Generation of Carbon Dioxide and Mobilization of Antimony Trioxide
by Fungal Decomposition of Building Materials

by

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For their love, support, patience and understanding throughout this endeavor, I dedicate this work to my family, daughter, and most of all, my loving wife.
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Generation of Carbon Dioxide and Mobilization of Antimony Trioxide by Fungal Decomposition of Building Materials

John D. Krause

Abstract

Fungal contamination of buildings poses numerous challenges to researchers, building owners and occupants. Public health agencies promote prevention and remediation of mold and water damage, but lack sensitive methods to detect hidden mold growth and a complete understanding of the biological mechanisms that make occupying moldy buildings a hazard. The widespread use of the fire retardant antimony trioxide (Sb₂O₃) on building materials and furnishings makes it inevitable that mold growth on treated materials will occur in some buildings with water damage. Several authors have speculation that microbial growth on materials treated with antimony trioxide could mobilize antimony through a volatile intermediate, trimethylstibine.

The purpose of this study was to determine if fungal growth on a commonly used building material that contains antimony trioxide, fiberglass ductboard, results in the mobilization and release of antimony compounds. Additionally, CO₂ generation rates
were determined during fungal growth on fiberglass ductboard and gypsum wallboard.

Results demonstrated a significant reduction of antimony concentration in fiberglass ductboard after fungal growth had occurred. Antimony emission rates and resulting concentrations of antimony oxide aerosols were estimated using an indoor mass balance mathematical model. Concentrations of CO$_2$ were also modeled within a wall cavity and static HVAC ducts to determine if fungal growth could elevate CO$_2$ levels above ambient concentrations.

Although volatile phase antimony was not detected in chamber experiments, probably due to rapid oxidation and high humidity, mobilization of antimony trioxide from fiberglass ductboard components was demonstrated in several experiments. Indoor Air modeling of a residence suggest that concentrations of antimony could, under worst case conditions, exceed the reference concentration (RfC) of antimony trioxide by 10 to 1,000 times. These results suggest that biomethylation and mobilization of antimony by mold growth on building materials could result in elevated occupant exposures to antimony compounds. Antimony is a known respiratory irritant that can be similar to arsenic in its toxicity.

Modeling results also suggest that elevated carbon dioxide concentrations due to fungal metabolic respiration are variable and dependent on environmental conditions. Measuring elevated carbon dioxide concentrations to detect hidden fungal growth was determined to not be a predictive assessment tool.
INTRODUCTION

Indoor mold contamination has been the subject of recent interest from the news media, legislators, insurance companies and homeowners. Mold exposures have been a subject of concern for U.S. public health agencies, including the Centers for Disease Control and Prevention (CDC), the National Institute for Occupational Safety and Health (NIOSH) and the U.S. Environmental Protection Agency (EPA) since the early 1990’s (EPA, 1992; Macher, 1999). The Bioaerosols Committee of the American Conference of Governmental Industrial Hygienists (ACGIH) defines

“biological contamination in buildings as the presence of (a) biologically derived aerosols, gases, and vapors of a kind and concentration likely to cause disease or predispose persons to adverse health effects, (b) inappropriate concentrations of outdoor bioaerosols, especially in buildings designed to prevent their entry, or (c) indoor biological growth and remnants of growth that may become airborne and to which people may be exposed” (Macher, 1999 p.1-1).

Due to the dearth of information on exposure and disease associated with living and working in indoor environments with fungal growth, regulatory action has been slow and inconsistent (IOM, 2004).
Fungi colonize wet building materials in the indoor environment causing material degradation, volatile organic compound (VOC) production, and release spores and hyphal fragments containing potent allergens and respiratory irritants (EPA, 2001; Gorny et al., 2002; Macher, 1999). Some physicians and building occupants claim that exposure to indoor fungi results in a myriad of symptoms and diseases (Fung & Hughson, 2003; Hodgson et al., 1998; Johanning et al., 1996). However, exposure to ubiquitous fungal spores and hyphae in the outdoor environment is unavoidable (Burge, 1995; EPA, 1992). Many people inhabit buildings with extensive mold growth and do not experience recognizable adverse health effects, while others seem to react severely. The specific allergens, irritants or toxins responsible for occupant reactions to fungi are under study, but no consensus has been reached on which agents are responsible for the reported symptoms. Outdoor air spore concentrations typically exceed indoor building concentrations with and without a history of water damage (Horner et al., 2004; Shelton, et al., 2002). Median outdoor fungal concentrations were 6 to 7 times higher than indoor levels in an analysis of over 12,000 air samples collected throughout the United States (Shelton, et al., 2002). Some emphasis has been made on the hazard that certain fungal species pose when growing indoors. However, no consensus has been reached on whether certain species pose a special hazard. Public health professionals examining the subject have advocated that indoor mold growth is a public health risk, irrespective of the species present (EPA, 2001; IOM, 2004; Macher, 1999; NYC DOH, 2000; OSHA, 2003). This conservative guidance attempts to protect all building occupants, but does not resolve the biological mechanisms contributing to occupant illness.
Modern buildings can suffer from water damage resulting in mold growth. Much of this damage occurs within wall cavities and hidden spaces such as heating, ventilation, and air conditioning (HVAC) ducts. Fungal bioaerosols generated within interstitial wall cavities can migrate into occupied areas of a building (Macher, 1999). HVAC ducts can spread airborne contaminants to the occupied areas exposing occupants to fungal bioaerosols generated in these hidden spaces (EPA, 1995). Currently there are no measurement methods to detect fungal growth present within hidden cavities that have been validated. Detecting hidden mold growth early could provide an important tool for protecting public health. Researchers have speculated that volatile emissions from mold growth may cause occupant illness. Gao et al. have conducted research on the volatile organic compounds produced by fungal growth and determined the concentrations within a building to be small, but possibly useful in detecting hidden mold growth (Gao et al., 2002; Macher, 1999). However, little information is available on volatile emissions of metalloids due to mold growth on building materials. The hazard from fungal growth on building materials known to contain toxic metalloids has been essentially ignored since the early 1900’s.

