, which were collected at 10-minute intervals to compensate for anticipated lower fungal loads expected during the fall). Samples were collected at an approximate height of 3 feet using aseptic techniques (cleaning and sanitizing work surfaces, wiping the Andersen sampler collection surfaces with alcohol between samples, and inverting sample plates before and after sampling). CMA and DG18 were used for the enumeration of fungi. All sample plates were incubated at room temperature (approximately 25°C). The taxa and rank of the collected microorganisms were determined by morphological characteristics.

To measure air concentrations of total spores, air samples were collected with an Air-O-Cell (Zefon Analytical Accessories, St. Petersburg, FL), which collects airborne particles through impaction onto a coated glass slide housed in a 37-millimeter (mm) plastic cassette. The cassette is connected via Tygon® tubing to a high-flow pump operating at a calibrated flow rate of 15 Lpm over a 5-minute period (10 minutes for the outdoor sample). Samples were analyzed for fungal spore counts by optical microscopy. Slides were 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 micrometers (µm) in diameter were considered possible fungal spores.

On November 19–20, 1998, air samples for culturable fungi were collected at 14 interior building locations on the second, third, and fifth floors and one outdoor location. At each sample location, three replicate samples of each nutrient medium were collected for culturable fungi (with the exception of the outdoor location, where six samples were collected). Samples were collected over a 2-day period. Area air samples for total spores were collected at 13 locations throughout the building on the same floors as the culturable fungi samples, as well as at an outdoor sampling location. In February and April 1999 and July 2000, air samples for culturable fungi and total spores were collected from the same areas, except that in February all indoor sites were on the third floor, two outdoor sites were sampled, and no samples for culturable fungi were collected. In April 1999 and July 2000, malt extract agar (MEA) was used instead of CMA as a culture medium. The number of indoor sampling locations during the three follow-up surveys ranged from 11 to 14. When feasible, the same rooms were sampled during each of the surveys.

Medical Evaluation

The medical evaluation included a questionnaire about work activities at the hotel, medical history, and health symptoms during the last month worked at the hotel and a blood test for antibodies to roridin, a mycotoxin produced by various fungi, including *Stachybotrys* species.¹ As explained to all participants, the blood test was not a standard clinical test. NIOSH investigators used the blood test as a research tool to determine if antibody testing might be a feasible way to assess exposure to mycotoxins in the workplace. The study was approved by the NIOSH Human Subjects Research Board and required informed consent from the participants.

Lists of former hotel employees were reviewed with hotel managers to ascertain which employees worked in areas contaminated by fungi and thus were considered exposed. Of the approximately 90 employees, 10–20 were so categorized; a lesser number worked exclusively in the bar/restaurant area and were considered unexposed. The NIOSH medical investigator attempted to contact these employees to seek their participation in the medical survey.

Serum was tested for immunoglobulin G (IgG), indicative of previous exposure, and immunoglobulin M (IgM), indicative

METHODS

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of recent exposure, to roridin. The following analytical method description is adapted from Trout et al.¹

Enzyme-linked immunosorbent assays (ELISA) for roridin-hemisuccinate-human serum albumin (RH-HSA) specific IgG antibodies were performed on each serum sample. Fifty microliters (μL) of a 50 microgram/milliliter solution of RH-HSA was placed in each well and incubated overnight at 4°C. Each well was then washed three times with phosphate buffered saline (0.02 M phosphate buffer, pH 7.4), containing 0.91 Molar sodium chloride and Tween, and a similar wash repeated between all subsequent steps. Aliquots (200 μL) of each diluted serum sample (1:10 in 5% BSA deionized water) were added to the wells and allowed to incubate at room temperature for 2 hours. After washing, 100 μL of goat anti human IgG or IgM (whole molecule) alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) were added to each well and incubated for 2 hours at room temperature. The plates were again washed and 100 μL of 0.6 mM disodium p-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO) substrate solution, diluted in alkaline glycine buffer (0.05 M glycine and 0.5 mM magnesium chloride, pH 10.4), was added. After 10–15 minutes, the reactions were read on an automated ELISA plate reader. Each serum was tested in triplicate. Seven stored sera obtained from unrelated NIOSH studies, from persons living in the same geographic area as the hotel but with no known contact to the hotel or excessively moldy environments, were used as assay controls. A response was defined as positive if the serum optical density was greater than the assay control sera's mean OD plus three standard deviations.

