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Research and Development of Risk Management Alternatives for Controlling Mold

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by

Marc Y. Menetrez, Timothy R. Dean, and Doris A. Betancourt
Office of Research and Development
National Risk Management Research Laboratory
Air Pollution Prevention and Control Division
Research Triangle Park, NC 27711

EPA Project Officer: Marc Y. Menetrez

U. S. Environmental Protection Agency
Office of Research and Development
Washington, DC 20460

Abstract

The U.S. Environmental Protection Agency, Air Pollution Prevention and Control Division (APPCD), Indoor Environment Management Branch (IEMB) has, since 1995, conducted research into controlling biological contamination in the indoor environment. Six areas of research have been addressed: (1) research and development studies to quantify the effects of moisture, relative humidity (RH), and dust and develop risk management alternatives for prevention and control of mold growth; (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials: (3) evaluation of antimicrobial treatments as control technologies; (4) field testing of sealants and encapsulents used in air duct systems; (5) characterization of emission rates and modeling of exposure through heating, ventilating, and air conditioning operation; and (6) improved methods of sampling and analysis of mold. The conclusions resulting from this body of research are listed to summarize the accomplishments and put into perspective the interrelationships of these areas of investigation in reducing human exposure to biological contamination in the indoor environment. Through a cooperative research agreement, the Research Triangle Institute (RTI) has played a major role in the development of the program described in this report. The RTI Research Triangle Park facility houses the Microbiological Laboratory (ML) in which the static and dynamic microbial test chamber are kept and staffed. As part of the opening of the new EPA Environmental Resource Center Building in 2002, APPCD/IEMB has established the Biocontaminant Laboratory (BL) for conducting applied risk management research. The BL is a multi-functional state-of-the-art biological/molecular research laboratory engaged in intra/inter laboratory cooperative research. A description of the RTI ML and APPCD/IEMB BL facility is included in this report.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

Sally Gutierrez, Director
National Risk Management Research Laboratory

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Introduction

The indoor environment has become an important area of research in recent years. The past twenty years have brought the recognition that an important factor in the health of people in the indoor environment is the dampness of the buildings in which they live and work [1–3], and the potential for mold colonization as depicted in Figures 1 and 2. Furthermore, it is now recognized that the principal biological organisms responsible for the health problems in these environments are the fungi rather than bacteria and viruses [4]. It has been estimated that upwards of 40% of all homes in North America contain fungal growth, while numbers in Northern Europe are in the range of 20–40% fungal contamination [5–6]. Although traditionally, fungi in this context have been viewed as a source of allergens (and in unusual circumstances, pathogens), data have accumulated to show that the adverse health effects resulting from inhalation of fungal spores is due to a variety of factors [7]. One characteristic associated with certain fungi, is the low molecular weight toxins (mycotoxins) they produce. Mycotoxins are important in human and animal health because of their production by toxigenic fungi associated with food and animal feed. In the indoor environment, mycotoxins tend to concentrate in fungal spores and thus present a potential hazard to those exposed who inhale them.

Figure 1. *Cladosporium,* and *Aspergillus*.

Figure 2. *Stachybotrys* on drywall.

Although only a small percentage of fungal species has been associated with adverse health effects, increased awareness and continuing research will likely result in the identification of many more pathogenic and toxigenic fungi. Organisms such as *Stachybotrys chartarum*, *Penicillium purpurogenum*, *Aspergillus versicolor*, and *Cladosporium* spp. are organisms that are frequently found in buildings that are heavily contaminated with mold and are potentially associated with adverse health effects in humans [8–9]. These health effects may include itchy eyes; stuffy nose; headache; fatigue; and, in severe cases, idiopathic pulmonary hemosiderosis (IHP) in infants [10–14]. In numerous cases the mold *S. chartarum* has been found to be associated with idiopathic pulmonary hemorrhage in infants [1–2]. It is also studied for toxin production and its occurrence in water damaged buildings. Growth of *S. chartarum* on building materials such as

drywall has been frequently documented. Indoor exposure to mold has also been linked to pulmonary disease, including allergies and asthma. Given this significant risk of exposure and frequency of occurrence, environmental factors regarding the growth of mold have been studied.

The U.S. EPA, Air Pollution Prevention and Control Division (APPCD), Indoor Environment Management Branch (IEMB) has conducted research since 1995, into controlling biological contamination in the indoor environment. The IEMB Biocontaminant Laboratory (BL) conducts research into biological contamination in the indoor environment. The goal of this research is the development of engineering guidelines for the prevention, mitigation, and control of biocontaminants which can be problematic in the indoor environment as shown in Figure 3. Mold contamination can also cause significant damage to buildings. The last ten years of research findings have contributed greatly to understanding the threat of mold and the development of effective solutions.

Biological contamination in the indoor environment is recognized as a major health concern [15]. Exposure to airborne biocontaminants or their metabolites can induce irritational, allergic, and infectious responses, including acute reactions such as vomiting; diarrhea; hemorrhage; convulsions; and, in some cases, death [1, 3, 15–17]. Reducing occupant exposure to indoor air pollutants is the primary goal of the majority of indoor air quality (IAQ) research. For many indoor biocontaminants (e.g., microorganisms), the main growth locations are the structural and finishing materials and furnishings of the building [18, 19]. The application of effective engineering controls within the building is essential to prevent biological pollution in the indoor environment.

It is well recognized that fungi can colonize and amplify on a variety of building materials if sufficient nutrients and moisture are present. Mold contamination has been associated with a variety of building and furnishing materials including carpet, ceiling tile, gypsum wallboard, flooring, insulation, and heating and air-conditioning components [18, 19].

The goal of this research publication was to provide industry, academia, and regulators with an organized presentation of the many developments accomplished in risk management research in the last decade within US EPA/APPCD/IEMB. It can ultimately provide engineering guidelines for the prevention, mitigation, and control of biological contaminants in the indoor environment.

The objectives were to: (1) provide a scientific basis for studying building material colonization by microorganisms, (2) conduct research on source management and climate control, (3) evaluate engineering solutions and

Figure 3. Mixture of *Cladosporium, Aspergillus,* and *Penicillium* on painted drywall.

control techniques, and (4) develop molecular characterization techniques for identifying biological contaminants.

Microbiology Research Facilities

In response to increasing examples of mold contamination and the potential threat to human health, the U.S. EPA, APPCD/IEMB has conducted research into controlling biological contamination in the indoor environment. This capstone report of "Research and Development of Risk Management Alternatives for Controlling Mold" covers the "where, why, and how" this research has been performed; the findings and conclusions that have been accomplished; as well as a preview of upcoming research.

Research Triangle Institute's Microbiology Laboratory

The Research Triangle Institute (RTI) microbiology laboratory (ML) (as shown in Figure 4) designs and conducts applied and basic research in environmental microbiology and aerobiology. Specializing in biological aerosols, they research biological contaminants isolated from the environment to identify environmental causes of illness and to recommend methods for preventing such biological contamination and its associated adverse health effects. RTI's microbiology research program is fully equipped to collect, characterize, study, inactivate, and control microbial populations. Particular emphasis is placed on cooperative research among microbiologists, aerosol scientists, engineers, and chemists.

Figure 4. Research Triangle Institute Microbiology Laboratory.

Laboratory and field research includes environmental microbiological assessment, environmental biological pollution studies, and antimicrobial/biocide efficacy evaluations. Projects include Homeland Security defense projects, evaluating equipment for detecting bioterrorism agents, and decontamination protocols.

RTI's microbiologists work in a uniquely designed microbiology laboratory applying expertise in: (1) methods development, (2) system or process evaluation, (3) environmental monitoring and exposure assessment, (4) technology transfer assistance, (5) bioaerosol penetration and containment, (6) aerosol sampler evaluations, (7) product evaluations and assessments, (8) materials biodeterioration, (9) bioburden assessments, (10) static and dynamic chamber studies, (11) antimicrobials/biocides assessments (gas-phase, aqueous, bound, and UVC irradiation), (12) antimicrobial "As-Used" evaluations, (13) cleanroom fabric microbial penetration evaluations, (14) biofiltration assessments, and (15) Cleaning effectiveness.

RTI's microbiology research program is home to a unique microbiology research laboratory. Functioning as a Class 10,000 or better cleanroom, the 1,500 ft^2 biological safety level 2 (BSL2) laboratory contains standard microbiological equipment such as steam autoclave; incubators; refrigerators; centrifuges; light, fluorescent, and phase contrast microscopes; colony counters; spectrophotometers; fluorometers; analytical balances; and biological safety cabinets. A low-temperature freezer houses an extensive collection of fungi and bacteria.

In addition, RTI has a full range of bioaerosol samplers including 1-, 2-, and 6- stage Andersen impactors, Mattson-Garvin samplers, glass impingers, and a high volume surface sampler (HVS3). They have the required facilities and expertise for the collection of membrane filter sam-ples. Located within the laboratory is a large

microbio-logical aerosol test facility, which contains a microbial dynamic test chamber with controlled air flow, temper-ature, and relative humidity conditions. Additional chambers include small, bioaerosol test chambers that are used to evaluate bioaerosol sampler collection efficiencies for recovery of airborne viruses. A walk-in environmentally controlled room houses 21 static chambers that are used primarily for ASTM 6329 assessments.

The RTI Microbiology Laboratory, including the static and dynamic microbial growth chambers, were developed through a cooperative agreement with EPA/APPCD/IEMB.

APPCD/IEMB Biocontaminant Laboratory

In 2002, the APPCD/IEMB Biocontaminant Research and Development Program created an in-house biocontaminant laboratory utilizing state-of-the-art analytical evaluation of biological contaminants by means of both cultural and molecular biology techniques. The analytical capabilities encompass both viable and non-viable mold and bacteria, fragments of organisms or spores, and biological particulate matter in indoor and ambient air. Samples of indoor and outdoor air can be analyzed quantitatively for species specific identification of mold, [and mycotoxins, and β-(1,3) glucan assays], bacteria, (and endotoxins), allergens, particulate matter (PM), and microbial volatile organic compounds (MVOC). Characterization of microbial populations can be processed from soils, waters, air, dusts, and materials. Microscopy, electron microscopy and photomicrography are performed. Allergen identification by antigen assay or other enzymelinked immunosorbent assays (ELISAs) are also performed.