Mold growth on building materials treated with the flame retardant antimony trioxide (CAS 1309-64-4; Sb$_2$O$_3$), such as fiberglass ductboard, is inevitable in modern buildings. The increased use of synthetic materials to insulate and construct buildings has resulted in the greater use of fire retardants. Research on the biomethylation of antimony (Sb) by microorganisms has demonstrated that fungal and bacterial growth can mobilize and release antimony as a toxic, volatile gas called trimethylstibine (Gürleyük et
al., 1997; Jenkins et al., 1998a). These findings raise the concern that mold growth on flame retardant-treated building materials may release antimony as a toxic gas, which rapidly oxidizes into less toxic, but still hazardous, particulate matter. Occupant exposure to antimony resulting from fungal decomposition of fire retardant treated building materials has not been previously studied. One purpose of this research study was to examine the possibility of antimony mobilization from a commonly used building material that often supports fungal growth when water damaged.
THE PARADOX OF MOLD EXPOSURE AND HEALTH EFFECTS

A paradox is defined as an apparent contradiction with common sense. Public health organizations recognize exposure to fungi, spores, volatile organic compounds and fungal products as a human health risk (EPA, 1992; IOM, 2004; Macher, 1999). The ubiquitous nature of fungi in the outdoor and indoor environments, in food, and industry highlights the paradoxical nature of this concept. Fungi constitute up to 25% of the biomass on this planet (McNeel and Kreutzer, 1996). Fungi are the primary decomposers in the environment, breaking down leaves, grass cuttings, garden compost, trees, and organic material in landfills (Burge, 1997). Comparatively, fungi contribute more biomass to soil than all other microorganisms. Many fungi are saprophytes, obtaining nutrition from dead plants and animals, contributing to decomposition and recycling of nutrients (Deacon, 1984; Kendrick, 1985). The earth would be a different place if fungi did not exist. Fungi are used to produce certain chemicals and flavoring agents used in household products and foods. Foods like cheese, beer and wine are dependent on fungi and yeast for their production and unique flavors (Burge, 1997; EPA, 1992; Kendrick, 1985).

When fungal growth is allowed to go unchecked, and certain environmental conditions exist, it can become a hazard to plants, animals, humans and buildings. Many common and exotic plant pathogens are fungi. Stored grains typically contain some small
amount of fungal components, and can be spoiled if fungal growth takes place. Some of
the most common infections in animals and humans are due to fungi, such as "athlete's
foot" and the dermal disease “ringworm” or Tinea Corporis. When the immune systems
of animals and humans are compromised they can succumb to debilitating or lethal
fungal infections. Certain fungal spores can cause opportunistic infections in
immunocompromised individuals (Fung and Hughson, 2003). Ingestion of toxic
metabolites produced by some fungi growing on grains and feeds, by animals or humans
can result in a variety of diseases. Epidemics of ergotism, known also as St. Anthony’s
fire, experienced by Europeans in the Middle Ages were due to ingestion of grains and
bread containing mycotoxins. Ergotism results in the loss of peripheral circulation,
gangrene, and death (EPA, 1992). Widespread equine epidemics in Poland, Hungary and
Russia in the early 1900’s killed thousands of horses from ingestion of hay contaminated
with *Stachybotrys chartarum*. This fungus was found to produce a series of potent toxins
called trichothocenes that resulted in the death of horses that fed on the hay (Lacey,
1985).

Many fungi can produce colonies that are visible to the unaided eye, often making
their growth on water damaged materials obvious. The health risk from indoor fungal
growth was recognized in ancient times and discussed in the Bible (Leviticus Chapter 14,
33-48), where a reference is made to mold growth in a home and measures to clean or
discard all affected materials are described. Not visible to unaided eye are the
bioaerosols they produce, but the odors they sometimes emit can be perceived.
Accurately measuring fungal bioaerosols and determining the biologically active components have been evasive goals for researchers and public health professionals. So far researchers have been unable to reach a consensus on useful biomarkers of exposure to environmental fungi in humans.

Our world would not be the same without fungi, but a variety of diseases and adverse health outcomes can be attributed to fungi. It is apparent that when fungi are provided an environment they can exploit, they can grow out of control and harm plants, animals, people, and buildings. The environmental conditions that enable fungi to grow are well documented, but the specific etiologic agents that may cause allergies, irritation and numerous non-specific symptoms in people have not been elucidated.
LITERATURE REVIEW

Health Effects of Exposure to Fungi

Evidence suggests that exposure to many fungal products can result in adverse health effects and decreased quality of life. In general, fungal aerosols and other bioaerosols found in damp environments, can cause allergy, infection, irritation, and toxicity. Determining the specific cause of these health effects has been impossible due to the presence of numerous microorganisms and bioaerosols in damp indoor environments. Chronic and/or repeated exposure to fungi has been associated with allergy, hypersensitivity pneumonitis, asthma symptoms in sensitized asthmatics, upper respiratory tract symptoms, wheeze and cough. Allergy and hypersensitivity disease are IgE-mediated immune responses to specific allergens, while hypersensitivity pneumonitis (HP) is an IgG and T cell-mediated response (EPA, 1992; IOM, 2004).

