

# Research and Development of Prevention and Control Measures for Mold Contamination

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## Key Words

Indoor air · Antimicrobial · Biocontaminant · Duct cleaning · Mitigation

## Abstract

The U.S. Environmental Protection Agency, Air Pollution Prevention and Control Division, Indoor Environment Management Branch has, since 1995, conducted research into controlling biological contamination in the indoor environment. In this paper four areas of research are discussed: (1) research and development of prevention and control measures for mitigation of indoor air pollutants by biocontaminants; (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials; (3) investigation and evaluation of antimicrobial treatments as control technologies to reduce ambient exposure; and (4) field testing of sealants and encapsulents used in air duct systems. The conclusions resulting from this body of research are listed to summarise the accomplishments and put into perspective the interrelationships of these areas of investigation in reducing human

exposure to biological contamination in the indoor environment.

## Introduction

Biological contamination in the indoor environment is recognised as a major health concern [1]. Exposure to airborne biocontaminants or their metabolites can induce responses including irritation, allergy and infection, and acute reactions such as vomiting, diarrhoea, haemorrhage, convulsions and, in some cases, death [1–5]. 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., micro-organisms), the main source reservoirs are the structural and finishing materials and furnishings of the building [6,7]. The application of effective engineering controls within the building is essential to prevent biological pollution in the indoor environment.

It is well recognised that fungi can colonise 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 [6,7].

The U.S. Environmental Protection Agency (EPA), Air Pollution and Control Division (APPCD), Indoor Environment Management Branch (IEMB) conducts ongoing research into biological contamination in the indoor environment. This paper focuses on research conducted during the period of 1995 to the year 2000. The goal of this research was the development of engineering guidelines for the prevention, mitigation and control of biocontaminants in indoor air. The objectives were to: (1) provide a scientific basis for studying material colonization by micro-organisms; (2) conduct research on source management and climate control; and (3) evaluate engineering solutions and control techniques.

### Research Programme

Four areas of research were identified by EPA/APPCD/IEMB for allocation of programme resources: (1) research and development of prevention and control measures for mitigation of indoor air pollutants by biocontaminants; (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials; (3) investigation and evaluation of antimicrobial treatments as control technologies to reduce ambient exposure; and (4) field testing of sealants and encapsulents used in air duct systems. Each of the four research areas is described below.

#### *Research and Development of Prevention and Control Measures for Mitigation of Indoor Air Pollutants by Biocontaminants*

This research included the development and characterisation of the static chamber test method (SCTM), utilising laboratory equipment, materials, and reagents to provide characterised environments, which allow scientific investigations of physical conditions and environmental factors favorable to the promotion of biological contamination in indoor spaces [8]. The SCTM was developed to access potential microbial growth on common building materials. Temperature and relative humidity (RH) are controlled to simulate the desired environmental conditions. Prior to chamber testing, materials can be treated by soaking to simulate a wetting event or treated with an antimicrobial to simulate mitigation practices [8].

With the use of the static environmental chambers developed for the SCTM, three varieties of fiberglass duct liner and ceiling tile materials were evaluated for their ability to support the growth of a fungus *Penicillium chrysogenum* [9]. The results suggest that dust accumulation should be properly controlled in any HVAC duct to prevent the growth of *P. chrysogenum* [9]. Wetting clean samples of HVAC duct liner materials was found not to increase amplification of the *P. chrysogenum* over levels seen without wetting [10]. Soiling duct liner samples with dust accumulated and previously harvested from HVAC systems exhibited a significant association with the growth of *P. chrysogenum* [10]. At moderate soiling levels ( $0.4\text{--}0.7\text{ mg cm}^{-2}$ ), growth occurred on fibrous glass ductboard and flexible ductboard, but not galvanised steel [11]. At heavy soiling levels ( $9\text{--}18\text{ mg cm}^{-2}$ ) growth was seen on all three types of duct liner [11].

The same static environmental chambers were used to study the impact of different levels of moisture and RH on the ability of ceiling tiles to support the growth of *P. glabrum*. Amplification occurred at RHs above 85–90%. Lower RH was demonstrated as effective in controlling fungal contamination on ceiling tiles [12].

Resistance to fungal growth was demonstrated to vary for newly purchased fiberglass duct liner (FDL) inoculated with *P. chrysogenum*. Of the three types of FDL tested, one demonstrated growth after inoculation and six weeks of static chamber isolation at 97% RH; in analogous testing, wetting FDL produced growth on two of the three types of lining, and soiling FDL with dust collected from residential heating and air-conditioning systems caused growth on all three types of lining, one of which contained a fungal biocide [13,14].