Laboratory capabilities include two standard BSL 2 hoods and two custom BSL 2 hoods for inoculation, deposition, and exposure studies; gas chromatography/mass spectrometry (GC/MS) (Agilent 6890 Gas Chromatograph, equipped with a 5973N Mass Selective Detector and 7683 Autosampler, DB-WAX polyethelene glycol bonded phase column) for performing MVOC studies; biological contaminant analysis performed by polymerase chain reaction (PCR) (Bio-Rad and ABI Laboratories, Thermal Cycler, and PCR detection system); and genetic sequencing analysis by ABI PRISM 3100 Genetic Analyzer, multi-color florescence based DNA capillary electrophoresis analysis system. Laboratory analysis includes vegetative and spore forms of bacteria and mold; allergen identification including dust mites, cockroach, dog and cat dander, and ragweed and tree pollen; human, animal, and plant virus and prion identification. Sample collection of viable and non-viable bioaerosols by air-filtration and Andersen viable and non-viable impactors, and filtration. BL personnel developed a method for measuring biological particulate material (BioPM), has studied bioaerosol penetration and containment, and conducted evaluations of wall simulation and distribution of contaminants. The laboratory is capable of analyzing biological samples for unknown pathogenic, toxic, immunosuppressant and carcinogenic contaminants.

With the crucial human health and safety issues of microbial contamination that affect the public, it is critical to understand our surroundings and how we interact. Biological contaminants—such as bacteria, fungi, viruses, protozoa, and algae as well as spores or other components of such organisms and products of organism growth such as mycotoxins—have been linked to a variety of adverse health effects. These effects include asthma and allergic reactions, infectious diseases, and a range of symptoms from sneezing to dizziness and digestive problems.

The research involving the collaborative work in both the RTI ML and the IEMB BL has resulted in a body of research publications which are summarized. The specific research accomplishments have also been grouped into areas of investigation. A discussion of the interrelationships of these areas of investigation is included. Reducing human exposure to biological contamination has been the overall objective through a comprehensive programmatic approach.

Research Program

Six areas of research were identified by EPA/APPCD/ IEMB for allocation of program resources: (1) research and development of risk management alternatives to prevent and control the growth of mold by studies to quantify the effects of moisture, relative humidity (RH), and dust, (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials, (3) evaluation of antimicrobial treatments as control tech-nologies, (4) field testing of sealants and encapsulents used in air duct systems, (5) characterization of emission rates and modeling of exposure through heating, ventilating, and air conditioning (HVAC) operation, and (6) improved methods of sampling and analysis of mold. Each of the six research areas is described below. How-ever, prior to the discussion of these research areas, the development of the static and dynamic microbial growth chambers are described. The development of these chambers has impacted all six research areas by providing unique research tools which facilitated many of these biological investigations.

Static Microbial Test Chamber

The static microbial test chamber (SMTC), depicted in Figure 5, is constructed from acrylic sheeting (measures 32 × 39 × 51 cm) with shelves for samples and a saturated salt solution on the chamber bottom to control the equilibrium RH (ERH) [20]. The SMTC was tested using ASTM 6329-98 [21]. This method was developed as part of on-going indoor air biocontaminant research. Multiple SMTCs were used to evaluate fungal growth on 2.5 to 3.8 cm square sections of fiberglass duct liner (FGDL) at various environmental conditions. The SMTC was de-veloped to assess potential microbial growth on a variety of common building materials. Temperature and RH are controlled to simulate the desired environmental con-ditions. Prior to chamber testing, materials can be treated by soaking to simulate a wetting event or treated with an antimicrobial to simulate mitigation practices [20 – 28].

Figure 5. Static Microbial Test Chamber.

Dynamic Microbial Test Chamber

To allow for experiments which involve air movement over contaminated surfaces and the release of biocontaminant particles into the air, research was conducted in the dynamic microbial test chamber (DMTC) depicted in Figure 6. The stainless steel and glass chamber is a room-sized 2.44 m^3 cube, designed and constructed under a cooperative agreement between EPA and RTI. Chamber air is conditioned by an air-handler unit (AHU) which keeps the chamber at a temperature of 18–32 °C, a controlled RH ranging from 55% to 95%, and an air circulation rate of 1.4 to 4.8 m^3/min. Air temperature and RH can be either raised or lowered depending on the requirement of the experiment.

The DMTC has been constructed to study the growth, emissions, and transport of biological contaminants. The DMTC allows for a variety of microbiological research to be performed involving biological growth on building materials, evaluation of emission and deposition of bio-aerosols, the impact of HVAC mechanical system com-ponents on biological contaminants, and in-duct tests of air cleaners. The chamber permits a contained and highly

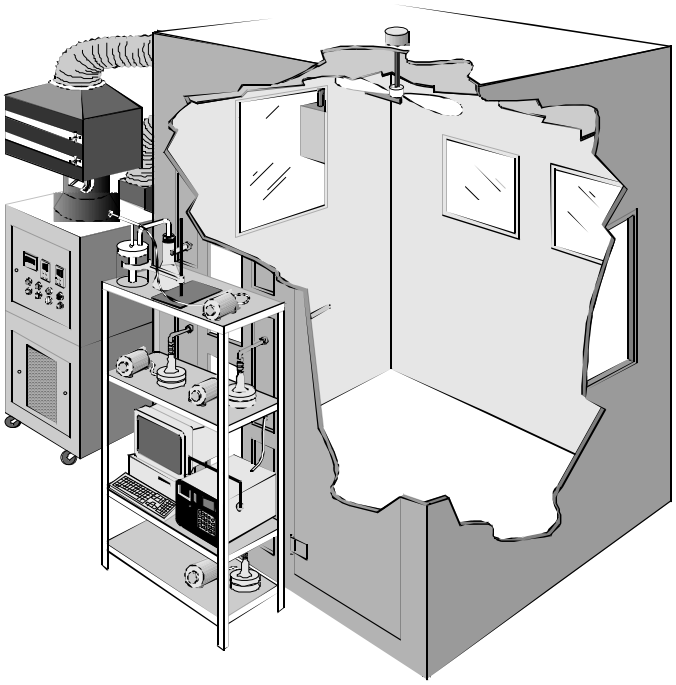

Figure 6. Cutaway Drawing of the Dynamic Microbial Test Chamber.

controlled approach to the study of bioaerosol characterization [29].

Research and Development of Risk Management Alternatives to Prevent and Control the Growth of Mold by Studies to Quantify the Effects of Moisture, Relative Humidity, and Dust

This research used a static chamber test method (SCTM) with the SMTC (see Figures 5 and 7) described above, and laboratory equipment, materials, and reagents to provide controlled environments, which allow scientific investigations of physical conditions and environmental factors favorable to the growth of biological contamination in indoor spaces [20].

With the use of multiple SMTCs and the development of the SCTM, three varieties of FGDL and ceiling tile materials were evaluated for their ability to support the growth of the fungus *Penicillium chrysogenum*.[22]. Fungal growth was evaluated on 2.5 to 3.8 cm square sections of FGDL. Wetting clean samples of FGDL materials was found to not increase amplification of the *P. chrysogenum* over levels seen without wetting [23]. Soiling FGDL samples with dust accumulated and previously harvested from HVAC systems exhibited a significant association with the growth of *P. chrysogenum* [23]. At moderate soiling levels (0.4–0.7 mg/cm^2), growth occurred on FGDL ductboard and flexible ductboard but not galvanized steel [24]. At heavy soiling levels (9–18 mg/cm^2), growth was seen on all three types of duct liner [24]. This suggests that dust accumulation should be properly controlled in any HVAC duct to prevent the growth of *P. chrysogenum* [22, 24].

The same SMTC environmental chambers (Figures 5 and 7) were used to study the impact of different levels of moisture and RH on the ability of ceiling tiles to support the growth of *Penicillium glabrum*. Amplification occurred at RH levels above 85%. Lower RH was demonstrated as effective in controlling fungal contamination on ceiling tiles [25].

Most of the existing standard test protocols for evaluating antimicrobial efficacy focus on applying the active chemical compound (antimicrobial or biocide) to the surface of a building material [26, 27]. VanOsdell, et al. [29], provides a practical hands-on evaluation protocol that is important to SCTM testing of materials under realistic environmental conditions (i.e., temperature and humidity) in which soiling with sterilized dust is a factor. The dust was obtained from the National Air Duct Cleaners Association (NADCA) and was gathered from actual HVAC systems which were cleaned by member companies. The use of this method enables the generation

Figure 7. Two Static Microbial Test Chambers.

of a quantitative endpoint for growth in a well-controlled environment with improved repeatability and comparability between tests and materials. This method was developed for evaluating fungal growth (as measured by sporulation) on indoor materials and has been used successfully to evaluate the ability of different types of materials to sustain the growth of *Penicillium glabrum*, *Aspergillus niger*, *A. versicolor*, and *P. chrysogenum* [20]. Resistance to fungal growth was demonstrated to vary for three types of newly purchased FGDL (FGDL, FGDL with biocide, and FDGL ductboard) inoculated with *Penicillium chrysogenum.* Of these types of FGDL tested, the FGDL ductboard demonstrated growth after inoculation and 6 weeks of static chamber isolation at 97% RH; in analogous testing, wetting FGDL produced growth on FDGL ductboard and FGDL, and soiling FGDL with dust collected from residential heating and air-conditioning systems caused growth on all three types of FGDL, including one of which contained a manufacturers applied fungal biocide [26, 27]. When considering these findings and the expected soiling which is produced by normal HVAC operation, the importance of maintaining low indoor RH is demonstrated to be imperative.

In another project utilizing the DMTC, the impact of RH, air velocity, and surface growth on the emission rates of fungal spores were measured. The DMTC (see Figures 6 and 8) was operated at 23.5 °C and 95% RH and utilized a separate AHU that forced conditioned air through a high efficiency particulate air (HEPA) filter and eight miniducts to simulate HVAC duct use with single pass air velocity rates of 250 cm/s. Each 28.0 × 84.0 cm miniduct contained a single sheet of FGDL material to be tested. Eight miniducts were used to simultaneously test three encapsulant coatings applied to: one set of three FGDL samples soiled with duct dust obtained from NADCA, three unsoiled FGDL samples, and two control samples, for a total of eight miniduct samples. HEPA filtered conditioned air passed over the surface of the FGDL samples, traveling horizontally within the miniduct apparatus, and exited the duct and returned to the external air-handler for filtration and conditioning. The research indicates that emission rates are inversely proportional to RH but directly related to air flow and surface loading [28].