Allergies in genetically predisposed people can result from exposure to the antigenic components of fungi. From a study of workers in U.S. office buildings, the overall self-reported prevalence of physician-diagnosed allergy to mold was 22%, with 18% in male respondents and 24% in female respondents (n = 2,435) (Crandall & Sieber, 1996b).
Asthma attacks can be initiated in people with allergic asthma and sensitivity to fungi (Fung and Hughson, 2003). The increased prevalence of asthma in the United States, and other industrialized countries, (CDC, 1998) has been speculated to be connected with indoor air pollution. Data clearly indicate that exposure to fungi plays a role in asthma. Several studies have documented the sensitizing potential of fungal allergens and relate the existence of asthma to the participants’ sensitization. Although the number of studies is too few, there are data that support a relationship between fungal allergen sensitization and symptoms of asthma. In 2000 the Institute of Medicine Committee on the Assessment of Asthma and Indoor Air concluded there was sufficient evidence to demonstrate that exposure to fungi could exacerbate symptoms in sensitized asthmatics. However, sufficient evidence was not available to determine if exposure to fungi caused the development of asthma (IOM, 2000). As more data becomes available about damp indoor environments, exposure to the microorganisms and the chemicals present, a clearer understanding should evolve.

The underlying causes for the drastic increase in asthma prevalence throughout the United States are still unknown. Self-reported prevalence of asthma increased 75% from 1980 to 1995 for the US population as a whole. This equates to a 5% increase per year, with the greatest increase among children age 0-4 years. This age group increased 160%, from 22.2 per 1,000 to 57.8 per 1,000 (CDC, 1998). The underlying cause of this drastic increase is unknown, but many have speculated it to be related to degraded indoor air quality. Some researchers have speculated the increased asthma prevalence is due to fungal contamination of indoor environments, while many others point to the overall
microbial burden present in damp indoor environments. Regardless of the speculation so far no consensus has been reached in light of the inconclusive and contradictory studies (IOM, 2000; IOM, 2004).

Speculation has been made about the role of mycotoxins produced by fungi growing indoors. Many fungi can produce secondary metabolites that are classified as “mycotoxins”. It is estimated that the number of fungal species capable of producing mycotoxins range from 100-150. Over 200 different mycotoxins have been described (EPA, 1992). Toxigenic fungi and their spores are ubiquitous, but documented cases of mycotoxicosis have been primarily due to extremely wet conditions and mold growth on specific materials (EPA, 1992; Sharma and Salunkhe, 1991). Mycotoxins are found on and in the spores of fungi that produce them, on the hyphae and in the dust from substrates where they have grown. Though not considered volatile, some mycotoxins have been shown to migrate into the substrates that mold grow on (IOM, 2004). Mycotoxins range in their toxicity and effects, from mild acute toxicity to potent carcinogenicity. Most mycotoxins are cytotoxic, resulting in cell death (EPA, 1992).

Concerns over the role mycotoxins may have played in a cluster of infant deaths due to acute idiopathic pulmonary hemorrhage (AIPH) arose after reports of a CDC investigation. Investigators initially concluded that an association existed between infant AIPH and exposure to the toxigenic fungus Stachybotrys chartarum, but later review by an external task force dismissed the association due to a lack of substantiating data (CDC, 1994; CDC, 2000; Montana et al., 1997).
Associations between exposure to mycotoxin-containing fungi and occurrence of disease in building occupants have been rare. The vast majority of reported illnesses due to mycotoxins have been due to ingestion of contaminated food or in agricultural settings when grain handlers have been exposed to dense clouds of dust from contaminated grains. A critical review of the literature by Fung and Hughson concluded that mycotoxin levels in most mold-contaminated buildings are not likely to result in a dose sufficient to cause measurable health effects (Fung & Hughson, 2003; Robbins et al., 2000).

Researchers have been unable to demonstrate a dose response relationship between toxin-containing spores and occupant illness, in part due to the inadequacy of measurement and analysis methods for exposures to fungal aerosols (i.e. spores, allergens, irritants, toxins and volatile metabolites) (Canadian Public Health Association, 1987; Cohen & Hering, 1995; Dales et al., 1997; EPA, 1992;). The ideal measure of exposure to fungal aerosols is personal sampling of the breathing zone, but until recently, personal samplers were not available to collect bioaerosols. Many drawbacks have been identified with area samples used to measure fungal spore exposures. In addition to the bias introduced by stresses placed on the spores during capture, desiccation and culture onto agar media, contribute to underestimating fungal spore concentrations (Macher, 1999). Studies have also demonstrated the presence of a “personal cloud” created by building occupants. Air concentrations of particles smaller than 10 μm (PM$_{10}$) measured in the breathing zones of subjects wearing personal particle monitors were compared to concurrent results from area samples. Results indicated that area samples underestimated
personal exposures to PM$_{10}$ by 50%. Most fungal spores are included in this size range of particles (EPA, 1992; IOM, 2000; Ozaynak et al., 1996).

The etiologic agents that may cause the myriad of symptoms described by various physicians, researchers and building occupants in water damaged buildings have not been determined. Some of the unsubstantiated symptoms claimed to be due to mold exposures including dyspnea (shortness of breath), airflow obstruction, mucous membrane irritation, inhalation fevers, lower respiratory illness, rheumatologic immune diseases, acute pulmonary hemorrhage in infants, skin symptoms, development of asthma, gastrointestinal disorders, fatigue, neuropsychiatric symptoms, and cancer. Despite case studies, anecdotal reports and media hype, researchers and public health agencies have not found a link between these symptoms and exposure to moldy indoor environments (IOM, 2004).

Despite the lack of studies and conclusive data, there is sufficient evidence in the literature that upper respiratory tract symptoms, exacerbation of asthma, wheeze, and cough are associated with both exposure to fungi in damp environments and exposure to damp environments in general. Exposure to damp environments, with or without the presence of visibly detectable mold, appears to carry similar health risks. Increased odds ratios for asthma-related health effects associated with dampness indicators were reported in at least thirteen studies published between 1993 and 1999. The symptoms described as “asthma-related” included wheeze, persistent cough, bronchial obstruction and asthma. Despite finding a positive association between these symptoms and exposure to damp
environments the committee recognized the likelihood of multiple exposures to bacteria, fungi and dust mite allergens (IOM, 2000).

