NIOSH HEALTH HAZARD EVALUATION REPORT

HETA #2004-0387-3071
Gro-West
Utica, New York

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

PREFACE

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

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

ACKNOWLEDGMENTS AND AVAILABILITY OF REPORT

This report was prepared by Nancy Clark Burton of HETAB, Division of Surveillance, Hazard Evaluations and Field Studies (DSHEFS). Field assistance was provided by Donnie Booher, Chad Dowell, Kevin L. Dunn, Ron Sollberger, and Ken Wallingford of DSHEFS. Analytical support was provided by DataChem Laboratories, Salt Lake City, Utah and EMLabs/P&K Laboratories, Cherry Hill, New Jersey. Desktop publishing was performed by Robin Smith. Editorial assistance was provided by Ellen Galloway. Health communication assistance was provided by Stefanie Evans.

Copies of this report have been sent to employee and management representatives at Gro-West and the OSHA Regional Office. The report may be viewed and printed from the following internet address: http://www.cdc.gov/niosh/hhe. Copies may be purchased from the National Technical Information Service at 5825 Port Royal Road, Springfield, Virginia 22161.

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

Highlights of the NIOSH Health Hazard Evaluation

The National Institute for Occupational Safety and Health (NIOSH) received a management request for a health hazard evaluation (HHE) at Gro-West Inc. in Utica, New York. The company was remediating mold in a house being renovated for use as a women and children's shelter. Gaseous chlorine dioxide (ClO_2) was used to treat the house after a roof leak had been repaired. NIOSH employees conducted an investigation in November-December 2004 to look at the effectiveness of the ClO_2 treatment in reducing microbial contamination and re-occupancy issues related to any remaining contamination.

What NIOSH Did

- We collected air samples for culturable bacteria and fungi, total fungi determined by microscopic count and polymerase chain reaction (PCR) assays, endotoxin, and (1→3)-β-D-glucan before and after ClO_2 treatment.
- We collected sticky tape samples on surfaces before and after ClO_2 treatment for fungal analysis.
- We collected wipe samples for chloride, chlorate, and chlorite ion decontamination by-products before and after ClO_2 treatment.

What NIOSH Found

- Culturable bacteria and fungi concentrations and total fungal spore counts of fungi (as determined by spore trap and PCR) were significantly decreased after the ClO_2 treatment.
- Microscopic analyses of tape samples collected from surfaces after treatment showed that the fungal structures were still present on surfaces after ClO_2 treatment.
- There were no significant differences in airborne endotoxin and (1→3)-β-D-glucan concentrations before and after ClO_2 treatment.
- There was an increase in chloride, chlorate, and chlorite ions after ClO_2 treatment which was expected since these compounds are some of the end products of ClO_2 disinfection.

What Managers Can Do

- Use additional clean-up techniques, such as air cleaners and cleaning surfaces using high efficiency particle air (HEPA) filter vacuums to reduce concentrations of spores and microbial components before re-occupancy.

What To Do For More Information:
We encourage you to read the full report. If you would like a copy, either ask your health and safety representative to make you a copy or call 1-513-841-4252 and ask for HETA Report #2004-0387-3071

Health Hazard Evaluation Report 2004-0387-3071
Gro-West
Utica, New York
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SUMMARY

In September 2004, the National Institute for Occupational Safety and Health (NIOSH) received a management request for a health hazard evaluation (HHE) at Gro-West Inc. in Utica, New York. Gro-West management submitted the HHE request because they were remediating mold in a house being renovated for future use as a shelter for women and children. NIOSH was asked to evaluate the gaseous chlorine dioxide (ClO_2) treatment process with respect to its effectiveness in removing microbial contamination. NIOSH investigators conducted an evaluation in November and December 2004.

Traditional and newer techniques for evaluating microbial contamination were used under field conditions to evaluate the ClO_2 treatment effectiveness. The evaluation was performed in a microbially contaminated house, which had an undetected roof leak for an extended period that resulted in large areas of visible microbial growth. Concentrations of culturable fungi and bacteria, total fungi determined by microscopic count and polymerase chain reaction (PCR) assays, endotoxin, and (1→3)-β-D-glucan were determined before and after the house was treated with ClO_2. Area air samples were collected and analyzed for volatile organic compounds (VOCs) present in the house before and after ClO_2 treatment to see which VOCs were generated by the ClO_2 treatment. Wipe samples of walls were collected for chloride, chlorate, and chlorite ion decontamination by-products before and after ClO_2 treatment.

Culturable bacteria and fungi concentrations and total fungal spore counts (as determined by spore trap and PCR) decreased significantly after the ClO_2 treatment. However, microscopic analyses of tape samples collected from surfaces after treatment showed that fungal structures were still present on surfaces after ClO_2 treatment. No significant differences in airborne endotoxin and (1→3)-β-D-glucan concentrations were measured in the house before and after ClO_2 treatment. An increase in chloride, chlorate, and chlorite ions occurred after ClO_2 treatment, which was expected because these compounds are some of the end products of ClO_2 disinfection. Due to the potential for health effects from residuals present after ClO_2 treatment, additional clean-up techniques, such as using air cleaners and cleaning surfaces using high efficiency particulate air (HEPA) filter vacuums to reduce concentrations of spores and microbial components, were recommended.

This evaluation showed that gaseous ClO_2 treatment effectively reduced the total fungi and the culturable bacteria and fungi in the air of the treated house after the source of moisture incursion has been addressed. Fungal spores, however, were still visible on sticky tape samples after ClO_2 treatment. The treatment process appeared to have no significant effect on the airborne concentrations of endotoxin and (1→3)-β-D-glucan. Additional investigation is needed to determine whether exposures to the fungal spores and bioaerosols remaining after the ClO_2 treatment are capable of producing adverse health effects.

Keywords: NAICS 813211 (Grantmaking Foundations), gaseous chlorine dioxide, mold remediation, fungi, bacteria, endotoxin, (1→3)-β-D-glucan, polymerase chain reaction, PCR

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INTRODUCTION

On September 15, 2004, the National Institute for Occupational Safety and Health (NIOSH) received a management request for a health hazard evaluation (HHE) from Gro-West, a nonprofit organization. The organization had purchased a Victorian house in Utica, New York, to be renovated for use as a shelter for women and children. The house needed mold remediation due to a roof leak, and several agencies had volunteered their services to help with the remediation. Other participating agencies included the U.S. Environmental Protection Agency (EPA), the City of Utica, and Sabre Technical Services, the company responsible for the chlorine dioxide (ClO_2) treatment. NIOSH was asked to evaluate the effect of the ClO_2 treatment process on microbial contamination and potential exposures related to reoccupancy after treatment.