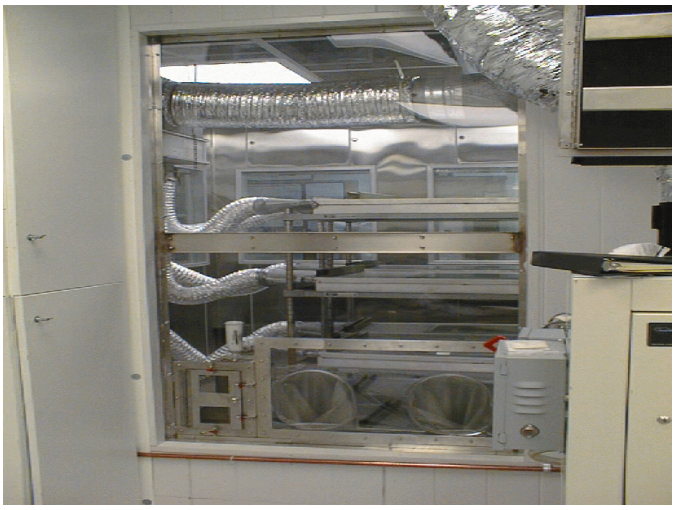

Figure 8. Dynamic Microbial Test Chamber.

In another set of experiments, SMTC and DMTC test results were generated under conditions of constant temperature, varying degrees of RH and conditions of wetting. Microorganisms (*Penicillium glabrum*, *Aspergillus niger*, *A. versicolor*, and *P. chrysogenum*) were used to evaluate the extent of biological growth upon building materials of differing moisture content. Dry and wet, used and new FGDL and ceiling tile materials were evaluated. Emphasis was on correlating the moisture content of building materials with microbial growth. Growth was determined to be a function of organism, RH, and the degree of soiling. The extent of soiling or dust deposited on FGDL and ceiling tile materials was also shown to be a significant determinant of growth [22–29]. The research showed that emission rates for these materials for *A. versicolor* and *P. chrysogenum* are inversely proportional to RH but directly related to air flow rate and surface loading [24–28, 30–32].

In another experiment, the environmental factors leading to the growth of *Stachybotrys chartarum* on building materials was investigated given the significant risk of exposure and frequency of reported occurrence. Commonly used building materials were sterilized, inoculated with *S. chartarum*, and exposed to controlled levels of relative humidity and wetting. A quantitative analysis of viable *S. chartarum* was performed on the building materials during a seven month period. The research indicates that, for environments with a relative humidity below total saturation, wetting was necessary for

visible growth to occur. Conversely, high levels of relative humidity without wetting did not initiate growth. Porous materials after becoming sufficiently wet and measuring saturation on a moisture meter, exhibited mold growth in every experiment conducted [33].

Duct Cleaning Effectiveness for Prevention and Control of Microbial Growth on Duct Materials

HVAC systems have been shown to act as a collection source for dust, and the accumulated dust can consist of such contaminants as mold, fungi, and bacteria. The potential health risks associated with exposure to these contaminants make removal of the dust a consideration, especially if improving indoor air quality is required. Because of their potential to rapidly spread contamination throughout a building, ventilation systems materials are of particular significance as potential microbial contamination sources. Portions of ventilation systems near cooling coils and drain pans are known to be exposed to high moisture levels for extended periods, and fibrous duct insulation materials are known to have become sources of microbial contamination in some buildings. The evaluation of duct cleaning as a means of control or prevention of microbial growth on insulated and galvanized duct surfaces has been conducted. Although duct cleaning is effective in removing accumulated dust and contaminants from the inner duct surface, the effects on air quality have not been substantiated. In addition, the abrasive action of rotary brushing used in duct cleaning could weaken the integrity of interior duct insulation. Possible erosion of duct insulation can however be avoided if a surface encapsulant is applied. Field evaluation of duct cleaning was performed as part of a larger project which focused on the use of antimicrobial encapsulants [18, 22, 21]. Most commercial applications of duct cleaning (rotary brushing or vacuuming) include the use of an antimicrobial encapsulant on FGDL. This improves the integrity or structure of the surface, helps to reunite any loose fibers which may have become partly dislodged by the actions of the cleaning process, and deposits a surface which should be hostile to potential microbial growth. The testing of three commercially available antimicrobial encapsulents/ sealants were monitored after being applied to a FGDL surface that was contaminated with mold and cleaned. The field experiment was conducted in the EPA test house, Cary, NC [23]. The results of the study of antimicrobial encapsulants efficacy is discussed in the next section, Evaluation of Antimicrobial Treatments as Control Technologies, and in the section titled Field Testing of Sealants and Encapsulants Used in Air Duct Systems.

As covered in the previous section, concurrent laboratory testing was performed which revealed that if dust is allowed to build on FGDL within the air distribution duct network, mold growth can become established at elevated RH levels even when an antimicrobial encapsulant is applied to duct surfaces [22, 23].

Understanding the cause of microbial contamination, the means of controlling or preventing microbial growth, and the consequential effects of the uncontrolled spread of microbial growth in typical operating conditions has been addressed [22–29]. To facilitate biological research on duct materials, the static and dynamic chambers were designed and constructed, and the methods of testing microbial growth under constant temperature and RH and conditions of static or dynamic air movement was developed [20, 22, 29]. The evaluation of fungal growth on FGDL and ceiling tiles were discussed above under Research and Development of Risk Management Alternatives to Prevent and Control the Growth of Mold by Studies to Quantify the Effects of Moisture, Relative Humidity (RH) and Dust. The impact of RH, air velocity, and surface growth on the emission rates of fungal spores from the surface of contaminated material have been studied and are addressed under the section titled Characterization of Emission Rates and Modeling of Exposure through HVAC Operation.

Findings confirm that fungal growth on FGDL is intrusive throughout the materials and that guidelines which recommend discarding microbially contaminated porous duct material should be followed [30–32]. Mechanical cleaning by HEPA air-vacuuming was able, at best, to reduce imbedded fiber soiling and temporarily decrease fungal levels. These fungal populations were able to reestablish growth within six weeks [31, 32].

Evaluation of Antimicrobial Treatments as Control Technologies

The efficacy of antimicrobial treatments (see Figure 9) to eliminate or control biological growth in the indoor environment can easily be tested on nonporous surfaces. However, the testing of antimicrobial efficacy on porous surfaces, such as those found in the indoor environment (i.e., gypsum board, HVAC duct-liner insulation, and wood products) can be more complicated and prone to incorrect conclusions regarding residual organisms and nonviable allergens [30–34]. Research to control biological growth using three separate antimicrobial encapsulants on contaminated duct-liner insulation has been performed in both field and laboratory testing. The results indicate differences in antimicrobial efficacy for the period of testing [34].

Figure 9. HVAC Duct Encapsulant Lining.

Three common HVAC antimicrobial encapsulants were evaluated for their use on FGDL in both laboratory and field application experiments. The antimicrobial encapsulants tested are manufactured for use in HVAC system components and on duct surfaces (see Figure 10). Coating I was a polyacrylate copolymer containing 9% barium metaborate and 0.16% iodo-2-propynyl butylcarbamate; Coating II was an acrylic coating containing decabromodiphenyl oxide and antimony trioxide; and Coating III was an acrylic primer containing phosphoric acid compounds with a phosphated quaternary amine complex [diethanolamine n-coco alkyl derivatives; 2,2'-(coco ankylimino)bisethanol]. Although the field test was inconclusive and truncated, the findings are discussed in the next section.

Laboratory SMTC experiments showed differences in

Figure 10. Encapsulant Application.

degrees of efficacy for the three antimicrobial coatings. Two antimicrobial coatings limited fungal contamination for the duration of testing. The effective coatings (Coatings I and III) contained zinc oxide/borates and a phosphated quaternary amine, respectively. The ineffective coating (Coating II) contained decabromodiphenyl oxide and antimony trioxide. Although all three antimicrobials are registered with the EPA, they were not equally effective, nor should they be expected to perform equally in field use [33, 34].

Methods of testing antimicrobial efficacy are needed to evaluate differences in products having or seeking EPA registration. The series of experiments described in Menetrez et al. (2002), are adequate tests for viable mold [34]. However, in addition to viable mold antimicrobial efficacy, additional testing may be needed for viable bacteria (bacteria cells and spores), nonviable mold (mycotoxins) and bacteria (endotoxins), as well as viruses and some forms of allergens (e.g., dust mites) to determine whether the manufacturers' claims apply [34–36].

Field Testing of Sealants and Encapsulants Used in Air Duct Systems

Under favorable conditions, biocontaminants are able to grow and multiply on a variety of building materials and indoor surfaces. All antimimicrobial manufacturers' claims should be verified through the testing of efficacy performance. Potential biocontaminants and their metabolites that are claimed to be controlled should be tested on the appropriate materials and under the appropriate conditions of use. Methods of testing for antimicrobial

efficacy are incomplete and may be misleading if (1) testing is performed only on nonporous materials, (2) testing involves a limited number (one or two) of microbial species, and (3) testing of microbial fragments is not included. Analytical methods are available to accomplish a comprehensive determination of antimicrobial efficacy and should be made part of the evaluation process.

The overall goal of antimicrobial efficacy research should be to contribute to developing better methods of understanding indoor air bioaerosol contamination and to enhance the ability to prevent and control indoor exposure to all forms of biocontaminants. Specific objectives include (1) establishing standardized test methods and procedures for evaluation, (2) developing techniques and equipment to conduct test evaluations, and (3) identifying materials, microbial populations, and constituent biological entities (viable and nonviable metabolites and microbial volatile organic compounds) to be tested with antimicrobial treatments [34].

Three commercially available biocidal encapsulants/sealants (as previously described in the section titled Evaluation of Antimicrobial Treatments as Control Technologies) were also field tested and monitored after being applied to fiberglass duct liner surfaces that were contaminated with mold and cleaned. The field experiment was conducted in the EPA test house, Cary, NC. Participating members of the NADCA rotary-cleaned and spray-coated according to the manufacturers specifications the fiberglass duct liner in the trunk-lines of the EPA test house with three popular brands of encapsulants/sealants. The encapsulant/sealant efficacy was field-tested under normal residential conditions for cooling and heating. The test environment was representative of the area for a HVAC system located in a residential crawl space. During the cooling season, the HVAC system was cool and had a high humidity when running (especially in the area of the cooling coils and drain pan where condensate flow was constant and the air remained near saturation) and had some intermediate condition when not running. The results suggest that dust and high humidity should be properly controlled in any HVAC system to prevent the growth of *P. chrysogenum* [23, 33, 34].