Until studies with greater power are performed, that are capable of quantifying occupant exposure to the causative agents of illness, an association will be difficult to determine, if one truly exists. Adding to the difficulty in differentiating between the effects of multiple exposures to bioaerosols in damp environments, researchers must account for exposure to ubiquitous fungal spores present in the outdoor environment. Indoor spore concentrations are typically lower than outdoors, even in some buildings with visible mold growth (Horner et. al., 2004; Macher, 1999; Shelton et al., 2002). Humans have been exposed to fungi throughout history and are constantly ingesting and inhaling mold and their spores without detectable adverse reaction. Is it possible that exposure to fungal bioaerosols produced by mold colonies growing indoors poses a greater risk than outdoor fungal bioaerosols?

The guidance currently provided by public health agencies including the CDC, US EPA, ACGIH, AIHA, OSHA, NIOSH and numerous state and city health departments is that indoor mold growth poses an unacceptable health risk and should be remediated immediately, regardless of the species present (AIHA, 2001; EPA, 2001; IOM, 2004; NYC DOH, 2000; OSHA, 2003). Guidance for precautions and the extent of remediation necessary to protect building occupants and remediation personnel is only now under development (NIOSH, 2002). The methods of remediation and post-
remediation conditions that constitute a successful and effective remediation are still under debate.

Studies of human populations living and working in buildings with fungal contamination (i.e. growth) have not demonstrated consistent correlations between measures of airborne spores or amounts of fungal growth, and the frequency or severity of occupant illness (IOM, 2004; Macher, 1999). The inability to detect correlations between airborne spore concentrations and health outcomes or occupant complaints is in part due to the inadequacy of air sampling instruments and analytical methods. The inconsistency of findings and the inability to determine a dose-response curve or threshold level of exposure has frustrated researchers and public health agencies.

Consensus is that human exposure to indoor fungi is predominantly from fungal spores via the inhalation route. The outdoor concentrations of these spores can vary by four orders of magnitude on a daily basis and indoor environments often have lower spore concentration measurements than outdoors, even in buildings with extensive fungal growth (European Collaborate Action, 1993; Horner et al., 2004; Shelton et al., 2002). For example, extensive sampling (n = 476) of an office building over 14 months did not reveal elevated fungal levels even though fungal contamination was found in the air ducts (Burge et al, 2000). If exposure to fungal spores were the sole cause of adverse health effects, and spore concentrations indoors are lower than outdoors, why should people suffer ill effect?
A guide post frequently described in the literature is comparison of indoor versus outdoor air concentrations and the rank order of species. This comparison initially seems relevant because fungi are naturally occurring organisms, most likely originating from outdoor sources that enter a building via natural or mechanical ventilation (Baughman & Arens, 1996; EPA, 1992). The assumption made is that if indoor spore concentrations are higher than outdoor concentrations of the same species, or if the biodiversity of species is shifted in the indoor environment, then an indoor reservoir is likely present. While this approach can sometimes help an investigator identify an area with mold damage, it cannot be used to demonstrate the absence of an indoor reservoir of fungal growth. Because of the temporal variability and infrequency of spore release from indoor reservoirs, air sampling from indoor environments can result in false negatives. The probability of air sampling methods not detecting the presence of fungal growth when it truly exists, also known as the method specificity, has not been reported for existing methods (Dillon et al., 1996).

The indoor to outdoor ratio approach is based on an assumption that exposure to aerosols from indoor fungal growth poses the same risk as exposure to aerosols from outdoor fungal growth. That is fungal growth occurring outdoors, in soil and on decaying leaves and trees, releases spores with the same composition, allergenicity and toxicity as fungal growth occurring on synthetic, treated and preserved man-made materials. If this assumption does not prove to be true and exposure to spores, hyphal fragments and volatile organic compounds (VOCs) from indoor fungal growth, poses a different risk
than exposure to outdoor fungal aerosols, then researchers may need to reconsider the relevance of the indoor to outdoor comparison.

Research at the University of Cincinnati has revealed that in addition to fungal spores, sub-micron size hyphal fragments can be released from colonies of fungal growth. These fragments are typically smaller than 1 µm in diameter and are probably not viable, meaning they cannot form a colony (Gorny et al., 2002). These two properties have significance in that microscopic analysis of spore-trap samples, taken to measure total spore concentrations indoors, cannot resolve or identify particles less than 1 µm, essentially making these hyphal fragments undetectable by conventional spore trap analysis methods. Because these fragments are not whole cells they are not likely to be viable. Commonly used impaction plate methods used to capture and culture viable spores cannot detect the presence of non-viable fragments. Until this report by Gorny, et al. the existence and magnitude of this fungal aerosol component was unrecognized by researchers, scientists and public health professionals performing building assessments.

This initial research on hyphal fragments also examined the allergenicity of these sub-micron hyphal fragments. The researchers concluded that fungal fragments are released independently of spores, with the number of fungal fragments exceeding spores by 2-3 orders of magnitude. These fungal fragments exhibited 2 to 5 times greater immunological reactivity than spores in monoclonal antibody assays. These data suggest fungal fragments may have greater allergenicity than spores and their contribution to occupant allergic reactions may be greater (Gorny et al., 2002). This research also
suggests that much of the surrogate exposure data (i.e. total and viable spore concentrations in air) may be missing a significant component of fungal bioaerosols.

The overall confusion and complexity of the exposure-dose-response paradigm may be due more to a lack of “resolution” than inconsistency of data. When the overall subject of “Damp Indoor Spaces and Health” was examined in a recently published report by the National Academy of Sciences Institute of Medicine, a very clear association was recognized between exposure to damp indoor spaces and increased health risks. However, because of inconsistent research methods and measures of “damp” environments and fungal exposures, a causal effect could not be ascertained from the numerous studies reviewed. The committee did find sufficient evidence in the literature that exposure to damp indoor environments was associated with upper respiratory tract symptoms, cough, wheeze and asthma symptoms in sensitized asthmatic persons. A similar association with visible mold was found, but it was recognized that other organisms and chemical agents could be contributing to the symptoms and illnesses reported (IOM, 2004).