After telephone consultations with the requestors and representatives of Sabre Technical Services, a site visit was made on November 29–December 6, 2004. An opening conference was held with the management of Gro-West and management and employees of Sabre Technical Services. Sabre Technical Services completed the ClO_2 treatment process on December 3, 2004. NIOSH investigators conducted environmental sampling for culturable fungi and bacteria, total fungi determined by microscopic count and polymerase chain reaction (PCR) assays, endotoxin; (1→3)-β-D-glucan, and volatile organic compounds (VOCs). Chloride, chlorate, and chlorite ions were measured before and after ClO_2 treatment.

BACKGROUND

The evaluation was performed in a 1890s Victorian house (see Figure 1) in Utica, New York. The house had three stories and a dirt/stone basement and had been purchased by a nonprofit organization because of its location and structure. The house had been occupied until approximately 6 months prior to the ClO_2 treatment. A major roof leak was identified as the source of the water incursion and was repaired after an asbestos abatement project was completed. Visible mold was present in most areas of the first, second, and third floors. Some rooms had bird and cat droppings present on the floor and in an old safe.

Sabre Technical Services employees were responsible for the ClO_2 treatment process. The entire structure was tented using the standard procedure for whole-house pesticide treatment (see Figure 2). The interior of the house was ventilated, heated, and humidified prior to the application of ClO_2 gas to maintain the tent under positive pressure and provide optimal conditions for the ClO_2 treatment. The positive pressure was verified by the use of pressure gauges.

The ClO_2 solution was created on site using household bleach (5%–6% sodium hypochlorite), 6-N- hydrochloric acid, 25% sodium chlorite, and distilled water. The Sabre ClO_2 gas generator used a sparging column into which the ClO_2 solution was pumped. Air from the tented house was pumped countercurrent from the ClO_2 solution in the sparging column, which picked up the ClO_2 from the solution. The ClO_2-laden air was then returned to the house. When the ClO_2 concentration reached the desired level (650 parts per million [ppm]), the pumping of the liquid solution into the gas generator was stopped. The ClO_2 concentration was monitored during the treatment, and additional ClO_2 was added to the house using this method to keep the ClO_2 at the desired level. The treatment process is based on achieving a total exposure of at least 9,000 ppm-hours (calculated as ppm × time). The air inside the house was neutralized using a negative air scrubbing system after the target exposure level and duration had been obtained. The spent liquid and remaining ClO_2 solution were neutralized with 10% sodium hydroxide.[1]

During the treatment process, ClO_2 concentrations were monitored by Sabre Technical Services employees inside the house on each floor and outside the house every 15 minutes. Samples were collected into a midget

impinger containing 5% potassium iodide phosphate buffer solution in conjunction with a Gilian sampling pump (Sensidyne, LP, Clearwater, FL) at 1 liter per minute (Lpm). Pre- and postcalibration of the impinger/pump were performed using a mini-Buck calibrator (A.P. Buck, Inc., Orlando, FL). The samples were analyzed using a sodium thiolsulfate titration method.[2,3] An average total exposure level of 10,351 ppm-hours was achieved for treatment. The average ClO_2 concentrations over the 12.5-hour treatment period for the basement, first floor, second floor, and third floor were 739 ppm, 902 ppm, 845 ppm, and 821 ppm, respectively. Relative humidity (RH) and temperature inside the house were measured in real-time using HOBO® data loggers (Onset Computer Corporation, Bourne, MA). The house was maintained at about 75°F temperature and 70% RH.

METHODS

After a walk-through survey of the house, NIOSH investigators chose eight sampling locations for the evaluation, two on each floor and the basement (see Figure 3 for a representative sample station). Floor fans that had been used to help ventilate the house were turned off or redirected to prevent interference with the collection of air samples. An outdoor sampling location was set up in the back yard of the house. A sampling/monitoring equipment facility was established in a tent in the back yard to support the evaluation.

Draeger® colorimetric detector tubes (Draeger Safety, Inc., Pittsburgh, PA) were used to determine the remaining concentration of ClO_2 48 hours after treatment. The 48-hour time period was selected because it was similar to that used for the ClO_2 decontamination of buildings contaminated with *Bacillus anthracis* during the 2001 anthrax attacks. The time period is used to allow any remaining ClO_2 gas inside the house to off-gas and react with any remaining organic materials.[4] The tent was kept under positive pressure during this time to prevent fungi and bacteria from entering the house from outdoors.

Microbial Sampling

Interior environmental sampling was conducted at the house on November 30, 2004, and December 5, 2004. The same environmental sampling protocol was used before and after ClO_2 treatment. As presented in Table 1, microbial contamination and other compounds were assessed using a series of standard monitoring techniques. Airborne culturable count was determined by collecting samples with an Andersen N-6 single-stage impactor (Thermo Electron Corporation, Waltham, MA) on malt extract agar and tryptic soy agar in triplicate. Air samples for total microbial counting were collected using an Air-O-Cell® spore trap sampler (Zefon International, Inc., Ocala, FL). Three parallel filter samples were collected from the air: one for PCR analysis, one for endotoxin, and one for (1→3)-β-*D*-glucan assay. PCR samples were collected on a 0.3 micrometer (µm) pore-size 37-millimeter (mm) polytetrafluoroethylene (PTFE) filter. The PCR analysis was conducted to determine spore equivalent count of 23 selected fungal species using standard protocols and primer sequences for biological agents as patented by the EPA.[5] Endotoxin samples were collected on 5.0-µm pore size 37-mm polyvinyl chloride (PVC) filters, and (1→3)-β-*D*-glucan samples were collected on 0.3-µm pore size 37-mm PTFE filters. The samples were analyzed by the *Limulus Amoebocyte* lysate (LAL) assay. Microscopic analysis of sticky tape samples collected from surfaces was performed to determine the level and form of fungal growth.