EVALUATION CRITERIA

OSHA requires an employer to furnish employees a place of employment that is free from recognized hazards that are causing or are likely to cause death or serious physical harm [Occupational Safety and Health Act of 1970, Public Law 91-596, sec. 5(a)(1)]. Thus, employers should understand that not all hazardous chemicals have specific OSHA exposure limits such as permissible exposure limits (PELs) and short-term exposure limits. An employer is still required by OSHA to protect its employees from hazards, even in the absence of a specific OSHA PEL.

EVALUATION CRITERIA (CONTINUED)

No exposure guidelines for mold in air exist, so it is not possible to distinguish between safe and unsafe levels of exposure. Nevertheless, the potential for health problems is an important reason to prevent indoor mold growth and to remediate any indoor mold contamination.⁴ Moisture intrusion along with nutrient sources such as building materials or furnishings allows mold to grow indoors, so it is important to keep the building interior and furnishings dry. NIOSH investigators concur with the EPA's recommendations to remedy mold contamination in indoor environments (www.epa.gov/iaq/molds/mold_remediation.html).⁵

Mold

Molds are normal inhabitants of the environment. Saprophytic varieties (which use nonliving organic matter as a food source) inhabit soil, vegetation, water, or any reservoir that can provide nutrients. Under the appropriate temperature and pH and with sufficient moisture and nutrients, molds can grow. The molds can become airborne and spread. In the outdoor environment, the levels of mold in the air vary by geographic location, climate, and surrounding activity. In an indoor environment with no unusual source of molds, the level of molds may vary with the cleanliness of the heating, ventilation, and air conditioning system and with the numbers and activity level of the building occupants. Generally, indoor mold levels are below outdoor levels, but the types of organisms found are similar.^{2,6,7}

The types and severity of symptoms related to exposure to mold in the indoor environment depend in part on the extent of the mold present, the extent of the individual's exposure, and the susceptibility of the individual (for example, whether he or she has pre-existing allergies or asthma). In general, excessive exposure to fungi may produce health problems by several primary mechanisms including (1) allergy or hypersensitivity, (2) infection, and (3) toxic effects. Additionally, molds produce a variety of volatile organic compounds, the most common of which is ethanol, that have been postulated to cause upper airway irritation.⁸ The potential irritant effects of volatile organic compounds from exposure to mold in the indoor environment, however, are not well understood.

Allergic responses are the most common type of health problem associated with exposure to molds. These health problems may

EVALUATION CRITERIA (CONTINUED)

include sneezing; itching of the nose, eyes, mouth, or throat; nasal stuffiness and runny nose; and red, itchy eyes. Repeated or single exposure to mold or mold spores may cause previously nonsensitized individuals to become sensitized. Molds can trigger asthma symptoms (shortness of breath, wheezing, cough) in persons who are allergic to mold. A review of the scientific literature concluded that exposure to molds in the indoor environment may make pre-existing asthma worse, but also concluded that there was not enough evidence to determine whether exposure to mold in the indoor environment could cause asthma.⁹ In its 2004 report, “Damp Indoor Spaces and Health,” the Institute of Medicine found sufficient evidence of an association between mold or dampness indoors and nasal and throat symptoms, asthma symptoms in sensitized asthmatics, wheeze, cough, and hypersensitivity pneumonitis in susceptible persons.¹⁰ Hypersensitivity pneumonitis is another allergic response that has developed in people following extensive short-term (acute) or long-term (chronic) exposure to molds. It is a very rare illness, which may resemble bacterial pneumonia, and typically involves respiratory symptoms (such as cough, wheezing, or shortness of breath) as well as other symptoms (such as extreme fatigue and low-grade fever).

People with weakened immune systems (immune-compromised or immune suppressed-individuals) may be more vulnerable to infections by molds. For example, *Aspergillus fumigatus*, a mold that has been found almost everywhere on every conceivable type of substrate, has been known to infect the lungs of immune-compromised individuals after inhalation of the airborne spores.¹¹ Healthy individuals are usually not vulnerable to infections from airborne mold exposure.