Using a broad base of numerous epidemiological studies, the IOM committee recognized an association between the presence of fungal growth and certain health effects, but due to the lack of exposure data, quantifiable biomarkers and consistent findings, a causal agent was not identified. Ironically, visible signs of indoor mold growth were more predictive of adverse health outcomes than measured indices of exposure such as airborne spore concentrations. This suggests two possibilities that are
not mutually exclusive. One is that other organisms that thrive in damp environments and chemicals released when materials become wet may be contributing to or causing the reported symptoms and illnesses. The other possibility is that exposure to other fungal aerosols, besides spores, may be the causative agent. In light of the recently described fungal hyphal fragments (Gorny et al., 2002), the possibility of one or more un-measured fungal aerosol contributing to adverse health effects seems to exist.

**Prevalence of Mold Growth in Buildings and on Building Materials**

The prevalence of mold growth in U.S. buildings has not been fully reported. How much mold growth constitutes a health hazard is also unknown. In the 1970's and 1980's microbial contamination was identified as the primary cause for poor air quality in only 5% of 529 indoor air quality (IAQ) investigations conducted by the National Institute for Occupational Safety and Health (NIOSH); The remaining 95% were attributed to inadequate ventilation, entrainment of outdoor air contaminants, contaminants in building fabric and unknown sources (Crandall & Sieber, 1996a). Assessment of 104 building evaluations in 1993 revealed many conditions that can support mold growth were identified such as poor condensate pan drainage (16%), dirty HVAC system components (36.5%), and water–damaged duct liner (1%). The findings of this NIOSH study suggested that poor maintenance of HVAC systems related to building occupant complaints. Poor maintenance of HVAC systems can lead to proliferation of microbial contamination resulting in the HVAC systems becoming a contaminant source for occupied spaces of the building (Crandall & Sieber, 1996a). However, in the last 10 years, microorganisms were determined to be the primary source
of indoor air contamination in as many as 35-50% of IAQ cases (Lewis, 1994). Current
trends in occupant complaints and public awareness suggests the problem may be
widespread, especially after seasonal storms, floods and hurricanes that cause both
structural damage to buildings and introduce the necessary water for mold spores to
germinate and grow. There is currently no monitoring program to track either the
number of homes, schools or workplaces with mold growth or complaints.

Mold growth on two of the major building materials currently used, gypsum
wallboard and fiberglass ductboard, has been well documented (Price et al., 1994;
Samimi and Ross, 2003; Van Loo et al., 2004; Reeslev et al., 2003; Hodgson et al., 1998;
Doll, 2002; Flappan et al., 1999; Johanning et al., 1996). Studies have shown that soiled
building materials are more susceptible to mold growth at lower moisture levels than new
materials (Chang et al., 1995; Gravesen et al., 1999; Samimi et al., 2003). The key
determinant of fungal growth on building materials is water availability, although
nutrients, pH, oxygen, and temperature can also effect fungal growth (Doll, 2002).

**Quantifying Mold Growth Using β-N-acetylhexosaminidase Activity**

Quantifying mold growth on building materials is impractical using most standard
mycological methods. One variable is the surface area of affected material, but the
density of mold growth can also vary drastically. An ideal indicator of mold growth is
biomass density. Using the total surface area of growth and mold biomass density, the
amount of mold biomass present in a building could be estimated.
Measuring the dry weight (i.e., mass) of laboratory cultures is sometimes performed, but samples must be handled carefully. Measuring the mass of samples taken from building materials cannot differentiate between mold biomass and other non-mold constituents. Instead, measurements of chemical indicators such as ergosterol or β-N-acetylhexosaminidase enzyme activity have become recognized as useful surrogates of mold biomass. Both of these markers are believed to be common to all known filamentous fungi, but some inter-species variability exists (Nielsen and Madson, 2000; Reeslev, et al., 2003; Schnurer, 1993).

Ergosterol has been used to monitor and estimate mold biomass in seeds, grains, decaying wood and building materials. The analysis of samples for ergosterol requires extensive sample extraction and clean-up along with laboratory analysis by gas chromatography/mass spectrometry (GC/MS), high performance liquid chromatography (HPLC) or spectrophotometry (Nielsen and Madson, 2000; Schnurer, 1993).

In general, there has been demonstrated a good correlation between ergosterol content, the degree of infestation assessed by visual inspection, inspection under a stereo microscope, and the measurement of β-N-acetylhexosaminidase enzyme activity. A correlation between β-N-acetylhexosaminidase enzyme activity and ergosterol measurements was reported by Nielsen and Madson, with $r^2 = 0.75$ (n = 54). Both methods were able to detect mold growth on samples before gross visible signs of colonization were observed (2000). Other studies have reported that mold biomass density correlated well with both ergosterol content ($r^2 = 0.968; P<0.001$) and β-N-
acetylhexosaminidase enzyme activity ($r^2 = 0.968$; $P<0.001$) (Miller, et al 1998; Reeslev et al., 2003). A separate study compared $\beta$-N-acetylhexosaminidase enzyme activity with microscopic analysis results on both new and contaminated building materials. The ability of enzyme activity to detect fungal growth was compared with results of microscopic analysis using paired samples. The enzyme activity method was estimated to have a sensitivity of $\sim$89%, a specificity of $\sim$100%, a positive predictive value of $\sim$100% and a negative predictive value of $\sim$95% (Krause et al., 2003).