The relative efficiency of the ClO_2 treatment for each of the measured microbial sample types was calculated as:

$$\text{Relative Efficiency} = \frac{\text{Concentration}_{\text{before}} - \text{Concentration}_{\text{after}}}{\text{Concentration}_{\text{before}}} \times 100\%$$

Volatile Organic Compounds

Thermal desorption tubes were used to qualitatively assess the presence of VOCs. These tubes were analyzed by the NIOSH laboratory using a Markes Unity/Ultra automatic thermal desorption system (Markes International Inc., New Haven, CT) and an HP5890A gas chromatograph with an HP5970 mass selective detector (Agilent Technologies, Inc. Santa Clara, CA) in accordance with NIOSH Method 2549.[6]

Chloride, Chlorate, and Chlorite Salt Ions

Gauze wipe samples (Dukal sterile gauze pads #1312, Dukal Corporation, Hauppauge, NY) were collected for chloride, chlorate, and chlorite ions (the end product of ClO_2 reactions) before and after the ClO_2 treatment. The samples were analyzed using ion chromatography according to a modified U.S. EPA Method 300.0.[7] The method was modified to accommodate the gauze collection material. The limits of detection per sample were as follows: chloride, 0.5 micrograms (µg); chlorate, 0.3 micrograms; and chlorite 0.1 micrograms (µg). The limits of quantitation (LOQ) per sample were as follows: chloride, 2 µg; chlorate, 1 µg; and chlorite, 0.4 µg.

Data Analysis

Data analysis was performed using the SAS statistical package version 9.1 (SAS Institute, Inc., Cary, North Carolina). Paired t-tests were performed to compare the average sample concentrations before and after the ClO_2 treatment. For samples with nondetectable concentrations, one half of the limit of detection was used in the analysis.[8] A significance level of 0.05 was used for all statistical tests.

EVALUATION CRITERIA

In evaluating the hazards posed by workplace exposures, NIOSH investigators use both mandatory (legally enforceable) and recommended occupational exposure limits (OELs) for chemical, physical, and biological agents as a guide for making recommendations. OELs have been developed by federal agencies and safety and health organizations to prevent the occurrence of adverse health effects from workplace exposures. Generally, OELs suggest levels of exposure to which most workers may be exposed up to 10 hours per day, 40 hours per week for a working lifetime without experiencing adverse health effects. However, not all workers will be protected from adverse health effects even if their exposures are maintained below these levels. A small percentage may experience adverse health effects because of individual susceptibility, a preexisting medical condition, and/or a hypersensitivity (allergy). In addition, some hazardous substances may act in combination with other workplace exposures, the general environment, or with medications or personal habits of the worker to produce health effects even if the occupational exposures are controlled at the level set by the exposure limit. Also, some substances can be absorbed by direct contact with the skin and mucous membranes in addition to being inhaled, which contributes to the individual's overall exposure.

Most OELs are expressed as a time-weighted average (TWA) exposure. A TWA refers to the average exposure during a normal 8- to 10-hour workday. Some chemical substances and physical agents have a recommended short-term exposure limit (STEL) or ceiling value where health effects are caused by exposures over a short period. Unless otherwise noted, the STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday, and the ceiling limit is an exposure that should not be exceeded at any time.

In the U.S., OELs have been established by federal agencies, professional organizations, state and local governments, and other entities. Some OELs are legally enforceable limits, while others are recommendations. The U.S. Department of Labor Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs) (29 CFR [Code of

Federal Regulations] 1910 [general industry]; 29 CFR 1926 [construction industry]; and 29 CFR 1917 [maritime industry]) are legal limits enforceable in workplaces covered under the Occupational Safety and Health Act.[9] NIOSH recommended exposure limits (RELs) are recommendations based on a critical review of the scientific and technical information available on a given hazard and the adequacy of methods to identify and control the hazard. NIOSH RELs can be found in the *NIOSH Pocket Guide to Chemical Hazards*.[10] NIOSH also recommends different types of risk management practices (e.g., engineering controls, safe work practices, worker education/training, personal protective equipment, and exposure and medical monitoring) to minimize the risk of exposure and adverse health effects from these hazards. Other OELs that are commonly used and cited in the U.S. include the threshold limit values (TLVs®) recommended by American Conference of Governmental Industrial Hygienists (ACGIH®), a professional organization, and the workplace environmental exposure limits (WEELs) recommended by the American Industrial Hygiene Association, another professional organization. The TLVs and WEELs are developed by committee members of these associations from a review of the published, peer-reviewed literature. They are not consensus standards. ACGIH TLVs are considered voluntary exposure guidelines for use by industrial hygienists and others trained in this discipline "to assist in the control of health hazards."[11] WEELs have been established for some chemicals "when no other legal or authoritative limits exist."[12]

Outside the U.S., OELs have been established by various agencies and organizations and include both legal and recommended limits. Since 2006, the Berufsgenossenschaftliches Institut für Arbeitsschutz (German Institute for Occupational Safety and Health) has maintained a database of international OELs from European Union member states, Canada (Québec), Japan, Switzerland, and the U.S. [http://www.hvbg.de/e/bia/gestis/limit_values/index.html]. The database contains international limits for over 1,250 hazardous substances and is updated annually.

Employers should understand that not all hazardous chemicals have specific OSHA PELs, and for some agents the legally enforceable and recommended limits may not reflect current health-based information. However, an employer is still required by OSHA to protect its employees from hazards even in the absence of a specific OSHA PEL. OSHA requires an employer to furnish employees a place of employment free from recognized hazards that cause 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, NIOSH investigators encourage employers to make use of other OELs when making risk assessment and risk management decisions to best protect the health of their employees. NIOSH investigators also encourage the use of the traditional hierarchy of controls approach to eliminate or minimize identified workplace hazards. This includes, in order of preference, the use of: (1) substitution or elimination of the hazardous agent; (2) engineering controls (e.g., local exhaust ventilation, process enclosure, dilution ventilation); (3) administrative controls (e.g., limiting time of exposure, employee training, work practice changes, medical surveillance); and (4) personal protective equipment (e.g., respiratory protection, gloves, eye protection, hearing protection). Control banding, a qualitative risk assessment and risk management tool, is a complementary approach to protecting worker health that focuses resources on exposure controls by describing how a risk needs to be managed [http://www.cdc.gov/niosh/topics/ctrlbanding/]. This approach can be applied in situations where OELs have not been established or can be used to supplement the OELs, when available.