Field study measurements of FGDL surfaces prior to coating averaged approximately 1,000 CFUs/10 cm^2 of fungal contamination. This concentration of mold contamination did not substantially change during the experiment. Part of the reason for this was attributed to the fact that after 4 months of monitoring, the cooling coils were found to be leaking condensate into the supply duct and needed to be replaced. The conveyance of moisture into the adjacent duct may have been the reason for the initial growth of mold. After replacing the defective cooling coils, only background concentrations of fungal contamination were found on FGDL surfaces. The reduced moisture level created by the new cooling coils lessened the potential for the growth of mold and effectively terminated the test house field experiment before differences in antimicrobial efficacy could be demonstrated. After monitoring the test house for 7 months (a complete cooling season) without evidence of fungal concentration exceeding background levels, the experiment was stopped [34, 35].

Dynamic chamber laboratory experiments of untreated FGDL removed from the test house as bulk samples indicated that the population of *A. versicolor* increased by 3 logs (1,000 fold) by the end of the first month and remained approximately level through the 3.5-month study [34]. A slight variation increase followed by a decrease in the *A. versicolor* population was observed for Coatings I and III on FGDL, for the period of 3.5 months. This was in contrast to the increased populations observed in samples with Coating II. The increase in fungi population for Coating II was comparable to that observed in the untreated samples, as compared with successful limiting of growth accomplished by Coatings I and III [34]. The laboratory testing of these three antimicrobial encapsulants are described in the previous section titled Evaluation of Antimicrobial Treatments as Control Technologies for laboratory testing.

Soiled FGDL experiments (described in the previous section) resulted in similar populations of *A. versicolor* for untreated and Coating II samples. The results were again similar for Coatings I and III, in which fungi populations were observed to increase in the first month, and then

decrease. The results indicates that antimicrobials can remain effective with moderate dust loading [34].

A comparison of mold growth on FGDL demonstrated in the SMTC results (conducted at 70%, 85%, 90%, and 94% RH) with DMTC (conducted at 94 % RH) results for the three types of antimicrobials agreed. Increased fungi growth for Coating II was comparable to that observed in the untreated samples, whereas limited growth was observed on Coatings I and III [34]. These studies demonstrated that SMTC fungi growth decreases with decreasing RH. Both SMTC and DMTC methods indicate greater effectiveness in controlling growth with Coatings I and III [34].

Characterization of Emission Rates and Modeling of Exposure through HVAC Operation

Biological contaminants are known to be indoor air pollutants which carry a substantial health risk with exposure [1, 3, 15–18]. Biological allergens (mold, bacteria, pollen, animal dander, dust mites, roaches) and their fragments in the size range of 0.3 to 10.0 µm make up a component of airborne particulate matter (BioPM), addressed by Menetrez, et al. [36, 37], and Foarde, et al. [38]. BioPM is composed of a large variety of viable and non-viable organisms, some of which can be infectious bacteria and fungi, as well as fragmented pieces of biological organisms which can be allergenic, toxic, immunosuppressant or can produce inflammatory responses [1, 3, 15–18]. To limit exposure to BioPM in the ambient and indoor environment, the development of control technologies are required. Antimicrobial agents and biocides have long been used to control, prevent, and remediate microbial growth for many different applications in the environment. This research deals with BioPM as the main physical mechanism for pulmonary exposure to biocontaminants. The objective of this research was to evaluate the sample collection and analysis of BioPM in the size range of 0.3 to 10.0 µm [32, 36–38]. An additional part of this work quantified the emission rates of mold (a substantial constituent of BioPM) and used a computer model to project its distribution throughout a school with a HVAC dominated indoor environment.

The importance of minimizing exposure to BioPM of indoor origin in the indoor environment is well established. However, the importance of minimizing exposure to BioPM in the ambient fraction of particulate matter with aerodynamic diameters less than 2.5 µm ($PM_{2.5}$) has not been well studied. In 1998, North American Research Strategy for Tropospheric Ozone and Aerosols (NARSTO) listed a number of ambient PM constituents that need further study. One of these is BioPM, now thought to be a previously unrecognized causative agent for adverse health effects.

Biological agents potentially play two important roles in influencing the adverse health effects that have been associated with $PM_{2.5}$ exposures. The first role is as a constituent of PM. The second is as an agent to exacerbate adverse health effects in sensitive individuals. In addition to mold, yeast, and pollen, bacteria are present in both ambient and indoor air from a large variety of human activities. Agricultural activities such as plowing or hog production, manufacturing operations such as cotton mills or grain storage, and waste treatment activities such as wastewater treatment release airborne bacteria. The size range of airborne bacteria is from 0.5 to 2.0 µm (*Bacillus* spp., *Pseudomonas* spp., *Xanthomonas* spp., and *Arthrobacter* spp.), and submicrometer fragments of these gram-negative organisms can contain toxins that are combined with their cell wall. These fragments are known as endotoxins and, when inhaled, have been shown to increase non-specific bronchial reactivity in asthmatics [33, 36–38, 40].

The evaluation and control strategy of BioPM had been complicated by the lack of methods that will allow us to quantitatively assess the BioPM fraction of $PM_{2.5}$. At issue is how much of occupant exposure originates indoors, how much is derived from outdoor sources, and what is the interaction. Because PM exposure indoors can originate from both indoor and outdoor sources, it was determined that information was needed to scientifically quantify the relationship between indoor and outdoor levels of PM aerosols [33, 36–40].

An investigation has been conducted into the feasibility of

developing sampling methods and analysis techniques for quantifying BioPM and its relative distribution indoors and outdoors. Andersen non-viable impactor samples of the indoor and outdoor air were collected over a 5-week period from three sites. Then the samples were analyzed for total protein, ragweed, and fungal antigens β-1,3 glucan and endotoxins. A preliminary assessment of a variety of sampling methods for the measurement of the BioPM fraction of indoor and outdoor PM was performed for the purpose of method optimization [33, 36–40]. The results indicated that sizable fractions of the fine PM sampled in both indoor and outdoor samples were of biological origin. These results are preliminary, and although they establish the presence of a biological component with indoor and outdoor fine PM, additional research is needed to (1) further develop the measurement method, (2) quantify the relationship between indoor and outdoor levels of the BioPM, and (3) determine the fraction of ambient and indoor PM that is biological [33, 36–40].

Biocontaminants such as mold spores are capable of being released into the indoor air from the site of growth and being transported in a viable or non-viable form. Exposure to toxic mold and the mycotoxins contained in the spore and vegetative body have been shown to produce adverse health effects resulting from inhalation, ingestion, and dermal contact. A study of the release of *Stachybotrys chartarum* spores from contaminated gypsum wallboard showed the effects of environmental conditions on the transport of viable and non-viable spores and fragments. The findings of *S. chartarum* spore emissions with low air velocity flow conditions were found to be directly proportional to airflow and indirectly proportional to relative humidity. These emission findings corroborate previous observations involving *Penicillium* and *Aspergillus* [33, 36, 37]. Viability of *S. chartarum* spore emissions was also discussed with respect to culturable and commonly used field measurement techniques [37].

Spore emission rates from FGDL for *Penicillium chrysogenum* and *Aspergillus versicolor* at four RH levels (95%, 85%, 75% and 65%) of conditioned input air and two velocities (35 and less than 10 cm/s) were used to test emission rates. Emissions increased as the air velocity increased across the contaminated FGDL surface. The published experiments also found that as the RH was lowered, the emission rate increased for both organisms. These relationships were investigated to determine whether the spore emission rate for *S. chartarum* would follow a similar pattern. The emission rates did confirm that *S. chartarum* spore emissions increase with increased airflow and as the humidity is lowered [37, 38].

A second set of experiments was designed to quantify emissions rates of four room-wall simulators (RWS) containing gypsum wallboard (see Figure 11), at 65% RH over an extended period of time. The RWSs (17.1 × 17.1 × 161.7 cm duct section) were constructed of 16 gauge stainless steel, and the front cover is made from glass to permit visual monitoring of growth on the test material. The top inlet and bottom discharge ends of the RWS transition to 2.5 cm diameter tubes. Each RWS was connected to a pressurized plenum fed by a blower drawing HEPA filtered and conditioned air from the DMTC. A single piece of gypsum board 107 × 42 cm (4494 cm^2) was scored length-wise to permit it to be folded into a three-sided trough that fit into and formed the interior walls of the RWS. Air from the plenum entered each RWS through the inlet transition, flowed vertically down along the glass and three-sided gypsum board wall, and discharged through the outlet transition. Bioaerosol samples were drawn from the RWS discharge air through the bottom end into the tubing which penetrated the DMTC wall and into the sampling equipment. Surface concentrations of spores

Figure 11. Room Wall Simulators.

on the gypsum wallboard in the four RWS were essentially the same. However, the age of the growth on the RWS 1, 2, and 3 was 2 months old when the emission experiments were initiated. RWS 4 was 3 months old and exhibited a higher sustained rate of emissions. RWS 1 through 3 started at levels between 500 and 1,000 CFU/m^2/hr and tapered off by day 2 or 3. RWS 4 started at the same initial level as RWS 1, 2, and 3 but continued to emit that same level of spores for the entire first week before the level started to decrease. Emission of spores at an RH below 65% and an air velocity of 35 cm/s exhibited rates of both culturable colony forming units (CFUs) and total spores per square meter that were sustained at a high level of emission for an 80 day period. For 30 days CFUs appeared to be about 10% of the number of total spores. After 30 days, the levels of culturable CFUs and total spores approach being equal. For the final 25 days, the percentage of culturable CFUs was lower than the second 30 days, but higher than the first 30 days [37].

To better understand the implications of the emission rates measured in these experiments, an IAQ model was used to estimate concentration and potential exposure, as has been done with other indoor air contaminants. The goal was to estimate the potential indoor concentration levels using a typical emission rate from these experiments and reasonable ventilation parameters. The model used was RISK IAQ Model for Windows [38], which is a completely mixed room model incorporating source/sink behavior that can generate concentration and exposure estimates as functions of time. The ventilation flows and pollutant emission rates were set at desired levels for each modeled room. For this study, a school was modeled having 10 rooms distributed over a floor area of 371.6 m^2 with 2.44-m ceilings and served by one HVAC system. The total HVAC airflow to the area was 56.6 m^3/min (2000 ft^3/min). The school was assigned three separate HVAC scenarios: (1) source with the HVAC always on and no outdoor air, (2) source with HVAC on from 0800 to 1700 hours, 9-hour duty cycle, and no outdoor air, and (3) the same 9-hour duty cycle as scenario 2 with the addition of an outdoor air intake level of 5% of the recirculating airflow to the HVAC system as is the case in a typical school. The source of emission was calculated from an area of mold contamination of 9.29 m^2 (100 ft^2), selected as representative of a modest area of contamination. The actual areas of mold contamination in a problem building would be expected to emit *S. chartarum* spores at a similar concentration per unit of contaminated surface area.