The impact of RH, air velocity and surface growth on the emission rates of fungal spores have been measured. The results indicate that emission rates are inversely proportional to RH but directly related to air flow and surface loading [15].

Most of the existing standard test protocol for evaluating antimicrobial efficacy focus on applying the active chemical compound (antimicrobial or biocide). VanOsdell et al. [16] has provided a practical hands-on evaluating protocol that is important to test materials under realistic environmental conditions (i.e., temperature, humidity and soiling). The use of this method enabled the generation 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 *P. glabrum*, *Aspergillus niger*, *A. versicolor* and *P. chrysogenum* [8].

A dynamic microbiological test chamber (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 bioaerosols, the impact of HVAC mechanical system components on biological contaminants, and in-duct tests of air cleaners. The chamber permits for a novel approach to the study of bioaerosol characterisation [16].

Static and dynamic chamber test data were generated under conditions of constant temperature and varying degrees of RH. Micro-organisms (*P. glabrum*, *A. niger*, *A. versicolor* and *P. chrysogenum*) were used to evaluate the extent of biological growth upon building materials of differing moisture content. Wetted, used and new FDL 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 FDL and ceiling tile materials was also shown to be a significant determinant of growth [9–16]. The results showed that emission rates for *A. versicolor* and *P. chrysogenum* are inversely proportional to RH but directly related to air flow rate and surface loading [11–15,17–19].

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

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 galvanised duct surfaces has been conducted, and the emission and transport of fungal spores from the surface of the contaminated duct materials has been evaluated. 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 and summarised below [9–16]. To facilitate

biological research on duct materials, the design and construction of the static and dynamic chambers, and the development of methods of testing microbial growth under constant temperature and RH, and conditions of static or dynamic air movement was executed [8,9,16]. The evaluation of fungal growth on FDL and ceiling tiles were discussed above. 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.

#### *Investigation and Evaluation of Antimicrobial Treatments as Control Technologies to Reduce Ambient Exposure*

Biological contaminants are known to be indoor air pollutants which carry a substantial health risk with exposure [1–6]. Biocontaminants and their fragments make up a component of airborne particulate matter (BioPM) [19], addressed by Menetrez et al. [20], in the size range of 0.3–10.0 µm. 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 [1–6]. 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 and with antimicrobial treatments as the main control technology. The overall objective of this research was to evaluate the sample collection and analysis of BioPM and to investigate antimicrobial efficacy as a technology for controlling exposure [19,21].

The importance of minimising exposure to BioPM of indoor origin in the indoor environment is well established. However, the importance of minimising exposure to BioPM in the ambient fraction of particulate matter with aerodynamic diameters < 2.5 µm (PM<sub>2.5</sub>) 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 unrecognised causative agent for adverse health effects.

Biological agents potentially play two distinct and important roles in influencing the adverse health effects that have been associated with PM<sub>2.5</sub> 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  $\mu\text{m}$  (*Bacillus* spp., *Pseudomonas* spp., *Xanthomonas* spp. and *Arthrobacter* spp.), and sub-micron fragments of these gram-negative organisms can contain toxins which 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 [20,22,23].

The evaluation and control strategy of BioPM had been complicated by the lack of methods that will allow us to assess quantitatively the BioPM fraction of  $\text{PM}_{2.5}$ . 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 quantify scientifically the relationship between indoor and outdoor levels of PM aerosols [20,23,24].

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 indoor and outdoor air were collected over a 5-week period from three local sites. Then, the samples were analysed for total protein, ragweed and fungal antigens  $\beta$ -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 optimisation [20,24,25].

#### *Field Testing of Sealants and Encapsulants used in Air Duct Systems*

Three different commercially available biocidal encapsulants/sealants were monitored after being applied to fiberglass duct liner surface that was contaminated with mold and cleaned. The field experiment was conducted in the EPA test house, Cary, NC. Participating members of the National Air Duct Cleaners Association rotary-cleaned and spray-coated the fiberglass duct liner in the trunk-lines of the EPA test house, according to the manufacturers specifications, 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 heating, ventilating and air-conditioning (HVAC/HAC) system located in a residential crawl space. During the cooling season, the HVAC/HAC system was cool and had a high humidity when on (especially where condensate flow was constant, in the area of the cooling coils and drip pan, where the air remained near saturation) and some intermediate condition when off. The results suggest that dust and high humidity should be properly controlled in any HVAC duct to prevent the growth of *P. chrysogenum* [10].