Specific molds can produce toxic substances called mycotoxins under the right conditions. Although illness associated with exposures (from inhalation and/or skin contact) to mycotoxins in agricultural or industrial environments has been reported, there is no conclusive evidence of a link between mycotoxin exposure in the indoor environment and human illness.¹²⁻¹⁴ Roridin is a macrocyclic tricothecene mycotoxin known to be produced by several different fungi including *Stachybotrys* species. Tricothecene mycotoxins are potent irritants and may bind proteins, thus eliciting an immune response. *De novo* biosynthesis of antibodies from mycotoxin exposure has been shown for some mycotoxins such as aflatoxins found in food.¹⁵ In addition, cows exposed

to mycotoxins in their feed have been shown to produce anti-mycotoxin antibodies.¹⁶ Roridin-A IgG antibodies, generated in animals, have been shown to be specific for macrocyclic tricothecenes.¹⁷

RESULTS

Industrial Hygiene Evaluation

Bulk Samples

The results of the bulk sample analyses are summarized in Table 1. Fungal concentrations from the bulk material samples ranged from 8.8×10^4 to 5.2×10^7 colony forming units per gram of material (CFU/g). Samples cultured on the different nutrient media (CMA and DG18) produced similar concentrations and taxonomic rankings. The predominant fungi identified included *Acremonium* sp., *Alternaria* sp., *Aspergillus niger*, *Aspergillus sydowii*, *Mucor* sp., *Penicillium* sp., *Phoma* sp., *Stachybotrys chartarum* (previously known as *Stachybotrys atra*), *Ulocladium chartarum*, and yeasts. Most of these genera have been implicated as allergens, and *Aspergillus*, *Penicillium*, and *Stachybotrys chartarum* have been additionally noted as mycotoxin producers. *Stachybotrys chartarum*, *Mucor* sp., and yeasts are characterized as hydrophilic (moisture-loving) fungi. *Stachybotrys chartarum* was identified in 4 of the 10 sampled rooms (Rooms 329, 417, 501, and 536). All of the bulk material samples revealed concentrations of fungi consistent with the existence of an active microbial reservoir.

The results of the microscopic analysis of the tape samples are summarized in Table 2. Concentrations of fungal structures are presented semiquantitatively, that is, as trace, a few, many, numerous, and massive. Identification was facilitated by the observation of spores, hyphae, and conidiophores. The predominant fungi identified included *Acremonium* sp., *Alternaria* sp., *Aspergillus*/*Penicillium*-like, *Chaetomium*-like, *Penicillium* sp., *Stachybotrys chartarum*, *Stachybotrys*-like, *Ulocladium* sp., and *Ulocladium*-like. *Stachybotrys chartarum* or *Stachybotrys*-like spores were identified in the samples collected from 6 of the 10 sampled rooms (Rooms 300, 317, 429, 501, 517, and 536). The results were relatively consistent with the bulk sample results from the same room, although some variations occurred.

The results of the analysis for mycotoxins from the bulk samples collected on November 20, 1998, are summarized in Table 3.

RESULTS

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Mycotoxins produced by *Stachybotrys chartarum* (or *Memmoniella echinata*) were identified in 8 of 18 samples. The complex trichothecenes, satratoxin and roridin, produced by *Stachybotrys chartarum*, were found in minute quantities in the positive bulk samples. *Memmoniella echinata*, closely related to *Stachybotrys chartarum*, produces the simple trichothecenes, trichodermol and trichodermin, which are considerably less toxigenic than the complex trichothecene, satratoxin. *Memmoniella echinata* also produces griseofulvins. Atranones, spirocyclic compounds (phenylspirodrimanes) produced by *Stachybotrys chartarum* and *Memmoniella echinata*, were identified in the samples collected from five rooms; a large amount was identified in the sample collected from Room 529. Satratoxin and roridin, exhibited as verrucarol upon hydrolysis, were identified in the samples collected from Rooms 517 and 529. Epidechlorogriseofulvin was identified in the samples collected from three rooms (Room 301, 317, and 329).

Air Samples

November 1998

Geometric mean culturable fungi concentrations indoors ranged from 294 to 2,690 CFU/m³ for the samples collected on CMA and from 319 to 2,745 CFU/m³ for the samples collected on DG18 (Table 4). Except for Room 206, the indoor fungi concentrations were above the geometric mean concentrations outdoors, 380 for CMA and 419 CFU/m³ for DG18. Concentrations in samples collected on the third and fifth floors were generally higher than those on the second floor. Outdoors and on the second floor, the predominant genus was *Cladosporium* for both nutrient media, comprising approximately 70% of the total. On the third and fifth floors, however, *Penicillium* sp. were predominant. *Aspergillus* sp. were identified in greatest numbers on the fifth floor, comprising as high as 17% of the total on CMA and 21% of the total on DG18. *Stachybotrys chartarum* was identified only on the CMA nutrient medium (which is expected, because CMA is selective for *Stachybotrys*), in Rooms 206, 317, 508, 517, and 529.