As a quantitative measure of fungal biomass, enzyme activity demonstrated a similar capability to ergosterol measurement. For specific strains of *Stachybotrys chartarum* (IBT 9695) and *Aspergillus versicolor* (IBT 16000) conversion factors were determined from experiments performed on agar medium. The study found a linear correlation between $\beta$-N-acetylhexosaminidase activity and the actual biomass density measured by weighing the mold growing on agar plates covered with cellophane. The conversion factors (CFs) were used to estimate the biomass density of molds grown on gypsum wallboard. The biomass densities estimated from ergosterol content and $\beta$-N-acetylhexosaminidase activity data gave similar results. Not surprisingly mold growth on gypsum wallboard showed significantly slower growth and lower stationary phase biomass density than on agar. The CF for *Stachybotrys chartarum* (IBT 9695) was reported as 8,275 fluorescence units per mg of biomass per cm$^2$. The CF for *Aspergillus versicolor* (IBT 16000) was reported as 12,370 fluorescence units per mg of biomass per cm$^2$. Because the conversion factors were determined from colonies grown on agar medium, the biomass may not be representative of fungi grown on other substrates such
as gypsum wallboard or fiberglass ductboard, limiting the accuracy of biomass density estimates. Despite the recognized limitation in very accurately estimating biomass density, enzyme measurements yielded the same information on biomass as ergosterol measurements over the time course of fungal growth in a study of the two species described above (Reeslev et al., 2003).

The existence of enzyme activity to biomass density conversion factors for *Stachybotrys chartarum* (IBT 9695) and *Aspergillus versicolor* (IBT 16000) was one of the factors for choosing these two fungi. By using isolates from these two species the conversion factors to estimate fungal biomass would have greater applicability. The other factor for choosing these two fungal species was that they have both been described in reports as growing on water damaged building materials and as possible contributors to adverse health effects (Ezeonu et al., 1994; Flappan et al., 1999; Gao et al., 2002; Hodgson et al., 1998; Johanning et al., 1996).

**Finding Hidden Mold Growth**

Detecting the presence of hidden mold growth located within wall cavities, plumbing chases, attics, crawlspace, and HVAC ducts is a daunting challenge for researchers, inspectors and building occupants. Locating hidden mold growth is critical to a building investigation because fungal spores and microbial volatile organic compounds (MVOCs) migrate from interstitial cavities and HVAC ducts into the occupied areas where exposure can occur (Macher, 1999). Air samples taken from occupied areas may sometimes detect hidden areas of mold growth, but the sensitivity of
such methods for detecting hidden mold growth is not reported. Even if air samples do indicate a hidden source is present, determining the exact location is beyond the capabilities of such methods.

Spore trap methods modified to sample from wall cavities have been used, but the interpretation of sample results is not well defined. Because internal insulation may interfere with spore collection and construction debris can obscure the sample, the method has many drawbacks. A search of the peer-reviewed literature did not reveal any studies that evaluated the ability of spore trap methods to detect mold growth within wall cavities. Without opening wall cavities, visually inspecting the area, and sampling surfaces for sources of mold growth, none of the methods currently available have been demonstrated to predictably find mold growth within the interstitial spaces of a building.

Use of Carbon Dioxide to Detect Metabolic Activity of Mold Growth

Fungi are known to generate a variety of gaseous by-products commonly known as microbial volatile organic compounds (MVOCs). Along with aerobic bacteria, fungi metabolize carbon-based nutrient sources and oxygen for energy, releasing carbon dioxide (CO₂), water and a variety of other compounds (Deacon, 1984; EPA, 1992; Foster, 1949). Much emphasis has been placed on measuring “signature” MVOCs produced by mold growth on building materials. While many researchers have identified several compounds typically produced by mold growth, none have been shown to be unique or predictive of species. Most of the compounds tentatively identifies as “indicator MVOCs” are also emitted from building products without mold growth (Gao
et al., 2002). No consensus has been reached on relevant MVOCs and interpretation of sample results are subjective. The role of MVOCs as possible respiratory irritants is also not yet clear (Pasanen et al., 1998).

On the other hand carbon dioxide is produced by all fungi when metabolically active. Production of CO$_2$ is often used in laboratory experiments as a direct indicator of metabolic activity. The absence, or reduction, of CO$_2$ is also used to demonstrate the lack of metabolic activity indicative of growth (Foster, 1949; Korpi et al., 1997). By drawing air from inside a wall cavity through a small diameter probe, CO$_2$ concentrations can be measured inside a wall cavity.

Research has demonstrated the usefulness of carbon dioxide monitoring for grain silos as an early detection system for spoilage from mold growth (Bhat et al., 2003). While not a specific indicator of mold growth, carbon dioxide can be used as a non-specific indicator of microbial metabolism, necessary for growth. To be useful as an indicator of fungal growth within building cavities an estimate of carbon dioxide production rates from fungal growth is necessary.

Carbon dioxide has been used as an indicator of ventilation rates in buildings when CO$_2$ in the building is at equilibrium. This indicator is based on assumptions that include a constant CO$_2$ production rate from human occupants, constant occupancy, constant air exchange rate, and no other significant sources of carbon dioxide production. In residential and commercial buildings, sources of CO$_2$, besides building occupants,
include combustion sources, dry-ice use, carbonated beverage stations and animals. Carbon dioxide emission rates from building materials such as gypsum wallboard, lumber, fiberglass insulation or commonly used finishing materials have not been reported in the published literature. One study of fungal growth on gypsum wallboard designed to determine MVOCs did report that without supply air CO\textsubscript{2} concentrations could reach 10\% (100,000 ppm) (Gao et al., 2002). Because building products are not a source of CO\textsubscript{2}, it can be used as an indicator of building ventilation (ASHRAE, 1990; NIST, 1994).