Chlorine Dioxide (ClO_2)

ClO_2 has been approved by the EPA as a disinfectant, sanitizer, and sterilant.[13] Gaseous ClO_2 is used as a disinfectant and sterilant in the paper, fruit, vegetable, dairy, poultry, and beef processing industries, as well as in industrial

wastewater processing.[14,15,16] Aqueous ClO_2 has been frequently used to treat drinking water and to bleach wood pulp in the paper industry. It has also been used to control mold in libraries.[17,18] Under a crisis exemption from the EPA, ClO_2 gas was used to treat *Bacillus anthracis* spores in 2001 and 2002 in contaminated buildings and the exterior of mail packages.[1,19] Additional studies have been completed on the efficiency of ClO_2 for inactivation of *Bacillus* endospores as surrogates for *B. anthracis* spores.[20,21,22,23] The generation of gaseous ClO_2 is presently being explored for the remediation of structures that have been affected by microbial growth. The advantage of using a gas is that it can penetrate into building cavities. The NIOSH REL, ACGIH TLV, and OSHA PEL for ClO_2 are the same at a level of 0.1 ppm as a TWA over the workshift.[9,10,11] The immediately dangerous to life and health level for ClO_2 is 5 ppm.[10]

Wilson and associates conducted a laboratory study investigating the effect of ClO_2 gas on the colonies of four fungal species (*Chaetomium globosum*, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, and *Stachybotrys chartarum*), ascospores (*Chaetomium globosum*), and mycotoxins produced by *S. chartarum*.[24] The investigators exposed fungal colonies grown on filter paper and purified ascospores to ClO_2 at concentrations of either 500 ppm or 1,000 ppm in a sealed chamber for 24 hours. Both concentration levels were found effective in rendering *Cladosporium cladosporioides*, *Penicillium chrysogenum*, and *Stachybotrys chartarum* colonies nonculturable after exposure to both ClO_2 concentrations. *C. globosum* colonies showed a reduction of 91% at the 500 ppm concentration and 87% at the 1,000 ppm of ClO_2.[24] The *C. globosum* ascospores were almost totally inactivated and spore count decreased, indicating that some ascospores were destroyed by the treatment. The ClO_2 did not detoxify the *S. chartarum* mycotoxins as determined by a yeast toxicity assay.

Mold

The types and severity of symptoms related to exposure to mold in the indoor environment depend in part on the extent of the mold present, the extent of the individual's exposure, and the susceptibility of the individual (for example, whether they have preexisting allergies or asthma). In general, excessive exposure to fungi may produce health problems by several primary mechanisms, including allergy or hypersensitivity, infection, and toxic effects. Additionally, molds produce a variety of VOCs, the most common of which is ethanol, that have been postulated to cause upper airway irritation. However, as discussed above, potential irritant effects of VOCs from exposure to mold in the indoor environment are not well understood. Evidence also shows that exposure to fungi may occur through fungal fragments that can contain allergens, toxins, and (1→3)-β-*D*-glucan.[25,26,27]

Allergic responses are the most common type of health problem associated with exposure to molds. These health problems may include sneezing; itching of the nose, eyes, mouth, or throat; nasal stuffiness and runny nose; and red, itchy eyes. Repeated or single exposure to mold or mold spores may cause previously nonsensitized individuals to become sensitized. Molds can trigger asthma symptoms (shortness of breath, wheezing, cough) in persons who are allergic to mold. A recent review of the scientific literature concluded that exposure to molds in the indoor environment may make preexisting asthma worse, but also concluded that there was not enough evidence to determine whether exposure to mold in the indoor environment could cause asthma.[28] In its 2004 report, "Damp Indoor Spaces and Health," the Institute of Medicine (IOM) 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.[29]

People with weakened immune systems (immune-compromised or immune-suppressed individuals) may be more vulnerable to infections by molds. For example, *Aspergillus fumigatus* is a fungal species that has been found almost everywhere on every conceivable type of substrate. It has been known to infect the lungs

of immune-compromised individuals after inhalation of the airborne spores.[30] Healthy individuals are usually not vulnerable to infections from airborne mold exposure.

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. 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 concurs with the U.S. EPA's recommendations to remedy mold contamination in indoor environments [www.epa.gov/iaq/molds/mold_remediation.html].[31,32] Additional information on health effects and mold remediation can be found in the Centers for Disease Control and Prevention (CDC) document "Mold Prevention Strategies and Possible Health Effects in the Aftermath of Hurricanes and Major Floods" [http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5508a1.htm].

Bacteria and Endotoxin

Gram-negative bacteria (GNB) and endotoxin (which are found in the cell wall of GNB) are ubiquitous in nature. Endotoxin is released when the bacterial cell is lysed (broken down) or when it is multiplying.[33,34] It is found in water, soil, and living organisms. Endotoxin has been found in various industrial settings and in nonindustrial environments associated with bacterial contamination, cooling towers, humidifiers, air conditioners, and other water-associated processes.[35,36,37,38,39]

In experimental studies, human volunteers exposed via inhalation to high levels of endotoxin experience airway and alveolar inflammation as well as chest tightness, fever, and malaise and have an acute reduction in lung function, as measured by the forced expiratory volume in one second (FEV_1).[40,41] Airborne endotoxin exposures between 45 and 400 endotoxin units per cubic meter (EU/m^3) have been associated with acute airflow obstruction, mucous membrane irritation, chest tightness, cough, shortness of breath, fever, and wheezing.[42,43,44,45] Chronic health effects that have been associated with airborne endotoxin exposures include asthma, chronic bronchitis, bronchial hyperreactivity, chronic airway obstruction, hypersensitivity pneumonitis, and organic dust toxic symndrome.[42] Some studies suggest that high environmental and occupational endotoxin exposures may be protective in terms of atopic sensitization.[46,47]

ACGIH has proposed using relative limit values (RLVs) rather than the more usual TLVs as a reference for endotoxin.[40] RLVs require collecting of samples from an area considered to represent background levels of endotoxin and analyzing at the same time as the samples of interest. The RLV is expressed in terms of a comparison between the exposed and background areas.[40] ACGIH proposes that health effects are consistent with endotoxin exposure, and if the endotoxin exposures exceed 10 times the simultaneously determined background levels, then the RLV action level has been exceeded.[40] In 1998, the Dutch Expert Committee on Occupational Standards recommended an exposure limit of 50 EU/m^3; this was later raised to 200 EU/m^3 to accommodate economic feasibility for the agricultural industry.[48]