Concentration profiles were run, and the exposure levels were calculated for an average person (breathing at 0.83 m^3/h and, for simplicity, staying in the room 24 h/day) for all scenarios. The model specified a building dominated by the HVAC system and having a building air exchange rate of 3.8 air changes per hour (ach). Various surfaces within a building, as well as components of a HVAC system may support microbial growth. The area of contamination was located within the school building, which was served by the HVAC system as defined by each of the three scenarios modeled. From the evaluation, we can conclude that running the HVAC system constantly will result in the maximum transport of spores and the greatest concentration of both total spores and CFUs and is the worst scenario of the three. A nine hour HVAC duty cycle, which is 37.5% of the time, was calculated by the model to have a proportional decrease in total spores and CFUs, and an air filter that removes 40% of the particulate matter from the air stream can also achieve a significant decrease in total spores and CFUs. This indicates that air filtration is able to dramatically reduce spore concentrations from unacceptably high levels to background levels. For this simulation using the RISK IAQ Model for Windows [38], mold spore concentrations were reduced from 680 to 60 CFU/m^3 by use of a 40% particle filter, effecting a one order of magnitude decrease. It should be stressed that the model treats the entire area of the school as a well mixed reactor, having equal concentrations regardless of the proximity to the source of contamination, and only considers entire spores in the 4 to 6 μm size range [37].

Understanding the factors that govern spore release, aerosolization, and transport allows prediction of potential exposure. *S. chartarum* spore emissions from gypsum board at low flow are directly proportional to airflow and inversely proportional to RH, which supports previous research with *Penicillium* and *Aspergillus*. The relationship between the culturable CFUs and total spores needs

further investigation. However it demonstrates the difficulty in providing a correlation between airborne field measurements (using culturable CFUs) and human exposure. Air filtration can be a helpful tool for minimizing exposure and containing mold contamination while remediation is performed [37].

Improved Methods of Mold Sampling and Analysis

Mold contamination exists in either solid (intact spores and fine non-viable particles) or gaseous state (microbial volatile organic compounds). Fine particulate matter is a significant pollutant both indoor and outdoors, and measurements of indoor exposures are needed for evaluating total long-term personal exposures to $PM_{2.5}$ originating from both ambient outdoor and indoor sources. Bacteria, fungi, viruses, and allergens are important components of outdoor and indoor aerosols. Desiccated, nonviable fragments of these organisms are also ubiquitous. These fragments can remain pathogenic, toxic, or allergenic (depending upon the specific organism or organism component) [36, 37, 39, 40]. The collection and characterization of these fragments were addressed as BioPM in the previous section, Characterization of Emission Rates and Modeling of Exposure Through HVAC Operation. The identification of gases produced by the metabolic activity of mold and the development of molecular identification techniques of mixed samples of mold spores are addressed in the following sections.

Evaluation of Microbial Volatile Organic Compounds

Analytical methods are available to comprehensively identify microbial populations and metabolites (particles and gases). A study by Menetrez et. al. [39] examined MVOC emissions from six fungi (*Aspergillus glaucus*, *Aspergillus versicolor, Cladosporium sphaerospermum, Penicillium chrysogenum, Penicillium italicum, Rhodotorula glutinis*) and one bacterial species (*Streptomyces* spp.) commonly found in indoor environments. Data were presented on peak emission rates from inoculated agar plates loaded with surface growth, ranging from 33.5 $\mu g/m^2$/24 hr for *Cladosporium sphaerospermum* (predominantly δ-Humulene, Tetramethyl tetrahydrofuran, and α-Humulene) to 515 $\mu g/m^2$/24 hr for *Rhodotorula glutinis* (predominantly 3-Methyl-2-butanol, Phenyl ethyl alcohol, and 2-Methyl-1-propanol). Furthermore, changes in MVOC emission levels over the growth cycle of two of the microorganisms are examined. In addition, a calculation of the impact of MVOC emissions on indoor air quality in a typical house is made, as well as an application of an exposure model used in a typical school environment [42].

A unique method was developed and utilized for the purpose of this research in which microbial growth was allowed on agar plates (glass) enclosed within glass blocks. Consecutive growth periods of 24 hr were followed by gas collection of analytes adsorbed on the Tenax-TA tubes which were thermally desorbed at 250 °C into a GC equipped with a flow splitter [42].

The measured emission rates reflect single-day emissions from the tested microorganisms. In a growth environment, fungi experience an exponential increase in biomass (normally in hours) followed by a period of time where biomass either remains constant, or drops because of autolysis. Thus, the MVOC peak emission rate variations observed may be a direct result of normal microbial metabolism. It was anticipated that observed emission variations between replicates would be significant. However, the actual MVOC peak emission rates were relatively consistent between replicates, possibly due to the 24-hour sampling time chosen for this investigation. The long sampling period may have minimized the influence of any short-time variations in MVOC emissions inside the chambers. The variation from the mean average for the test microorganisms as a group was ±28%; the largest single variation was 76%, and the lowest was 5.4% [44].

Rhodotorula glutinis yielded the highest average peak emission rate (515 $\mu g/m^2$/24 h); whereas, *Cladosporium sphaerospermum* produced the lowest (33.5 $\mu g/m^2$/24 h). The average peak emission rate for all the microorganisms studied was 206 ± 57.8 $\mu g/m^2$/24 h, based on a total colonized surface area of 1 m^2 [42].

Experiments were performed to evaluate peak emission rate variations over the life of the biocontaminant

colonies. Agar plates inoculated with *Penicillium chrysogenum* and *Streptomyces* spp. were introduced into the glass chambers, followed by headspace collection. Headspace samples were collected when the colonies were new (2-4 days), mature (6-14 days), and middle-aged (14-18 days). MVOC emissions increased to a maximum at the later sample periods [42].

Except for *Penicillium italicum*, the primary MVOC emissions from each microorganism were alcohols and ketones. Percent emissions were calculated by dividing each MVOC's mass concentration by the total mass quantified from each biocontaminant after background subtraction. Alcohols and ketones, as well as reduced sulfur compounds (e.g., dimethyl disulfide, dimethyl trisulfide), have been identified previously as odiferous compounds associated with biocontaminated indoor environments. These compounds account for 32–93% of the total MVOC emissions found in individual samples. The reduced sulfur compounds are classified as heterocompounds in this article, which also include the nitrogen-containing compounds identified throughout the experiment (e.g., 2-acetylthiazole, methylpyrazine). Predominant emissions from *Penicillium italicum* were identified as sesquiterpenes, consistent with the many sesquiterpene compounds. The identification of α-humulene in the headspace of *Cladosporium sphaerospermum* suggests its possible role as a microbe-specific growth indicator, yet the emission of this sesquiterpene by *C. sphaerospermum* growing on building materials needs to be ascertained before it is identified as such [42].

Volatile metabolic by-products from seven common indoor biocontaminants have been studied in an attempt to establish MVOC peak emission rates from each individual microorganism and to evaluate their impact on overall IAQ. Many of the MVOCs identified in this study have been reported; however, identification of δ-humulene and α-humulene in the headspace of *Cladosporium sphaerospermum* suggests their possible role as microbe-specific growth indicators [42] which could be used as an indicator of indoor mold growth.

To better understand the implications of the emission rates measured in these experiments, an IAQ model was used to estimate concentration and potential exposure as has been done with other indoor air contaminants. The goal was to estimate the potential indoor concentration levels for using a typical emission rate from these experiments and reasonable ventilation parameters [38]. As in other experiments, the RISK IAQ Model for Windows was used, which assumes a completely mixed room model conditions and incorporating source/sink behavior that can generate MVOC concentration and exposure estimates as function of time [38].

For this study, a school was modeled having 10 rooms distributed over a floor area of 371.6 m^2 with 2.44-m ceilings and served by one HVAC system. The source of MVOC emissions was calculated from an area of mold contamination of 9.29 m^2 (100 ft^2), selected as representative of comparable emission rates.

Emission modeling for the different scenarios of HVAC operation demonstrated concentrations of MVOCs similar to those reported in the literature for known problem buildings [38]. The modeling results suggest that, as more MVOC emissions are identified, future modeling might serve as an aid in assessing the impact of MVOCs on indoor environments by providing field investigators a tool that can be used to assess the potentially deleterious effect of microbiological metabolic by-product emissions to overall IAQ [42].

Development of Rapid Multiplex PCR

Characterization of mold has previously been limited to visual identification or morphology [43, 44]. However, questions of accuracy and reproducibility have revealed a need to go beyond visual identification and growth morphology methods. The use of molecular biology has brought about significant change in microbiology which we have focused on the characterization of mold.

The growth of filamentous fungi (mold) in the indoor environment occurs in a dynamic setting. Environmental conditions are constantly changing allowing a vast diversity of different organisms to establish a stronghold and flourish in the built environment. These different organ-

isms produce a myriad of MVOCs, toxins, and allergenic components. Although only a small percentage of fungal species has been directly associated with adverse health affects, increased awareness and continuing research will likely result in the identification of many more pathogenic and toxigenic species. In order to properly assess the fungal exposure to building occupants and the implement an effective remediation strategy, it is imperative that fungal screening, isolation, and characterization be performed.

Research has been conducted utilizing molecular methods of fungal identification and characterization that are rapid, easy to perform, and cost sensitive. Molecular characterization research uses the PCR as the backbone of these applied strategies. The PCR allows for the amplification of virtually unlimited quantities of specific target DNA from the organism of interest. This amplified DNA can then be used to identify the organism or coupled to other technologies such as restriction fragment length polymorphism (RFLP) analysis, or genetic sequencing to unambiguously identify the organism of interest.

Reducing the time necessary for accurate fungal identification is an important aspect of rapid exposure estimation. To reduce the time necessary to extract and purify fungal DNA suitable for the PCR, research was performed to simplify existing technologies while maintaining high quality results [45]. It was determined that a simple mechanical disruption of the fungal spores followed by a phenol:chloroform-ethanol concentration produced highly purified DNA suitable for molecular analysis. The procedure, taking only one hour to complete, greatly enhances the rapidity of the subsequent molecular identification regime [45].