**Studies of Carbon Dioxide Production from Mold Growth**

Korpi reported that carbon dioxide from fungal growth in house dust revealed a rapid increase in CO\textsubscript{2} levels after the third day at 96\%-98\% relative humidity (RH). Carbon dioxide concentration in a sealed container reached 11\% after 25 days. The mass of mold present in the house dust was not determined, but the microbial concentration was estimated by serial dilution plating of the dust onto agar and colony formation. Unlike previous reported studies, the carbon dioxide production rate did not correlate with MVOC production in samples tested under different conditions. Samples from fungi in house dust at 96-98\% RH emitted lower MVOC levels than samples measured under 84-86\% RH. These results were inversely proportional to CO\textsubscript{2} generation rates. This finding was attributed to a lower adsorption of MVOCs by the Tenax TA sampling media at the higher RH. Reduced collection efficiency was hypothesized to be the reason for these paradoxical results (Korpi et al., 1997).
Carbon dioxide production rates from house dust at 96-98% RH were reported to initially increase, after being placed in an environment capable of supporting spore germination and colony growth, but declined over time. Seven days after introduction to the chamber carbon dioxide production was ~560 µg CO₂ per gram of dust per hr; at 14 days 480 µg CO₂/ per gram of dust per hr; and at 21 days ~420 µg CO₂ per gram of dust per hr (Korpi et al., 1997).

Carbon dioxide generation rates from fungal growth on grains have been reported for different species. A positive correlation was found between volatile metabolites and fungal biomass, as measured by ergosterol content, but no correlation was found between colony forming units (CFU) and volatile metabolites. The correlation between CO₂ and ergosterol was 0.65 (n = 72, P < 0.05). However, for each species of fungi the correlation was generally higher. Little difference was detected in carbon dioxide from growth on wheat versus oats (Borjesson et al., 1992).

These studies, while not directly applicable to fungal growth on building materials, support the conclusion that carbon dioxide is produced by fungi during growth and is positively correlated with fungal biomass at comparative stages of growth. Variables that influence CO₂ production included moisture availability (i.e. relative humidity), temperature, fungal species, and the nutrient composition of the substrate (Borjesson et al., 1992; Korpi et al., 1997; Vice, 2000).
Mold Growth on Gypsum Wallboard

Modern buildings are often constructed using synthetic materials not previously used. Gypsum drywall was invented around 1917, but did not become a predominant building material until after World War II (US Gypsum, 2005). Gypsum drywall or wallboard essentially replaced plaster on lathe for interior wall surfacing. The calcium sulfate gypsum core, along with a variety of binders and strengthening materials, is sandwiched between two cellulose coatings. Gypsum drywall is very susceptible to mold growth once it is made wet by water damage or long-term exposure to high humidity. US Gypsum, a major producer of gypsum wallboard, has begun adding the antimicrobial agent sodium pyrithione to inhibit mold growth in a new product called Humitek™ (US Gypsum, 2003a; US Gypsum, 2003b).

Construction practices, transportation and building maintenance can expose gypsum drywall to wet and humid conditions that encourage mold growth. Due to the porous nature of gypsum drywall, cleaning the fungal growth from affected surfaces is neither practical nor effective.
The shift from plaster to gypsum drywall has resulted in buildings with a greater susceptibility to mold growth when they become wet. The extent of interstitial wall cavities that can harbor hidden mold growth encompasses the perimeter and demising walls of most homes, schools and office buildings. There are currently no effective methods to ascertain if a wall cavity is harboring mold growth or is free of mold growth.

Gypsum drywall is often part of the exterior building envelope and half of its surfaces are hidden from view. These interstitial wall cavities are susceptible to water damage and mold growth if a leak occurs. Since the interior spaces are difficult to access for visual inspection, extensive mold growth can occur before any indicators are observed.
in the building. If a water damage incident occurs, such as a plumbing leak or flood, these interstitial cavities can be difficult to dry, creating hidden harbors for mold growth.

**Mold Growth on Fiberglass Ductboard**

Air conditioning ducts were initially constructed of metal interior surfaces with external insulation. Internally insulating HVAC ducts with fiberglass insulation was begun to remedy two problems; (1) it prevents sweating of ducts due to cold exterior surfaces; and (2) it reduces noise transmission from turbulent air flow and fan operation. Predictably, the internal fiberglass surfaces also capture dust and absorb water carried over from the air handling unit. Fiberglass insulation was eventually made more rigid and coated with a thin foil exterior sheathing, creating ductboard. Ductboard is now produced by several manufacturers and is used extensively for construction of HVAC ducts in residential, commercial and school buildings. The material is faster to install, requiring only a razor knife and foil tape, where metal ducts require metal shears, saws, and screws.

Air conditioning ducts constructed of fiberglass ductboard create pathways for air that can serve as a home to mold growth, but are inaccessible for inspection. HVAC systems with leaky ducts can overwhelm their ability to control moisture, resulting in conditions favorable to mold growth (Cummings et al., 1996). Dust and debris introduced during construction and system operation ensure that adequate inoculum and nutrients are present to support spore germination and fungal growth. Growth of fungal colonies on fiberglass ductboard and other forms of internal fiberglass insulation are
common (Ahearn et al., 1992; Chang et al., 1995; Morey and Williams, 1990; 1991; Price et al., 1994; Samimi and Ross, 2003; Van Loo et al., 2004). Metal duct surfaces can resist fungal growth even under conditions that allow fungal growth on fiberglass liner (Chang et al., 1996). The fiberglass liner not only captures nutrient dust and water, the binders present in the fiberglass can serve as a nutrient source once growth is established. The addition of antifungal treatments to fiberglass duct materials in recent years highlights the susceptibility of this material to fungal growth, but studies have demonstrated their limited effectiveness (Samimi and Ross, 2003).