Beta glucans

β-glucans are polysaccharides found in the cell walls of fungi as well as some bacteria and plants. (1→3)-β-D-glucans are the most abundant of the β-glucans. They have been used as a marker for fungal biomass. The results of epidemiological studies are mixed.[49] Some have shown that increased levels of (1→3)-β-D-glucans are associated with an increased number of health symptoms and other investigations have not.[49,50,51] Inhalation of (1→3)-β-D-glucans has also been associated in some studies with airway inflammation.[49,52,53,54]

RESULTS

Culturable Fungi

Table 2 shows the results for microbial sampling before and after ClO_2 treatment. Initial concentrations of culturable fungi in the house were extremely high. All plates were overgrown with a laboratory estimate of over 400 colonies per plate, which, after adjusting for multiple particle impaction, yields an estimate of over 1,000,000 colony-forming units per cubic meter (CFU/m^3). After treatment, the geometric mean for culturable fungi was 252 CFU/m^3. Paired t-tests comparing the culturable fungal concentrations before and after treatment showed a statistically significant difference ($p = 0.0001$). The average relative efficiency of the treatment against culturable fungi was about 97%. The predominant fungal types in the house before ClO_2 treatment were *Aspergillus niger*, *Aspergillus versicolor*, *Cladosporium* sp., *Mucor* sp., and *Penicillium* sp.; whereas after ClO_2 treatment, *Aspergillus versicolor*, *Penicillium* sp., and *Sporobolomyces* sp. dominated. Figures 4 and 5 show the effect of the ClO_2 treatment on the same area of visible microbial contamination before and after ClO_2 treatment. The data for the outside samples are presented in Table 3. The geometric means for the outside samples were 548 CFU/m^3 before treatment and 144 CFU/m^3 after treatment. The predominant genera/class for the outside samples prior to treatment were *Basidiomycetes*, *Cladosporium* sp., *Penicillium* sp., and *Epicoccum nigrum;* after treatment they were *Aspergillus fumigatus*, *Aureobasidium pullulans*, *Basidiomycetes*, *Cladosporium* sp., *Epicoccum nigrum, Penicillium* sp., and *Pithomyces chartarum*.

Total Spore Counts

The geometric mean for indoor total spore counts determined from Air-O-Cell samples was 73,454 spores per cubic meter (S/m^3) before the ClO_2 treatment and 1,552 S/m^3 after treatment; this difference was statistically significant ($p = 0.0052$). The average relative efficiency against total fungi was 97.6%. *Aspergillus/Penicillium*, *Stachybotrys*, *Basidiospores*, *Cladosporium*, and *Chaetomium* were the most commonly detected fungal spores before treatment in the spore trap samples. After treatment, *Ascospores*, *Aspergillus/Penicillium*, *Basidiospores*, and *Cladosporium* were found in the air samples. Outside concentrations of total fungi were 3,556 S/m^3 before treatment and 444 S/m^3 after treatment. The predominant genera/classes for the outside samples both before and after treatment were *Ascospores*, *Basidiomycetes*, *Cladosporium*, and *Aspergillus/Penicillium*. *Curvularia* and *Mxomycetes* were also detected before treatment and *Torula* after treatment.

Polymerase Chain Reaction Anaylsis for Fungal Spores

The geometric means for the PCR analysis before and after ClO_2 treatment were 5,535 and 332 spore equivalents per cubic meter (SE/m^3), respectively. These concentrations were significantly different ($p = 0.0249$), and the average relative efficiency was 90.5%. The five most commonly detected fungal species in the house using PCR analyses were *A. versicolor*, *Eurotium (Aspergillus) amstelodami*, *Cladosporium cladosporioides*, *Penicillium brevicompactum*, and *S. chartarum*. Figure 6 shows the relative efficiency of the treatment processes for these five species. *A. versicolor* and *S. chartarum* showed the highest relative efficiency (approximately 100%), followed by *E. (Aspergillus) amstelodami* (95%), *P. brevicompactum* (90%), and *C. cladosporioides* (85%). Outside fungal spore concentrations were 375 SE/m^3 (before) and 76 SE/m^3 (after).

Beta glucans

The geometric means for (1→3)-β-D-glucan samples before and after ClO_2 treatment were below the limit of detection (LOD) and 736 picograms per cubic meter (pg/m^3), respectively. Paired t-tests comparing the (1→3)-β-D-glucan concentrations before and after treatment showed no significant difference ($p = 0.06$).

Culturable Bacteria

The bacterial species detected were highly variable between the sampling locations in the house and included both Gram-negative and Gram-positive organisms. Most commonly found species both before and after treatment were *Aeromonas caviae*, *Bacillus mycoides*, *Bacillus sphaericus*, *Brevibacillus brevis*, *Brevibacterium casei*, *Brevundimonas vesicularis*, *Chryseobacterium indologenes*, *Comamonas testosteroni*, *Enterococcus durans*, *Flavimonas orzyhabicans*, *Micrococcus luteus*, *Pseudomonas fluorescens*, *Psychrobacter phenylpyruvicus*, *Rhizobium radiobacter*, *Sphingomonas paucimobilis*, *Staphyococcus xylosus*, and *Streptomyces*. The majority of these bacteria are environmental species; the others are associated with animals. The geometric means for indoor culturable bacteria samples before and after ClO_2 treatment were 1,077 and 158 CFU/m^3, respectively. These concentrations were significantly different ($p = 0.01$) resulting in an average relative efficiency of 84.9%.

Endotoxin

Endotoxin concentrations before and after ClO_2 treatment were 10.32 and 18.59 EU/m^3, respectively. Paired t-tests comparing endotoxin concentrations before and after treatment showed no significant difference ($p = 0.23$). Outside endotoxin levels were 0.74 EU/m^3 before and 21.92 EU/m^3 after the gas application.