## **Discussion**

The significant technical findings of the research discussed within this paper include:

- Establishing that SCTM was adapted into ASTM Standard 6329-98, "Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chambers" [8]. 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.
- Determining that water incursion or standing water is not required for growth on materials [10–13]. 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 [11–13].
- Confirming that, under equilibrium conditions, RH and moisture content correlate well with mold growth (depending on the moisture requirements of the test organisms) [11,12]. However, under non-equilibrium conditions, mold growth correlates better with increasing moisture content than with relative humidity [11–15].
- Finding that variations in the characteristics of alike building materials can impact the fungal resistance of that material [10–14,21]. Both new and used materials are capable of supporting mold growth, but generally used materials (soiled) were more susceptible [12–14].
- Establishing that reducing the moisture content of wet materials (within 3 days) before fungal growth became established provided effective source management [11,15]. However, established microbial



growth may continue even after the moisture content of a particular material is lowered below that required to initiate growth [15].

- Development of a method for artificially soiling materials [9]. Allowing fungal growth characterisation or antimicrobial efficacy testing methods to simulate realistic environmental conditions will result in laboratory experiments which more closely resemble real-world applications [9].
- Development of the Dynamic Microbial Test Chamber (DMTC), a room-sized dynamic chamber, designed and constructed under a co-operative agreement between EPA and the Research Triangle Institute (RTI), that can test ducts (mini-ducts) scaled to simulate duct velocities and duct materials in the mini-duct apparatus [16].
- 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 modelled using RISK IAQ Model for Windows [19]. The modelled 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 [19].
- Acutely impacting control and remediation practices by showing that RH is inversely related to fungal spore emissions [9,12,19]. 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 [19].
- Confirming that fungal growth is intrusive throughout porous materials and that guidelines which recommend discarding microbially contaminated porous duct material should be followed [17–19]. Mechanical cleaning by 'high-efficiency particulate arresting' (HEPA) air-vacuuming was able, at best,

to reduce embedded fibers soiling and temporarily decrease fungal levels. These fungal populations experienced re-growth within six weeks [18,19].

- Demonstrating that significant fractions of fine particulate matter in the indoor and outdoor environments are biological in origin [10,23,24].
- Finding that significant variation in antimicrobial efficacy of encapsulants to limit or eliminate biological growth indicates a need for widespread product testing and for the development of an efficacy testing protocol [21].

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

## Conclusions

The four areas of research identified by EPA/APPCD/IEMB for allocation of programme resources were: (1) research and development of prevention and control measures for mitigation of indoor air pollutants by biocontaminants; (2) duct cleaning effectiveness for prevention and control of microbial growth on duct materials; (3) investigation and evaluation of antimicrobial treatments as control technologies to reduce ambient exposure; and (4) field testing of sealants and encapsulants used in air duct systems. These areas of research were investigated, and the most significant findings are summarised 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 and the static and dynamic microbial test chambers for determining antimicrobial efficacy, and determining the most effective technique to handle and mitigate contaminated materials will ultimately improve the ability to control biological contaminants and to reduce human exposure.

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**Marc Menetrez**

06/18/2003 12:47 PM

To: Michael Sarles/RTP/USEPA/US@EPA

cc:

Subject: Re: The R & D Of Prevention and Control Paper

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**Michael Sarles**

06/18/03 09:47 AM

To: Marc Menetrez/RTP/USEPA/US@EPA

cc:

Subject: The R & D Of Prevention and Control Paper

Marc, I presume this is a journal article. I'd like to know what journal you're shooting for so I can use the proper editorial guidelines.

Mike



**Marc Menetrez**

06/23/2003 08:41 AM

To: Michael Sarles/RTP/USEPA/US@EPA

CC:

Subject: Re: Your R&D into Mold Paper

Mike:

This paper has already gone through the process. It had peer review, and everyone has already signed off (including Wellon, a few years ago). A rewrite was done, and I wanted as a courtesy to send it by you. You already have every form and number.

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06/20/03 09:19 AM

To: Marc Menetrez/RTP/USEPA/US@EPA

CC:

Subject: Your R&D into Mold Paper

Are there OMIS and GPRA numbers associated with this work? (I'm doing the paperwork now.)

Mike