The indoor geometric mean fungal structure (spores and hyphal fragments) concentrations (1,772 to 296,513 fungal structures per cubic meter [FS/m³], median 16,201 FS/m³) were above the outdoor concentration, 5,269 FS/m³, with the exception of rooms 508 (1,772 FS/m³) and 517 (1,812 FS/m³) (Table 5). Median concentrations on the third floor (63,400 FS/m³) and fifth floor

RESULTS

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(38,500 FS/m³) were higher than the one on the second floor (5,575 FS/m³). Outdoors, the predominant fungal structures identified were basidiospores; *Cladosporium* plus *Aspergillus*/*Penicillium* accounted for approximately 20% of the total. In all other sample locations inside the building, *Cladosporium*, *Aspergillus*/*Penicillium*, and *Stachybotrys* together comprised over 50% (up to 98% for Room 323) of the total fungal structure concentrations. On the third and fifth floors, *Aspergillus*/*Penicillium* was the predominant fungal structure observed on the sample filters. *Stachybotrys* was identified in all sampled rooms (1% to 35% of the sample, median 4%) except Room 323. It was not found outdoors.

February 1999

Geometric mean fungal structure concentrations ranged from 134 to 702 (median 258) FS/m³ indoors, compared with 544 and 645 FS/m³ outdoors (Table 5). Outdoors, *Cladosporium* and *Aspergillus*/*Penicillium* together accounted for 38% and 48% at the two sampling sites; indoors, their combined percentages ranged from 4% to 40% of the sample, with a median of 29%. *Stachybotrys* was not found outdoors or at six of the indoor sampling locations; its percentage in those samples in which it was found ranged from 1% to 10%.

April 1999

The 13 rooms sampled for culturable fungi were also sampled in November 1998. Except for Room 317, which had geometric mean culturable fungi concentrations of 772 for MEA and 843 CFU/m³ for DG18, all rooms had concentrations less than the outdoor concentrations of 141 for CMA and 164 CFU/m³ for DG18. *Stachybotrys* was found only in Rooms 206, 301, and 308, and constituted less than 2% of the fungi at each site (Table 5). On the MEA samples, *Cladosporium* plus *Aspergillus*/*Penicillium* comprised over 55% of the fungi at each site, including the outdoor location. On the DG18, samples, *Cladosporium* plus *Aspergillus*/*Penicillium* comprised over 85% of the fungi at each indoor site and 65% outside. With each culture medium, *Penicillium* comprised 98% of the fungi in room 317.

Geometric mean fungal structure concentrations ranged from 181 to 508 (median 321) FS/m³ indoors, compared with 598 FS/

RESULTS

(CONTINUED)

m³ outdoors (Table 5). Outdoors, *Cladosporium* plus *Aspergillus*/*Penicillium* accounted for 23% of the sample; indoors, their combined percentages ranged from 19% to 44% of the sample, with a median of 25%. *Stachybotrys* was found at only two sampling sites, in Room 300 at 3% and in Room 501 at 4%. (It was also present in Room 301 in February 1999; Room 501 was not sampled at that time.)

July 2000

Nine of the 12 rooms sampled for culturable fungi were also sampled in November 1998 and April 1999. The other three were not sampled on either previous survey. None of the 12 rooms had a geometric mean fungal concentration greater than 125 CFU/m³ on either culture medium (Table 4). The geometric mean outdoor concentrations were 260 CFU/m³ for DG18 and 219 CFU/m³ for MEA. *Stachybotrys* was not found in any location. On the MEA samples, *Cladosporium* and *Aspergillus*/*Penicillium* together constituted 18%–73% of the fungi at the indoor locations and 72% outdoors. On the DG18 samples, *Cladosporium* and *Aspergillus*/*Penicillium* together comprised 40%–84% of the fungi at the indoor sites, and 65% outside. Room 317 was not sampled.

Geometric mean fungal structure concentrations ranged from 74 to 404 (median 184) FS/m³ indoors, compared with 4,760 FS/m³ outdoors (Table 5). Outdoors, *Cladosporium* and *Aspergillus*/*Penicillium* together accounted for 10% of the sample; indoors, their combined percentages ranged from 11% to 44% of the sample, with a median of 32%. *Stachybotrys* was not found at any sampling site, including Rooms 301 and 501.