Tape Sampling

As shown in Table 4, tape sampling results showed the presence of spores, hyphae, and conidiophores of *Aspergillus*, *Cladosporium*, *Penicillium*, *Scopulariopsis*, and *S. chartarum* on the surfaces before treatment. After treatment, it was still possible to identify spores, hyphae, and conidiophores of *Aspergillus*, *Cladosporium*, and *Penicillium* using microscopic techniques at the same levels of contamination as found before ClO_2 treatment.

Volatile Organic Compounds

The thermal desorption tube samples for VOCs were collected on the second floor, which contained the highest degree of visible microbial contamination. Four samples were collected before and after ClO_2 treatment. Major compounds detected on all samples included toluene, hexane, heptane, xylene, and numerous other C_9-C_{12} aliphatic hydrocarbons, plus C_9-C_{10} alkyl benzenes. Three of the four samples collected after the ClO_2 treatment also contained traces of nitrogen compounds and chlorinated compounds such as chloropicrin (trichloronitromethane), methyl dichloroacetate, and dichlorobromo-methane. A possible source of the nitrogen compounds was an old safe used by a cat as a litter box.

Chloride, Chlorate, and Chlorite Salt Ions

The results for the chloride, chlorate, and chlorite salt ions are presented in Table 5. There was a significant increase in these ions using paired t-tests ($p = 0.032$; $p = 0.016$; $p = 0.018$, respectively) after the ClO_2 treatment. This trend was expected because these ions are the end products of the gaseous ClO_2 reaction.

DISCUSSION

In this evaluation, the relative efficiencies obtained using ClO_2 for culturable fungi and bacteria, total fungal spore counts from spore traps, and total fungal spore counts from PCR analyses ranged from 84.9% to 97.6%. These results indicate that the ClO_2 treatment decreased both the culturable and total counts of airborne microorganisms. However, there was no measured effect on the concentrations of their components, i.e., (1→3)-β-*D*-glucan and endotoxin.

The outside fungal concentrations after the treatment process may have been lower than anticipated due to snowfall prior to and during the sampling period. However, the initial

culturable fungal concentrations indoors were much higher than outside concentrations, and the species profile in the indoor air differed from that in outdoor air. Therefore, the decrease in the indoor fungal air concentrations cannot be explained solely by the decrease in the outdoor fungal air concentrations due to outside environmental factors.

Before the treatment, the levels of total fungi, culturable fungi, and endotoxin found in the house were similar or lower when compared to those found in two recent studies performed in flooded homes in New Orleans. Rao and associates reported a geometric mean of 2.8×10^5 spores/m^3 for total fungi, 0.7×10^5 CFU/m^3 for culturable fungi, and 22.3 EU/m^3 for endotoxin in moderately (n = 5) to heavily flooded homes (n = 15) in New Orleans.[55] Chew et al. found the following ranges in three homes before renovation: total fungi, $0.8–6.3 \times 10^5$ spores/m^3; culturable fungi, $0.22–5.2 \times 10^5$ CFU/m^3; and endotoxin, 17–139 EU/m^3.[56]

In this study, the respective concentrations were about as follows: total fungi, 0.7×10^5 spores/m^3; culturable fungi, 10×10^5 CFU/m^3; and endotoxin, 18.6 EU/m^3. Park and fellow researchers reported lower endotoxin levels, geometric mean 0.64 EU/m^3 (range, 0.02–19.8 EU/m^3) from bedrooms of 15 homes located in the greater Boston, Massachusetts, area.[57] Outdoor levels of endotoxin before treatment were comparable to the ones in California outdoor air (0.44 EU/m^3) and Denmark (0.33 EU/m^3).[58,59]

The (1→3)-β-*D*-glucan concentrations in this evaluation were similar to those reported in other studies that used LAL for (1→3)-β-*D*-glucan analysis.[49] The reported concentrations ranged up to 19 nanograms per cubic meter (ng/m^3) in indoor environments. In this study, the mean (1→3)-β-*D*-glucan concentration was 0.736 ng/m^3 after treatment. Bacteria concentrations in the house were much higher (1,077 CFU/m^3) than those found in nonproblem office buildings in the United States (average 102 CFU/m^3).[60] Levels of bacteria in microbially contaminated homes are not readily available for the United States.

Traditionally, monitoring for bioaerosols has consisted of culturing and microscopic counting of fungi and bacteria using short-term samples.[61] Newer PCR technologies have many advantages for indoor environments including quick turnaround of sample results, accurate identification and reproducibility, and the ability to detect nonviable fungi and fungal spores. The technology also allows for a longer sampling time to get a better understanding of environmental exposures.[62] The PCR method was used in this evaluation along with traditional culture and microscopic counting techniques to assess the efficiency of ClO_2 treatment against fungi. The predominant fungal species in the house were similar using all three methods. *Aspergillus, Penicillium*, and *Cladosporium* species were among the five most common detected by the three methods before treatment. *Stachybotrys* was detected both by microscopic counting and PCR, but not with cultivation. The agar media used for the culturable fungi sampling was not specific for *Stachybotrys,* and it is possible that the spores did not grow or were overgrown by other fungi. For the relative efficiency of ClO_2 treatment, these methods showed similar trends, but the highest efficiency was found with the culture-based technique. Both the total microscopic count and the PCR count obtained for air samples decreased significantly after ClO_2 treatment. This could be a result of the direct reduction of spores in the air or reduction of spores on surfaces that would serve as the source for the airborne spores. However, the sticky tape samples collected from surfaces did not show a reduction in the number of spores and hyphae after ClO_2 treatment. The discrepancy between the total microscopic count and PCR count could be caused by deactivation of DNA or inhibition of the PCR assay by the ClO_2 gas. Previous studies have shown that environmental contaminants in the indoor environment can inhibit PCR analyses, which may also give a false negative result.[63,64,65] Buttner and fellow investigators also identified the issue of inhibition of PCR for environmental samples in their surface disinfection study using

gaseous ClO_2 and foam decontaminant.[20] They also found that DNA and other compounds capable of producing immune responses were still present after treatment.

CONCLUSIONS

This evaluation showed that gaseous ClO_2 treatment can be used to kill fungi and bacteria in a field setting after the source of moisture incursion has been addressed. The treatment also reduced the total fungi in the air of the treated house. The fungal spores were still visible on sticky tape samples after ClO_2 treatment using microscopic techniques. The treatment process appeared to have no significant effect on the airborne concentrations of endotoxin and (1→3)-β-D-glucan. Additional investigation is needed to determine whether exposures to the fungal spores and bioaerosols remaining after the ClO_2 treatment are capable of producing adverse health effects.