When attempting to identify numerous organisms, multiple PCR reactions are needed, increasing both the time and cost of identification. Additional PCR research has focused on developing and optimizing a multiplex PCR that is capable of identifying four organisms in a single reaction. Research efforts have developed a simple method to identify multiple fungal species, *Stachybotrys chartarum*, *Aspergillus versicolor*, *Penicillium purpurogenum*, and *Cladosporium* spp. by performing multiplex PCR and distinguishing the different reaction products by their mobility during agarose gel electrophoresis [46]. Positive identification made by multiplex PCR can be made within 24 hours following receipt of the samples [46].

Additional research has been carried out by coupling the PCR to RFLP patterns. Following the amplification of the target sequence, the amplified DNA is enzymatically broken down, or digested, into smaller components. The specific smaller components are a direct result of the DNA sequence of each of the organisms. Identification can then be made by analysis of the different reaction products following their mobility during agarose gel electrophoresis. The results indicate that organisms belonging to the genuses *Stachybotrys*, *Cladosporium*, *Aspergillus*, and *Penicillium* can accurately be identified using their mobility patterns following treatment with only four digestion enzymes [47]. These identifications are unambiguous, allowing for rapid and positive identification of fungal contaminants [45–47].

PCR coupled to genetic sequencing has also been a major research focus. The overarching goal of this research is to positively identify as many organisms at a time as rapidly as possible. An environmental sample may contain hundreds of fungal spores and fragments: therefore, following PCR with multiple organisms, the amplified DNA is inserted into a mobile genetic element and inserted into bacterial cells. Each bacterial cell will contain only a single piece of PCR amplified DNA or the DNA from only a single organism of the environmental sample. The bacterial DNA can then be recovered and genetic sequencing carried out specifically for the PCR amplified fragment. The individual genetic sequences are then used to positively identify each organism present in the sample. Initial research results have shown the methodology capable of identifying *Stachybotrys*, *Cladosporium*, *Aspergillus*, and *Penicillium* species from a mixed culture environment and the generation of a patent application for this technology [45–47].

Fungal sampling and collection technology from various building materials has also produced results that save time and are inherently more accurate and cost effective. The

objective of this research was to develop a sample test method for porous building materials (ceiling tile) to detect and quantify mold growth utilizing a masticator blender. By comparing results to previously used technologies, statistical analysis showed that the masticator method yielded significantly higher estimates of the mold population [48]. This research showed that homogenizing samples of bulk ceiling tiles with the masticator blender is an effective method to recover mold spores from porous surfaces and can be a helpful tool when applied to antimicrobial efficacy testing.

Discussion

The significant technical findings of the research discussed in this report are summarized and listed below. They often overlap into more than one of the six research areas. An example of this is the development of the static and dynamic chambers which were utilized in all six research areas addressed in this report. The discussions of each of the six research areas and the technical findings are only meant to summarize the referenced publications and not to restate technical results from those publications. The findings listed below fall into one or more of the following areas: (1) research and development of risk management alternatives to prevent and control the growth of mold by studies to quantify the effects of moisture, RH, and dust, (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials, (3) evaluation of antimicrobial treatments as control technologies, (4) field testing of sealants and encapsulents used in air duct systems, (5) characterization of emission rates and modeling of exposure through HVAC operation, and (6) improved methods of sampling and analysis of mold.

Technical Findings

Static Microbial Growth Chamber

- Establishing that SCTM was adapted into ASTM Standard 6329-98 [21]. The SCTM defines how to conduct microbial testing in a well-controlled environment. The SCTM is essential to understanding mechanisms and improving the repeatability and comparability of data [20, 21].

Dynamic Microbial Growth Chamber

- Developing the DMTC, a room-sized dynamic chamber, designed and constructed under a cooperative agreement between EPA and RTI, that can test HVAC ducts (mini-ducts) scaled to simulate horizontal duct velocities and duct materials in the mini-duct apparatus [29], or vertical low velocity air flow against gypsum wallboard with room wall simulators [37].

Research and Development of Risk Management Alternatives to Prevent and Control the Growth of Mold by Studies to Quantify the Effects of Moisture, Relative Humidity, and Dust

- Determining that water incursion or standing water is not required for growth on materials [23–26]. For some species of mold, humidity alone can provide sufficient moisture to permit growth on building materials (material and organism dependent), relative to the hygroscopicity of the material [24–26].
- Developing a method for artificially soiling materials [22]. Allowing fungal growth characterization or antimicrobial efficacy testing methods to simulate realistic environmental conditions will result in laboratory experiments that more closely resemble real-world applications [22].
- Confirming that, under equilibrium conditions, RH and moisture content correlate well with mold growth (depending on the moisture requirements of the test organisms) [24, 25]. However, under nonequilibrium conditions, mold growth correlates better with increasing moisture content (in duct liner, ceiling tile and gypsum wallboard) than with RH [24–28].
- Finding that variations in the characteristics of similar building materials can impact the fungal resistance of that material [23–27, 34]. Both new and used materials are capable of supporting mold growth, but generally used materials (soiled) were more susceptible [25–27].
- Establishing that reducing the moisture content of wet materials (within 3 days) before fungal growth became established provided effective source management [24, 28]. However, established microbial growth may continue even after the moisture content of a particular material is lowered below that required to initiate growth [28].
- Finding that growth of *S. chartarum* was not detected in environments with a relative humidity below total saturation and no wetting occurred. Wetting was necessary for visible growth to occur. Porous materials, after becoming sufficiently wet, exhibited mold growth at all relative humidity levels tested [33].
- Acutely impacting control and remediation practices by showing that RH is inversely related to fungal spore emissions [22, 25, 32]. Lowering uncontrolled humidity is almost always a recommended practice which will lead to increased airborne contamination. This strategy points out the need for containment of contaminated areas to prevent the spread of contaminants [32].

Duct Cleaning Effectiveness for Prevention and Control of Microbial Growth on Duct Materials

- Confirming that fungal growth is intrusive throughout porous materials and that guidelines that recommend discarding microbially contaminated porous duct

material should be followed [30–32]. Mechanical cleaning by HEPA air vacuuming was able, at best, to reduce imbedded fiber soiling and temporarily decrease fungal levels. These fungal populations experienced regrowth within six weeks [31, 32].

- Finding suggest that dust and high humidity should be properly controlled in any HVAC system to prevent the growth of mold [23, 33, 34].

Evaluation of Antimicrobial Treatments as Control Technologies

- Finding that significant variation in antimicrobial efficacy of encapsulents to limit or eliminate biological growth indicates a need for widespread product testing and for the development of an efficacy testing protocol [34].
- Developing a method for artificially soiling materials, as previously discussed, allowed for fungal growth conditions of antimicrobial efficacy testing to simulate realistic environmental real-world applications [22].

Field Testing of Sealants and Encapsulents Used in Air Duct Systems

- Finding differences in degrees of efficacy for three antimicrobial coatings. Two antimicrobial coatings limited fungal contamination for the duration of testing. The effective coatings contained zinc oxide/borates and a phosphated quaternary amine. The ineffective coating contained decabromodiphenyl oxide and antimony trioxide. Although all three antimicrobials are registered with the EPA, they were not equally effective and would not be expected to perform equally in field use [34, 35].

Characterization of Emission Rates and Modeling of Exposure Through HVAC Operation

- Finding that fine PM sampled in both indoor and outdoor samples were of biological origin. This establishes the presence of a biological component with indoor and outdoor fine PM [36–39, 41].
- Determining that the emission rates of fungal spores from the surface of contaminated material result from a complex interaction of factors. Emission rates differ between organisms and are inversely proportional to RH but directly related to air flow and surface loading. Potential indoor concentrations were modeled using RISK IAQ Model for Windows [37, 38]. The modeled levels related well to the values reported in the literature for known problem buildings, suggesting that, once microbial emission rates are well enough understood, models may be useful in predicting exposure and, eventually, risk for individual organisms [37].
- Understanding the factors that govern spore release, aerosolization, and transport allow prediction of potential exposure. *S. chartarum* spore emissions from gypsum board at low flow are directly proportional to airflow and inversely proportional to RH, which supports previous research with *Penicillium* and *Aspergillus*. The relationship between the culturable CFUs and total spores needs further investigation but suggests one reason that correlation between airborne field measurements (using culturable CFUs) and possible exposure is so difficult. Air filtration can be a helpful tool for minimizing exposure and containing mold contamination while remediation is performed [36, 37, 40].

Improved Methods of Sampling and Analysis of Mold

- Identifying δ-humulene and α-humulene (among the many MVOCs from mold growth that have been reported) in the headspace of *Cladosporium sphaerospermum* suggests their possible role as microbe-specific growth indicators [42].
- Establishing that simple mechanical disruption followed by standard phenol:chloroform-ethanol concentration produces highly pure fungal DNA suitable for use in subsequent molecular biology applications [45].
- Developing a method of multiplex PCR that is capable of identifying 4 environmentally relevant fungi (*Stachybotrys chartarum*, *Aspergillus versicolor*, *Penicillium purpurogenum*, and *Cladosporium* spp.). The method is preferable due to savings in time and cost with increased identification accuracy [46].
- Developing a method of PCR followed by RFLP to

generate DNA patterns that enable the identification of medically relevant fungal organisms [46, 47].

- Developing a fungal screening technology that is capable of identifying potentially hundreds of organisms from a single PCR reaction. The methodology has been shown to work with *Stachybotrys*, *Cladosporium*, *Aspergillus*, and *Penicillium* species from a mixed culture environment and has resulted in the initiation of patent rights [46, 47].
- Developing a method of sampling and collecting fungal spores from porous building materials. The method, involving mastication, provides more complete analysis and accuracy by homogenizing the sample rather than relying on surface sampling [50].

The findings listed above cover a broad expanse of research related to detecting and controlling mold contamination. Additional work is needed to further reduce human exposure to biological contaminants.

Conclusions

The six areas of research identified by EPA/APPCD/ IEMB for allocation of program resources were (1) research and development of risk management alternatives to prevent and control the growth of mold by studies to quantify the effects of moisture, relative humidity and dust, (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials, (3) evaluation of antimicrobial treatments as control technologies, (4) field testing of sealants and encapsulents used in air duct systems, (5) characterization of emission rates and modeling of exposure through HVAC operation, and (6) improved methods of sampling and analysis of mold. These areas of research were investigated, and the most significant findings are summarized in the discussion section above. Advances in research and development of prevention and control as well as mitigation practices for a variety of molds were achieved. Understanding the growth requirements of mold, developing the test methodology ASTM Standard 6329-98 [21] and the static and dynamic microbial test chambers for determining antimicrobial efficacy, and determining the most effective technique to identify, handle, and mitigate contaminated materials will ultimately improve the ability to control biological contaminants and reducing human exposure.