Medical Evaluation

Most of the targeted employees were unreachable at the time of the NIOSH investigation as the hotel was closed. In all, eight employees participated in the survey, including the manager and facilities manager. Participants included five women and three men. They worked at the hotel for one to 14 years, with a median of 4 years. Six were exposed to mold (based on the work location criterion); two were unexposed bar/restaurant-area workers. The exposed employees had housekeeping, maintenance, or supervisory jobs.

RESULTS

(CONTINUED)

Exposed employees reported various symptoms, some of which improved away from work; none of the employees had a symptom pattern suggestive of hypersensitivity pneumonitis. Two employees, one exposed (not the manager) and one unexposed, had an elevated IgG titer to roridin. No employee had specific IgM antibodies.

DISCUSSION

Bulk samples collected during the initial site visit confirmed visual suspicions of fungal reservoirs on gypsum board surfaces. Concentrations ranged from 8.8×10^4 to 4.6×10^7 CFU/g on samples collected from the inside surface of exterior walls, and the predominant genera included *Acremonium*, *Alternaria*, *Penicillium*, *Phoma*, and *Ulocladium*. *Penicillium* was the predominant genus identified (concentration of 4.6×10^7 CFU/g) on the gypsum board sample collected in Room 517 adjacent to the vending machine. *Aspergillus sydowii* was the predominant species identified (from total concentrations greater than 1×10^5) on both filter samples from the unit ventilators. *Stachybotrys chartarum* was the predominant species identified (from total concentrations greater than 4×10^6) from one of the two samples collected from vinyl wallpaper in Room 501. Analysis of tape samples revealed similar results regarding the predominant genera, including *Acremonium*, *Alternaria*, *Penicillium*, and *Ulocladium*. *Stachybotrys* was identified as the predominant genus in the samples collected in Rooms 429 and 501.

The results of air sampling conducted November 19–20, 1998, clearly indicate the dissemination of fungi. Geometric mean concentrations of culturable airborne fungi indoors ranged up to 2,690 CFU/m³. All of the concentrations on the third and fifth floors were greater (as much as five times) than those measured outdoors. Additionally, the taxonomic ranking differed between the indoor and outdoor sample locations. Outdoors, the airborne culturable fungi load was predominantly *Cladosporium* sp., whereas indoors on the third and fifth floors *Penicillium* sp. were the predominant fungi. On the fifth floor, *Aspergillus* sp. comprised a greater proportion of the total load compared to other sampled floors and to the outdoors. *Stachybotrys chartarum* was found in low quantities on samples collected from five locations on the sampled floors. The nonculturable fungi samples revealed similar results. Geometric mean concentrations approached 3,000 FS/m³ (Room 329); all of the sample concentrations indoors were above the

DISCUSSION (CONTINUED)

outdoor concentration. Although *Aspergillus/Penicillium* comprised most of the total load, *Stachybotrys* appeared in many more sample locations and was a larger percentage of the total load than in the culturable fungi air samples.

The air sampling surveys of February and April 1999 and July 2000, which were after the mold remediation, documented the decrease of airborne fungi to levels lower than outdoors. The percentages of *Cladosporium* and *Aspergillus/Penicillium* together in the outdoor samples remained relatively constant in the culturable air samples, but varied in the total structure samples. Also, the total fungi concentrations varied within each type of sample. Indoors, the percentages of *Cladosporium* and *Aspergillus/Penicillium* in the samples remained relatively stable over time, but were somewhat lower in July 2000. Both the percentage of sampling locations at which *Stachybotrys* was found and its percentage in those samples declined over time.

Management and consultant reports of the history of water incursion into the building indicated significantly less moisture on the second floor rooms compared to all others. Differences in the architecture of the exterior wall could account for the reduced water incursion on the second floor. The outside wall on the second floor did not have a smooth transition to the floor above; an overhang existed between the second and the third floor. This overhang did not allow water that had entered the exterior wall cavities on the upper floors to penetrate the second floor. The NIOSH investigators' observations made during the initial walk-through inspection did not reveal significant fungal reservoirs like those observed on the other floors; therefore, bulk samples were not collected from the second floor. The fungal concentrations and taxonomic rankings on the air samples collected in two second-floor rooms were closer to those outdoors than were the results from most of the rooms on the other floors. The second-floor results do not suggest unidentified fungal reservoirs on that floor. This is consistent with the limited reports of water incursion in the second floor rooms.