RECOMMENDATIONS

Based on the information collected during this evaluation, the following recommendations are provided should this remediation approach be used in the future:

1. Because of the potential for health effects from residuals present after ClO_2 treatment, additional clean-up techniques, such as using air cleaners and cleaning surfaces using high efficiency particulate air (HEPA) filter vacuums to reduce concentrations of spores and microbial components, is recommended.

2. To document the effectiveness of the ClO_2 treatment of microbially contaminated houses, environmental sampling techniques, including the collection of samples for culturable microorganisms as well as endotoxin and (1→3)-β-D-glucan before and after the treatment process, are recommended.

3. The potential for by-products from the treatment process to interfere with the PCR analysis needs further research.

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Table 1. Sampling and Analysis Methods

HETA 2004-0387 Gro-West Utica, NY

Analyte	Sampler	Media	Analysis Method	Average Sampling Time (minutes)	Flow Rate (Lpm)*
Air Samples					
Culturable fungi	Andersen N-6	MEA	Cultivation (ID based on colony morphology)	3	28.3
Total fungi	Air-O-Cell	Slide	Microscopic Counting (ID based on spore morphology)	3	15
PCR fungi	3 piece Cassette	Filter (0.3 µm PTFE§)	Real-time PCR† (DNA)	320	2
(1→3)-β-D-glucan	3 piece Cassette	Filter (0.3 µm PTFE)	LAL‡	320	2
Culturable bacteria	Andersen N-6	TSA	Culturability (ID by MIDI- Gas Chromatography)	3	28.3
Endotoxin	Cassette	Filter (5 µm PVC)¶	LAL	320	2
Surface samples					
Total Fungi	Sticky Tape	Slide	Microscopic Counting	N/A**	N/A
Other Agents					
Salts (Chlorite, Chloride, Chlorate Ions)	Gauze Wipe		Ion Chromatography	N/A	N/A
Volatile Organic Compounds	Thermal Desorption Tube	Charcoal	Chromatography	15	0.1

*Lpm – liters per minute
§PTFE – polytetrafluoroethylene
†PCR – polymerase chain reaction
‡LAL – *Limulus* amebocyte lysate
¶PVC – polyvinyl chloride
**N/A – not applicable

Table 2. Geometric Mean and Range for Indoor Bioaerosol Concentrations Before and After Chlorine Dioxide (ClO_2) Treatment
HETA 2004-0387 Gro-West Utica, NY

Analysis	Number of Samples Before and After Treatment	Geometric Mean (Range)		Relative Efficiency (%) ± Standard Deviation
		Before ClO_2 Treatment	After ClO_2 Treatment	
Culturable fungi (CFU/m^3*)	24 + 24	> 1,000,000	252 (129–435)	97.40 ± 0.45
Total fungi (S/m^3†)	8 + 8	73,454 (16,311–195,289)	1,552 (978–2,267)	97.55 ± 2.45
PCR fungi (SE/m^3‡)	8 + 8	5,535 (943–23,598)	332 (118–706)	90.45 ± 11.2
(1→3)-β-D-glucan (pg/m^3§)	3 + 3	< 125 (Limit of Detection)	736 (580–1,100)	
Culturable bacteria (CFU/m^3)	6 + 6	1,077 (718–1,319)	158 (82–353)	84.93 ± 7.74
Endotoxin (EU/m^3)¶	8 + 8	10.32 (1.31–34.19)	18.59 (4.45–41.4)	

*CFU/m^3 – Colony forming units per cubic meter
†S/m^3 – Spores per cubic meter
‡SE/m^3 – Spore equivalents per cubic meter
§pg/m^3 – Picograms per cubic meter
¶EU/m^3 – Endotoxin units per cubic meter

Table 3. Geometric Mean and Range for Outdoor Bioaerosol Concentrations Before and After Chlorine Dioxide (ClO2) Treatment
HETA 2004-0387 Gro-West Utica, NY

Analysis	Number of Samples Before and After Treatment	Geometric Mean (Range)	
		Before ClO_2 Treatment	After ClO_2 Treatment
Culturable fungi (CFU/m^3)*	3 + 3	548 (380–710)	144 (94–188)
Total fungi (S/m^3)†	1 + 1	3,556	444
PCR fungi (SE/m^3)‡	1 + 1	375	76
Culturable bacteria (CFU/m^3)	3 + 1	1,197 (1,119–1,319)	67 (35–94)
Endotoxin§ (EU/m^3)	1 + 1	0.74	21.92

*CFU/m^3 – Colony forming units per cubic meter
†S/m^3 – Spores per cubic meter
‡SE/m^3 – Spore equivalents per cubic meter
§EU/m^3 – Endotoxin units per cubic meter

Table 4. Fungal Identification from Tape Samples using Optical Microscopy

HETA 2004-0387 Gro-West Utica, NY

Sample Location	Sample Site	Fungal ID	Category*
Before ClO_2† Treatment			
Basement	Galvanized Duct	*Penicillium*	A few
Basement	Wood Board over Utility Sink	*Cladosporium*	A few
First Floor	Door Frame between Dining Room and Porch	*Penicillium*	A few
First Floor	Painted Fireplace Mantle	*Aspergillus* *Cladosporium* *Penicillium*	A few A few Many
First Floor	Wallpaper Between Two Front Windows	None	None
Second Floor	Shelving between Windows in Pink Room	*Aspergillus* *Stachybotrys chartarum*	A few A few
Second Floor	Plaster Wall under Wallpaper Near Front Window	*Aspergillus*	A few
Second Floor	Yellow Room Wall by Safe	*Aspergillus* *Cladosporium* *Penicillium* *Scopulariopsis*	Many Many Massive Massive
Third Floor	Sink Countertop in Back Room	*Aspergillus* *Chaetomium* *Cladosporium* *Penicillium*	Many A trace A few A few
After ClO_2 Treatment			
Basement	Galvanized Duct–Basement	*Aspergillus* *Zygomycetes*	Many A few
Basement	Wood Board–Basement over Utility Sink	*Aspergillus* *Cladosporium*	Many Many
First Floor	Painted Fireplace Mantle	*Aspergillus* *Penicillium*	A few Numerous
First Floor	Door Frame between Dining Room and Porch	*Penicillium*	Many
First Floor	Wallpaper Between Two Front Windows	None	None
Second Floor	Pink Room on Mold Growth	*Gliocladium*-like	Massive
Second Floor	Plaster Wall under Wallpaper Near Front Window	None	None
Second Floor	Plaster Wall Near Front Window	None	None
Third Floor	Sink Countertop in Back Room	*Aspergillus*	Many