Future Research

Future areas of microbiological research are being planned. Progress in these areas of research will be subject to change and re-evaluation over time. However, these six research areas are a logical next step from past and present accomplishments.

Microbial Resistant Building Materials Product Evaluation - Gypsum Wallboard

When building materials becomes exposed to moisture by weather events, leaks in the building envelope, or inadequate control of relative humidity, absorption and transport of moisture through that material often renders it susceptible to the growth of biological contaminants. Microbial colonization and the rapid growth and dispersion of mold can expose building occupants and produce severe illnesses including pulmonary, immunologic, neurological and oncogenic disorders. Removing substrates from building materials or incorporating antimicrobial agents in the manufacture of building products may prevent mold growth and the spread of contaminants. The manufacture of microbial resistant building materials (such as wallboard, ceiling tiles and flooring) can inhibit or prevent mold growth. Limiting or preventing mold growth by the manufacture of microbial resistant building materials creates a product which can sustain temporary adverse conditions and is less likely to become a source of biological contamination, or need replacement than the current products.

Possible methods of gypsum wallboard improvement are being studied, including treatment with antimicrobials, ozone, and heat during the manufacturing process. The ability to remove viable mold from the inner sections of the wallboard may impair the ability of mold to germinate and grow following an isolated water incident. This reduction in mold growth could prevent contaminated wallboard from having to be removed and land filled, fitting well with the sustainability focus supported by the EPA.

The manufacture of microbial resistant gypsum wallboard has been initiated by a number of companies producing building material. Each company has established their own individual manufacturing strategy for producing this material. The resultant building material could potentially have a longer product life and be both environmentally friendly and less likely to need replacing than the current products.

The evaluation of gypsum wallboard would test (1) microbial growth, (2) moisture absorption, and (3) VOC emission. Established methods would be used to form the basis of evaluation. Each product evaluation result would then be evaluated.

Gypsum wallboard has been the selected to be the first building material to be evaluated by this process. Evaluation of joint compound and tape should be addressed in the future. The impact to the building product industry and the consumer public can be significant, with both gaining advantages through the sale of better products.

Microbial Resistant Building Materials Product Evaluation - Ceiling Tiles and Flooring

Ceiling tiles and flooring that have a greater ability to withstand moisture and prevent mold growth will be less problematic and in need of replacement than the current products. Possible methods of ceiling and flooring improvement should be studied, including treatment with antimicrobials, ozone, and heat during the manufacturing process. The ability to remove viable mold from the inner sections of the building products may impair the ability of mold to regrow following an isolated water incident. This reduction in mold growth could prevent contaminated ceiling and flooring systems from having to be removed and landfilled, fitting well with the sustainability focus supported by the EPA.

The evaluation of ceiling tiles and carpet flooring would test (1) microbial growth, (2) moisture absorption, and (3) VOC emission. Established methods would be used to form the basis of evaluation.

Acoustic ceiling tiles and carpeting used as building materials should be evaluated by this grading process. The impact to the building product industry and the consumer public can be significant, with both gaining advantages through the sale of better products.

HVAC Biological Film (Biofilm) Research

The presence of moisture on HVAC system cooling coils and drip pan from condensate flow establishes conditions favorable for microbial growth. The established microbial growth can then be responsible for releasing gases (MVOCs, Dirty Socks Syndrome) or particles (BioPM) into the conditioned airstream. The presence of a microbial film on the cooling coils is also responsible for loss of heat transfer efficiency and over-all component operation and is the possible cause of condensate "blow-by" into the supply air duct. The characterization of fungal organisms and their byproducts (MVOCs and BioPM) that are responsible for this condition and the most effective form of treatment (UV irradiation) would give building owners, building occupants, and building remediators accurate information for identifying and dealing with this problem.

The use of UV to destroy any biofilm that has been established on HVAC surfaces and not allow re-growth is the most efficacious manner of long-term treatment. The use of UV to deactivate airborne biological contaminants transported within existing HVAC systems adds additional benefit to this treatment alternative. Also, the prevention of biofilm build-up on cooling coil surfaces increases thermal transfer efficiency and decreases HVAC system energy use at the same time that it prevents organisms from establishing a foothold in the indoor environment, increasing the sustainability of current building systems.

MVOC characterization of gases and PCR identification of particles will be used to determine the best way to identify biofilms. An HVAC system that has an established biological film will be treated with UV radiation. Measurements of biological contaminants and heat transfer efficiency from before and after treatment will be conducted. Laboratory and field demonstrations will be performed concurrently.

Asthma Triggers

Research will focus on developing optimization techniques for collection and analysis of fine and ultra fine biological particles (fungal, bacterial, pollen, animal dander, and dust mite and cockroach allergens), which make up the entire range of biological particulate matter that are known asthma triggers. Improving methods of sampling for these size fractions with filter or impactor collection (such as the Andersen non-viable sampler) and subsequent analysis via scanning electron microscopy, polymerase chain reaction, mycotoxin presence, and gravimetric measurements can result in a better understanding of the potential exposure to viable and non-viable particles that are biological asthma triggers.

Homeland Security: Tests on Thermal Destruction Using Biocontaminated Building Materials (*Bacillus anthracis* surrogates): Bench Scale and Rotary Kiln Incinerator

A significant amount of contaminated building material may need to be disposed of after a bio-terrorism attack. The efficacy of disposal of building materials contaminated with biological agents by incineration is complicated by matrix effects associated with the contaminant and the material. This project is examining the destruction of surrogate biological agents inoculated on several common building materials including ceiling tiles and wallboard. A laboratory-scale reactor and a rotary kiln are being used in this project to examine building material, heating temperature, and residence time affecting the destruction of surrogate biological contaminants, including *Bacillus subtilis* and *Geobacillus stearothermophilus*, both surrogates for *Bacillus anthracis*. Work is focusing on the thermal destruction of biological contaminants as well as stack sampling to monitor containment of contaminant organisms during incineration. The results from these studies can be used to evaluate incineration technologies for appropriateness for disposal of contaminated building materials.

Reduced Infectious Disease

The spread of infectious disease in humans can be attributed to communication by touch and inhalation of the infectious agents (virus and bacteria). Because air conveyance through the HVAC can transport infectious agents along with other particles throughout a building, one infectious person can spread viable organisms to many other through the conveyance of conditioned air.

Research will focus on developing optimal air treatment techniques of fine and ultra fine biological particles (viral, bacterial) which are responsible for the spread of infectious disease. Biological surrogates of viral and bacterial organisms will be inoculated into the air stream traveling through an HVAC system. Improving methods of treatment can result in less transmission of infectious disease as well as better biological indoor air quality.

Research will involve UV irradiation to destroy viral and bacterial microorganisms on a surface and in a moving stream of air. Although the ability of UV to destroy bacillus spores and mold has been demonstrated, the antiviral or anti-pneumonia efficacy of UV on surfaces and in a moving airstream is largely unknown. The use of UV to clean air within an existing HVAC systems can have other beneficial effects such as preventing fungal growth and decreasing the energy use.

References

1. Vesper, S.J.; Vesper, M.J., 2002. Stachylysin may be a cause of hemorrhaging in humans exposed to *Stachybotrys chartarum. Infection and Immunity*. **70**(4):2065–2069.
2. Dearborn, D.G.; Yike, I.; Sorenson, W.G.; Miller, M.J.; Etzel, R.A., 1999. Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio. *Environ. Health Perspect*. **107**:S495–S499.
3. Vesper, S.J.; Dearborn, D.G.; Yike, I.; Allen, T.; Sobolewski, J.; Hinkley, S.F.; Jarvis, B.B.; Haugland, R.A., 2000. Evaluation of *Stachybotrys chartarum* in the house of an infant with pulmonary hemorrhage: Quantitative assessment before, during, and after remediation. *J. Urb. Health*. **77**:68–85.
4. Scheel, C.M., 2001. Possible sources of sick building syndrome in a Tennessee middle school. *Arch. Environ.. Health*. **56**(5):413–418.
5. Brunekreef, B.; Dockery, D.W.; Speizer, F.E.; Ware, J.H.; Spengler, J.D.; Ferris, B.G., 1989. Home dampness and respiratory morbidity in children. *Am. Rev. Respir. Dis*. **140**:1363–1367.
6. Nielsen, K.F., 2003. Mycotoxin production by indoor molds. *Fungal Genet Biol*. **39**:103–117.
7. Murtoniemi, T.; Nevalainen, A.; Suutari, M.; Toivola, M.; Komulainen, H.; Hirvonen, M.R., 2001. Induction of cytotoxicity and production of inflammatory mediators in TAW264.7 macrophages by spores grown on six different plasterboards. *Inhal. Toxicol*. **13**(3):233–247.
8. Meklin, T.; Haugland, R.A.; Reponen, T.; Varma, M.; Lummus, Z.; Bernstein, D.; Wymer, L.J.; Vesper, S.J., 2004. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. *J. Environ. Monit*. **6**:615–620.
9. Vesper, S.J.; Varma, M.; Wymer, L.J.; Dearborn, D.G.; Sobolewski, J.; Haugland, R.A., 2004. Quantitative polymerase chain reaction analysis of fungi in dust from homes of infants who developed idiopathic pulmonary hemorrhaging. *J. Occup. Environ. Med*. **46**(6):596–601.
10. Kuhn, D.M.; Ghannoum, M.A., 2003. Indoor mold, toxigenic fungi, and *Stachybotrys chartarum*: Infectious disease perspective. *Clin. Microbiol. Rev*. **16**:144–172.
11. Mahmoudi, M., Gershwin, M.E., 2000. Sick building syndrome. III. *Stachybotrys chartarum. J. Asthma*. **37**:191–198.
12. Gent, J.F.; Ren, P.; Belanger, K.; Triche, E.; Bracken, M.B.; Holford, T.R.; Leaderer, B.P., 2002. Levels of household mold associated with respiratory symptoms in the first year of life in a cohort at risk for asthma. *Environ. Health Perspect*. **110**:A781–A786.
13. Etzel. R.A., 2003. *Stachybotrys*. *Curr Opin Pediatr*. **15**:103–106.
14. Andersen, B.; Nielsen, K.F.; Jarvis, B.B.; 2002. Characterization of *Stachybotrys* from water-damaged buildings based on morphology, growth, and metabolite production. *Mycologia*. **94**:392–403.
15. Dearborn, D.G.,; Iwona, Y.; Sorenson, W.G.; Miller, M.J., 1999. Overview of investigations into pulmonary hemorrhage among infants in Cleveland, Ohio: *Environ. Health Perspect*. **107**(Suppl. 3):495–499.
16. Sudakin, D.L., 2000. *Stachybotrys chartarum*: Current knowledge of its role in disease. *Medscape*, www.medscape.com/viewarticle/408038 (accessed February 2006).
17. Novotny, W.; Dixit, A., 2000. Pulmonary hemorrhage in a infant following 2 weeks of fungal exposure. *Arch. of Ped. and Adolescent Med.* **154**:271–275.
18. Gravesen, S.; Nielsen, P.A.; Iversen, R.; Nielsen, H.F., 1999. Microfungal contamination of damp buildings-examples of risk constructions and risk materials. *Environ. Health Perspect*, **107**(Suppl. 3):505–508.
19. Andersen, B.; Nissen, A.T., 2000. Evaluation of media for detection of *stachybotrys* and *chaetomium* species associated with water-damaged buildings. *Int. Biodeterioration and Biodegradation*. **46**:111–116.
20. Foarde, K.K..; VanOsdell, D.W.; Chang, J.C.S., 1996. Static chamber method for evaluating the ability of indoor materials to support microbial growth, ASTM STP 1287, *Characterizing Sources of Indoor Air Pollution and Related Sink Effects*. ASTM International, West Conshohocken, PA. pp 87–97.
21. ASTM 6329-98, 2003. *Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chambers*. ASTM International, West Conshohocken, PA.
22. Foarde, K.K.; VanOsdell, D.W.; Chang, J.C.S., 1996. Evaluation of fungal growth on fiberglass duct materials for