Mycotoxins specific to *Stachybotrys chartarum* (complex trichothecenes and atranones) and *Memnoniella echinata* (griseofulvins) were identified in 44% of the bulk samples. The scientific community does not have a clear understanding of when mycotoxins are produced by a fungal species. Nonetheless, these findings indicate that *Stachybotrys chartarum* and *Memnoniella echinata* were actively producing mycotoxins.

DISCUSSION (CONTINUED)

Stachybotrys chartarum was observed in only four of the rooms from which bulk samples were collected, but *Stachybotrys chartarum* and *Stachybotrys*-like spores were observed in eight of the rooms in which sticky tape samples were collected. This information, combined with the identification of mycotoxins specific to *Stachybotrys chartarum* and *Memnoniella echinata*, suggests the existence of active reservoirs of these two species. Additionally, the identification of *Stachybotrys chartarum* in air samples (on both culturable and nonculturable fungi samples) documents the dissemination of spores into the air. It has been suggested that the mycotoxins from these species are found in the spores¹⁸; therefore, exposure to the fungal spores and their mycotoxins via inhalation is plausible.

The hotel floors sampled by NIOSH were selected because they had not undergone remediation efforts when the NIOSH investigation started. Sampling these floors provided an opportunity to assess conditions as they likely were before remediation of the hotel as a whole. These results can be extrapolated to that period after the peeling away of wallpaper yet prior to remediation. These results are not indicative of exposures that may have existed prior to peeling away of the wallpaper when guests occupied the hotel. However, given the level of contamination observed, it is plausible that some spores may have been released into the occupied areas prior to the wallpaper removal. The NIOSH surveys evaluated the hotel environment and hotel employees but not exposures or work practices associated with remediation and reconstruction activities.

The medical survey included only a small number of former employees, including fewer than half of those judged exposed to the areas of fungal contamination. Furthermore, the survey was conducted weeks after the exposures of interest and acute symptoms occurred. These circumstances, combined with media reports about the hotel's mold problem, could have resulted in the collection of inaccurate and unrepresentative information. The blood tests did not distinguish between exposed and unexposed employees. The small and potentially unrepresentative sample of employees, however, precludes drawing any conclusions about an association, or lack thereof, between fungal exposures at the hotel and either symptoms or immunologic response to mycotoxin exposure among employees. Although one employee had an illness consistent with hypersensitivity pneumonitis, with onset during the time he was exposed to fungi at the hotel, a specific microbiologic

DISCUSSION (CONTINUED)

etiology was not established during his diagnostic evaluation (which was not part of the NIOSH investigation).

CONCLUSIONS

The NIOSH investigation during November 1998 clearly documented the presence of active fungal reservoirs behind vinyl wallpaper of exterior hotel walls on the third, fourth, and fifth floors. This contamination was most prevalent at the ends of the hotel wings. Additionally, air sampling indicated the dissemination of fungal spores and hyphae into hotel rooms. The identified fungal genera included *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Phoma*, *Stachybotrys*, and *Ulocladium*. Mycotoxins were also identified in bulk samples. Follow-up surveys after tear-out, build-out, and re-occupancy indicated that the fungal contamination had been satisfactorily remediated.

Inhalation exposures to various fungal structures, and consequently their mycotoxins, were possible at the time of the NIOSH investigation. It is reasonable to assume that similar exposure conditions were present prior to removal of wallpaper during remediation. Although one employee developed an illness that seemed unexplained other than by exposures to fungi at the hotel, the extent to which other employees had symptoms related to these exposures was not determined.

RECOMMENDATIONS

1. Employees should notify management of any conditions, such as odors, visible mold, or signs of water leakage that suggest the possibility of fungal contamination. These reports should be appropriately investigated in a timely manner.
2. Any inspection for fungal contamination that involves invasive procedures (such as removing wallpaper or drilling holes) should be done by trained personnel utilizing appropriate exposure controls, work practices, and personal protective equipment.⁵
3. Contaminated areas should be closed to guests and hotel employees until the problem is resolved.
4. Remediation work should be done by knowledgeable personnel (preferably a contractor who specializes in such work) using appropriate practices, including isolation of the

RECOMMENDATIONS

(CONTINUED)

affected area by means of physical barriers and ventilation controls, measures to prevent contamination of the building's ventilation system, proper handling and disposal of contaminated materials, thorough housekeeping in work areas (using damp mopping and vacuums equipped with high-efficiency particulate air [HEPA] filters), and personal protective equipment.⁵