* massive>numerous>many>a few>a trace

† ClO_2 – chlorine dioxide

Table 5. Chlorate, Chloride and Chlorite Salt Wipe Concentrations Before and After Chlorine Dioxide (ClO_2) Treatment
HETA 2004-0387 Gro-West Utica, NY

Sample Location	Sample Site	Chlorate (µg/sample)*	Chloride (µg/sample)	Chlorite (µg/sample)
Before ClO_2† Treatment				
Basement	Particle Board Wall	Trace	17	ND
Basement	Metal Side of Furnace	ND	29	ND
1st Floor	Wallpaper under Stained Glass window	Trace	14	ND
1st Floor	Wallpaper near Fireplace	ND	ND	ND
2nd Floor	Marble fireplace Mantle	ND	33	34
2nd Floor	Wood door into Pink Room	ND	11	ND
3rd Floor	Landing Wall	ND	3.6	ND
3rd Floor	Far Wall in Right Room	ND	11	ND
After ClO_2 Treatment				
Basement	Particle Board Wall	96	910	10
Basement	Metal Side of Furnace	6.6	170	ND
1st Floor	Wallpaper under Stained Glass Window	260	2,400	4.9
1st Floor	Wallpaper near Fireplace	16	120	Trace
2nd Floor	Marble Fireplace Mantle	230	4,000	120
2nd Floor	Wood Door into Pink Room	94	920	6.1
3rd Floor	Landing Wall	17	620	ND
3rd Floor	Far Wall in Right Room	170	5,200	Trace
LOD‡		0.3	0.5	0.1
LOQ§		1	2	0.4

*µg/sample – microgram per sample
†ClO_2 – chlorine dioxide
‡LOD – limit of detection
§LOQ – limit of quantitation

Figure 1. Victorian House

Figure 2. Tented House

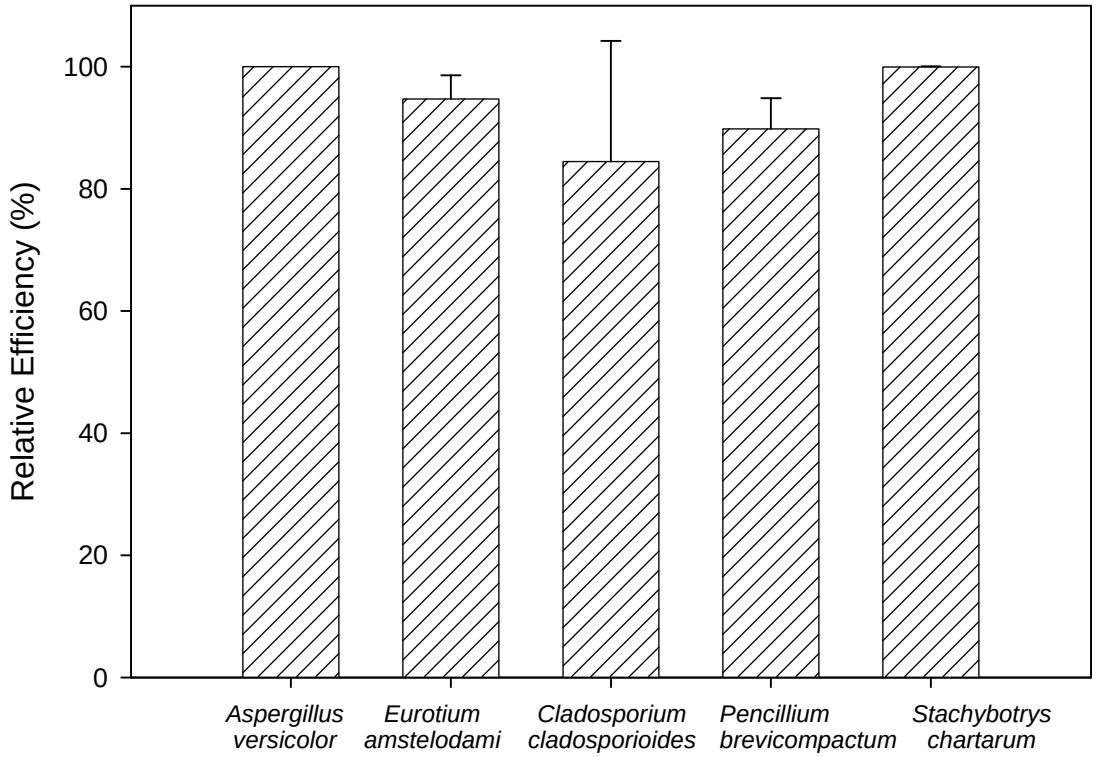
Figure 3. Sampling Station on 1st Floor

Figure 4. Pink Room before Treatment

Figure 5. Pink Room after Treatment

Figure 6. Relative Efficiency of Treatment (Average and Standard Deviation) for Five Most Common Fungal Species Detected in the PCR Analyses

HETA 2004-0387 Gro-West Utica, NY



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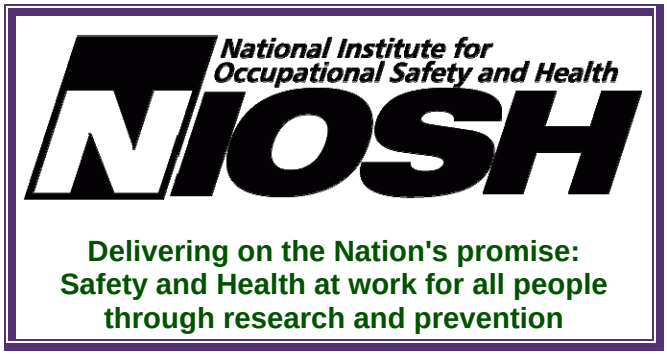

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