various moisture, soil, use, and temperature conditions. *Indoor Air*. **6**:83–92.

23. Foarde, K.K.; VanOsdell, D.W.; Chang, J.C.S., 1996. Amplification of *Penicillium chrysogenum* on three HVAC duct materials. *Indoor Air '96: Proc. of the 7th Int. Conference on Indoor Air Quality and Climate*. Nagoya, Japan, **3**:197–202.
24. Chang, J.C.S.; Foarde, K.K.; VanOsdell, D.W., 1995. Assessment of fungal *(Penicillium chrysogenum*) growth on three HVAC duct materials. *Environ. Int*. **22**:425–431.
25. Chang, J.C.S.; Foarde, K.K.; VanOsdell, D.W., 1995. Growth evaluation of fungi (*Penicillium* and *Aspergillus spp*.) on ceiling tiles. *Atm. Environ.* **29**:2331–2337.
26. Foarde, K.K.; VanOsdell, D.W.; Chang, J.C.S., 1995. Susceptibility of fiberglass duct lining to fungal (*Penicillium chrysogenum*) growth. *Proc. of the Air and Waste Manage. Assoc. Specialty Conference, Engineering Solutions to Indoor Air Quality Problems*.
27. Chang, J.C.S.; Foarde, K.K.; VanOsdell, D.W., 1995. Evaluation of fungal growth (*Penicillium glabrum*) on a ceiling tile. in Morawska L.; Bofinger N.D.: Maroni M. (eds): *Indoor Air - An Integrated Approach*. The Int. Soc. of Indoor Air Quality and Climate (ISIAQ), Gold Coast, Australia, pp 265–268.
28. Foarde, K.K.; VanOsdell, D.W.; Menetrez, M.Y., Chang, J.C.S. 1999. Investigating the influence of relative humidity, air velocity, and amplification on the emission rates of fungal spores. *Indoor Air 99*. **2**:507–512.
29. VanOsdell, D.W.; Foarde, K.K.; Chang, J.C.S., 1996. Design and operation of a dynamic test chamber for measurement of biocontaminant pollutant emission and control, ASTM STP 1287, *Characterizing Sources of Indoor Air Pollution and Related Sink Effects*. ASTM International, West Conshohocken, PA. pp 44–57.
30. Foarde, K.K.; VanOsdell, D.W.; Chang, J.C.S., 1997. Effectiveness of vacuum cleaning on fungally contaminated duct materials. *Proc. of the Air and Waste Manage. Assoc. Specialty Conference, Engineering Solutions to Indoor Air Quality Problems* **VIP-75**:325–335.
31. Foarde, K.K.; VanOsdell, D.W.; Myers, E.A., Chang, J.C.S., 1997, Investigation of contact vacuuming for remediation of fungally contaminated duct materials. *Environ Int* **23**:751–762.
32. Foarde, K.K.; VanOsdell, D.W.; Owen, M.K.; Chang, J.C.S., 1997, Fungal emission rates and their impact on indoor air. *Proc. of the Air and Waste Manag.e Assoc. Specialty Conference, Engineering Solutions to Indoor Air Quality Problems* **VIP-75**:581–592.
33. Menetrez, M.Y.; Foarde, K.K.; Webber, T.D.; Dean, T.R.; Betancourt, D.A., 2004 Growth responses of *Stachybotrys chartarum* to moisture variation on common building materials. *Indoor and Built Environ*. DOI: 10.1177/1420326X3043979 **13**:183–188.
34. Menetrez, M.Y.; Foarde, K.K., 2002, Testing antimicrobial efficacy on porous materials. *Indoor and Built Environ*. DOI:10.1159/000066014 **11**:202–207.
35. Foarde, K.K.; VanOsdell, D.W.; Menetrez, M.Y., 2000. Investigation of the potential antimicrobial efficacy of sealants used in HVAC systems. *Proc. of the Air and Waste Manage. Assoc. Specialty Conference, Engineering Solutions to Indoor Air Quality Problems*.
36. Menetrez, M.Y.; Foarde, K.K.; Ensor, D.S., 2001. An analytical method for the measurement of nonviable bioaerosols. *J. Air & Waste Manag. Assoc*. **51**:1436–1442.
37. Menetrez, M.Y.; Foarde, K.K., 2004. Emission exposure model for transport of toxic mold. *Indoor and Built Environ*. DOI: 10.1177/1420326X04041038 **13**:75–82.
38. Sparks, L.E., 1996. *IAQ Model for Windows, RISK Version 1.0, User Manual*, EPA-600/R-96-037 (NTIS PB96-501929), US EPA, Office of Research and Development, Research Triangle Park, NC.
39. Foarde, K.K.; Ensor, D.S.; Menetrez, M.Y. 2000. Indoor/outdoor ratios of biological PM: A preliminary study (Extended abstract). *Proc. Air and Waste Manage Assoc Specialty Conference, PM2000: Particulate Matter and Health - The Scientific Basis for Regulatory Decision-Making*, Charleston, SC, **VIP-94**.
40. Menetrez, M.Y.; Foarde, K.K.; Ensor, D.S., 2000. Fine biological PM: understanding size fraction transport and exposure potential (Poster). *Proc. Air and Waste Manage Assoc Specialty Conference, PM2000: Particulate Matter and Health - The Scientific Basis for Regulatory Decision-Making*,, Charleston, SC, **VIP-94**.
41. Menetrez, M.Y.; Foarde, K.K.; Ensor, D.S., 2000 Comparison of analytical methods for the measurement of non-viable biological PM. *Proc. of the Air and Waste Manage. Assoc. Specialty Conference, Engineering Solutions to Indoor Air Quality Problems*.
42. Menetrez, M.Y.; Foarde, K.K., 2002, Microbial volatile organic compound emission rates and exposure model. *Indoor and Built Environ*. DOI:10.1159/000066016. **11**:208–213.
43. Burge, H.A., 1995 Bioaerosols in Residential Environments. *Bioaerosols Handbook*, Chapter 21. CRC Press, Boca Raton, FL, pp 579–591.
44. Willeke K., Macher J.M., 1999. Air sampling: bioaerosols, assessment and control. American Conference of Governmental Industrial Hygienists, Kemper Woods

Center, Cincinnati, OH. pp 11/1–25.

45. Dean, T.R.; Betancourt, D.; Menetrez, M.Y., 2004. A rapid DNA extraction method for PCR identification of fungal indoor air contaminants. *J. Microbiol Methods*. **56**:431–434.
46. Dean, T.R.; Roop, B.; Betancourt, D.; Menetrez, M.Y., 2005. A simple multiplex polymerase chain reaction assay for the identification of four environmentally relevant fungal contaminants. *J. Microbiol Methods*. **61**:9–16.
47. Dean, T.R.; Kohan, M.; Betancourt, D.; Menetrez, M.Y.; 2005. A simple polymerase chain reaction/restriction fragment length polymorphism assay capable of identifying medically relevant filamentous fungi. *Molecular Biotechnology*. **31**:21–28.
48. Betancourt, D.A.; Dean, T.R.; Menetrez, M.Y., 2005. Method for evaluating mold growth on ceiling tile. *J. Microbiol Methods*. **61**:343–347.

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EPA Project Officer is Marc Y. Menetrez, Mail Drop E305-03, Phone (919) 541-7981, e-mail menetrez.marc@epa.gov

16. ABSTRACT

The report discusses research conducted since 1995 into controlling biological contamination in the indoor environment. The areas that have been addressed are (1) research and development studies to quantify the effects of moisture, relative humidity (RH), and dust and develop risk management alternatives for prevention and control of mold growth; (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials: (3) evaluation of antimicrobial treatments as control technologies; (4) field testing of sealants and encapsulents used in air duct systems; (5) characterization of emission rates and modeling of exposure through heating, ventilating, and air conditioning operation; and (6) improved methods of sampling and analysis of mold. The conclusions resulting from this body of research are summarized and the interrelationships of these areas of investigation in reducing human exposure to biological contamination in the indoor environment are put into perspective. As part of the opening of the new EPA Environmental Resource Center Building in 2002, the EPA's Air Pollution Prevention and Control Division, Indoor Environment Management Branch has established the Biocontaminant Laboratory (BL) for conducting applied risk management research. The BL is a multi-functional, state-of-the-art biological/molecular research laboratory engaged in intra/inter laboratory cooperative research. A description of the BL facility is included.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Air Pollution	Pollution Control	13B
Biological Laboratories	Stationary Sources	14B
Fungi		06C
Stachybotrys chartarum		06F
Penicillium chrysogenum		06M
HVAC		13A
Wallboard		13C

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