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APPENDIX: TABLES

Table 1
Results of Bulk Sample Analyses
Springdale Best Western Hotel, Springdale, Ohio
November 2, 1998

Sample Location	Location Description	Nutrient Media ^a	Fungi ^b	
			[CFU/g] ^c	Taxa Rank ^d
Room 300	Gypsum board from exterior-facing wall	CMA DG 18	4.8x10 ⁶ 4.1x10 ⁶	Pen>>Ulo Pen>>Ulo
Room 301	Gypsum board from exterior-facing wall	CMA DG 18	3.5x10 ⁶ 2.6x10 ⁶	Acr>>Alt>A syd>Pen Acr>>Alt>A syd>Pen
Room 301	Unit ventilator filter	CMA DG 18	1.4x10 ⁶ 1.1x10 ⁶	A syd>Pen>Cla>Muc A syd>Pen>Cla>Muc
Room 317	Gypsum board	CMA DG 18	3.5x10 ⁶ 3.6x10 ⁶	Pho>>Alt Pho>>Alt
Room 329	Gypsum board	CMA DG 18	4.6x10 ⁷ 4.5x10 ⁷	Pho>>Sta Pho>>Sta
Room 400	Gypsum board	CMA DG 18	1.9x10 ⁶ 1.6x10 ⁶	Pen>>Alt Pen>>Alt
Room 417	Gypsum board	CMA DG 18	1.6x10 ⁵ 8.8x10 ⁴	Pen>Sta>Acr Pen
Room 429	Gypsum board	CMA DG 18	8.4x10 ⁵ 1.2x10 ⁶	Ulo>>Acr Ulo>>Acr
Room 501A	Wallpaper	CMA DG 18	4.0x10 ⁶ 5.1x10 ⁶	Sta>>Acr>>Cla Sta>>Acr>>A ng
Room 501B	Wallpaper	CMA DG 18	1.0x10 ⁶ 1.9x10 ⁶	Ulo>>Pen Ulo
Room 517	Gypsum board adjacent to ice machine	CMA DG 18	4.6x10 ⁷ 5.2x10 ⁷	Pen Pen
Room 517	Gypsum board next to ventilator	CMA DG 18	1.0x10 ⁶ 1.3x10 ⁶	Alt>>Pen Alt
Room 536	Unit ventilator filter	CMA DG 18	8.0x10 ⁵ 5.9x10 ⁵	A syd A syd>>Pen
Room 536	Gypsum board	CMA DG 18	6.5x10 ⁶ 4.8x10 ⁶	Acr>>Sta>>Pen Acr>>Sta

^a CMA: cornmeal agar; DG18: dichloran glycerol

^b Acr: *Acremonium*

Alt: *Alternaria*

A ng: *Aspergillus niger*

A syd: *Aspergillus sydowii*

Muc: *Mucor*

Pen: *Penicillium*

Pho: *Phoma*

Sta: *Stachybotrys chartarum*

Ulo: *Ulocladium chartarum*

Yea: yeasts

^c CFU/g: colony-forming units per gram

^d >: Greater than; >>: Much greater than

APPENDIX: TABLES

(CONTINUED)

Table 2
Results of Microscopic Analyses of Tape Samples
Springdale Best Western Hotel, Springdale, Ohio
November 2, 1998

Sample Location	Location Description	Taxonomic Ranking ^a
Room 300A	Exterior wall next to unit ventilator	Trace (Ulo>Asp/Pen-l~Acr~Cha-l~Cla-l~Sta-l)
Room 300B	Exterior wall	Many (Acr~Pen~Sta~Ulo)
Room 301A	Exterior wall	Many (Alt~Pen~Ulo)
Room 301B	Exterior wall next to window	Numerous (Acr~Ulo>Asp/Pen-l)
Room 317	Interior wall opposite vending machine	Numerous (Pen>Sta-l)
Room 329	Exterior wall next to unit ventilator	Numerous (Alt~Ulo)
Room 400	Exterior wall	Massive (Pen)
Room 429	Exterior wall next to unit ventilator	Massive (Sta>Ulo)
Room 436	Ceiling of south wall	Many (Acr~Ulo)
Room 501A	Exterior wall	Numerous (Sta~Acr)
Room 501B	Exterior wall	Few (Asp/Pen-l>Sta-l)
Room 501C	Exterior wall	Numerous (Ulo)
Room 517A	Exterior wall	Few (Acr>Asp/Pen-l~Sta-l~Ulo-l)
Room 517B	Exterior wall	Numerous (Ulo)
Room 536	Exterior wall	Many (Alt>Asp/Pen-l~Sta-l~Ulo-l)

^aAcr: *Acremonium*

Alt: *Alternaria*

Asp/Pen: *Aspergillus/Penicillium*

Cha: *Chaetomium*

Pen: *Penicillium*

Sta: *Stachybotrys chartarum*

Ulo: *Ulocladium chartarum*

l: like

>: Greater than

APPENDIX: TABLES

(CONTINUED)

Table 3
Results of Analyses of Gypsum Board Bulk Samples for Mycotoxins
Springdale Best Western Hotel, Springdale, Ohio
November 20, 1998

Sample Location	Taxonomic Rank ^a
Room 300A	ND ^b
Room 300B	ND
Room 301A	Atranones
Room 301B	Epidechlorogriseofulven>>atranones
Room 303	ND
Room 317	Trace amounts of epidechlorogriseofulvin
Room 326	ND
Room 329	Epidechlorogriseofulvin
Room 500	Atranones
Room 501A	ND
Room 501B	Atranones
Room 502	ND
Room 515	ND
Room 517	Atronones>>tricothecene
Room 521	ND
Room 529	Atranones>>trace tricothecene
Room 534	ND
Room 536	ND

^a >>: Much greater than

^b None detected

APPENDIX: TABLES

(CONTINUED)

Table 4
Results of Air Sampling for Culturable Fungi
Springdale Best Western Hotel, Springdale, Ohio
November 19–20, 1998 through July 25–26, 2000

Room	Concentration (colony forming units per cubic meter) ^a					
	Cornmeal or Malt Extract Agar ^b			Dichloran Glycerol		
	Nov 1998	Apr 1999	Jul 2000	Nov 1998	Apr 1999	Jul 2000
201	881	c	c	826	c	c
205	c	c	44	c	c	47
206	294	72	82	319	90	95
300	2153	80	c	2745	129	c
301	d	60	35	d	91	44
308	949	42	56	1148	59	53
317	1966	772	c	1069	843	c
323	d	87	75	d	82	77
329	2690	70	53	1483	76	59
334	c	c	116	c	c	115
500	1430	74	c	1599	79	c
501	2106	70	56	2438	104	48
508	411	82	99	726	99	59
517	841	68	98	1016	71	79
529	1892	93	125	2302	90	109
534	c	c	106	c	c	65
536	607	114	c	2440	148	c
Outside	380	141	219	419	164	260

^aGeometric mean of replicate samples

^bCornmeal agar was used in November 1998; malt extract agar was used in April 1999 and July 2000

^cSample not collected

^dSample was overloaded with fungal growth and could not be counted

APPENDIX: TABLES

(CONTINUED)

Table 5
Results of Air Sampling for Spores and Hyphae
Springdale Best Western Hotel, Springdale, Ohio
November 19–20, 1998 through July 25–26, 2000

Room	Fungal Structures per Cubic Meter ^a				Percent <i>Stachybotrys</i>			
	Nov 98	Feb 99	Apr 99	Jul 2000	Nov 98	Feb 99	Apr 99	Jul 2000
201	5575	b	b	b	1	b	b	b
205	b	b	b	119	b	b	b	0
206	b	b	319	240	b	b	0	0
300	36128	569	227	b	17	1	3	b
301	90600	702	219	74	4	4	0	0
307	b	346	b	b	b	0	b	b
308	6422	316	181	163	3	10	0	0
317	8576	134	311	b	35	6	0	b
322	b	414	b	b	b	6	b	b
323	108538	161	339	175	0	0	0	0
328 (hallway)	b	245	b	b	b	0	b	b
329	296513	258	389	404	2	0	0	0
330	b	215	b	b	b	0	b	b
334	b	b	b	220	b	b	b	0
336	b	187	b	b	b	0	b	b
500	16201	b	322	b	2	b	0	b
501	60739	b	327	265	9	b	4	0
508	1772	b	344	164	4	b	0	0
517	1812	b	338	192	9	b	0	0
529	42685	b	508	112	28	b	0	0
534	b	b	b	291	b	b	b	0
536	42859	b	383	b	7	b	0	b
Outside (SW)	5269	645	598	4760	0	0	0	0
Outside (SE)	b	544	b	b	b	0	b	b

^a Geometric mean of replicate samples

^b Sample not collected

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