**Figure 2: Photographs of Mold Growth on Fiberglass Duct Liner**

Adoption of fiberglass ductboard has been less widespread than the shift from plaster on lathe to gypsum wallboard, but is common in residential construction. The use of fiberglass ductboard in schools and office buildings is less than seen in residential buildings. However, internal fiberglass liner in metal ducts is commonly used in
commercial buildings, schools and hospitals, creating a similar condition. The widespread use of this material has resulted in many buildings with an HVAC duct system prone to mold growth. Here the mold spores are undisturbed by cleaning efforts and nutrient-laden dust is constantly being deposited. Moisture can be supplied by carryover from improperly operating or installed air handling units. The internal surface area of fiberglass ducts serving a 325 m$^2$ (3,600 ft$^2$) home, delivering 1,500 cubic feet per minute (CFM) of conditioned air could exceed 58 m$^2$ (624 ft$^2$). In extreme cases mold growth can be present throughout such a duct system. A standardized example duct system, fabricated of fiberglass ductboard, was used to illustrate the amount of internal surface area potentially available for fungal growth in a residence (ACCA, 1995). This example system was used for indoor contaminant modeling in this research because it is an ideal system designed to all industry standards and depicts a standard system that could be used in a typical residence.

These two major changes in building materials have increased the susceptibility of modern buildings to microbial contamination by introducing thousands of square feet of materials that readily support mold growth when water damage occurs.

Mechanisms of Fungal Growth and Decomposition of Materials

*Gypsum Wallboard*

The cellulose coating of manufactured ductboard provides an ideal substrate for most fungi. Fungi that can break down cellulose, by producing the enzyme cellulase, readily colonize and decompose water damaged gypsum wallboard. The gypsum core
serves as an effective “sponge”, making the moisture it stores, readily available to fungi colonizing its surface. Growth appears to initiate on either the front “face” or rear “backing”, but can eventually spread to the gypsum core (Doll, 2002).

Colonization of water damaged gypsum wallboard by mesophilic fungi such as *Stachybotrys, Chaetomium, Fusarium* and *Aspergillus versicolor* is well documented (Ezeonu et al., 1994; Flappan et al., 1999; Gao et al., 2002; Hodgson et al., 1998; Johanning et al., 1996). These fungi typically colonize after water intrusion, floods or prolonged water leaks. Fungi that require less water can grow on gypsum wallboard when it is exposed to high relative humidity (>85% RH) for prolonged periods of time (Baughman & Arens, 1996).

Studies documenting the specific changes that occur in the various components that make up gypsum wallboard have not been published, but it has been observed that the structural integrity can be lost, even if the material is dried completely. How the growth and decomposition by fungi affects the fire rating and thermal insulation properties are not well described by product manufactures.

*Fiberglass Ductboard*

Molds produce and excrete acids during digestion of their nutrient substrate (Deacon, 1984; Foster, 1949; Jentschke et al., 2001; Roos and Luckner, 1984). The acidification of the substrate can help to break down organic and inorganic materials, including cellulose, adhesives, binders and even steel. Along with production of organic
and inorganic acids such as formic acid, citric acid and acetic acid, some fungi excrete protons (H\(^{+}\) ions) as part of a plasma membrane transport system. In the presence of ammonium (NH\(_{4}^{+}\)) extrusion of protons (H\(^{+}\)) is coupled with its cellular uptake. By exchanging H\(^{+}\) for NH\(_{4}^{+}\), the fungi maintains internal electro-neutrality, but causes high concentrations of H\(^{+}\) in the substrate, effectively lowering the pH. Cultures of *Penicillium cyclopium* were found to reduce the pH of the substrate to below 2.0 before exceeding their capacity to maintain internal pH (Deacon, 1984; Little and Staehle, 2001; Roos and Luckner, 1984). Numerous other fungi have been found to exhibit this linkage between NH\(_{4}^{+}\) uptake and H\(^{+}\) excretion, resulting in the acidification of soil and other growth substrates (Amrane et al., 1999; Jentschke et al., 2001).

Fungal colonization of fiberglass has been reported in numerous case reports and comprehensively described in many controlled laboratory studies (Ahearn, 1992; Chang, 1996; Morey and Williams, 1990; 1991; Price et al., 1994; Samimi, 2003; Van Loo et al., 2004). Fiberglass has been reported to contain fibrous glass, phenol, cured reaction products of hexamethylenetetramine and formaldehyde (HCHO) which is an established inhibitor of many microorganisms (Chang et al., 1995). However certain species of fungi have demonstrated an ability to utilize formaldehyde as a sole carbon source at concentrations up to 100 ppm. The breakdown product of urea, also found in fiberglass insulation, is ammonia. Both *Aspergillus fumigatus* and *Aspergillus versicolor* were found to use urea as a nitrogen source (Van Loo et al., 2004).
There are inherent characteristics of fiberglass that make fungal colonization possible. Fiberglass is hygroscopic and tends to absorb both water and nutrients. Thus, fiberglass insulation within HVAC systems can retain sufficient moisture to support spore germination and fungal growth. The urea-based resins commonly used to bind the glass fibers, may be broken down by extra-cellular acids to ammonia, a useful nutrient source for many fungi (Roos and Luckner, 1984; Van Loo et al., 2004).

A recent study was performed on the resistance of fiberglass ductboard to mold growth that contained a biocide. Results indicated that when the material surface was coated with a nutrient source, such as construction dust, and exposed to high humidity growth was inhibited. However once water accumulated on the material no inhibition was detectable (Samimi and Ross, 2003).

In a 1992 U.S. EPA document on Indoor Biological Pollutants, a brief mention was made of the ability of fungi to metabolize toxic solids into a gaseous state. The authors referred to the conversion of arsenic to trimethylarsine, causing arsenic poisoning (EPA, 1992). Still no consideration was made of the effect that fungal growth would have on the flame retardant applied to building materials.

In a 1995 US EPA research and development report on HVAC Systems as Emission Sources Affecting Indoor Air Quality: A Critical Review, it was recognized that “microbial agents metabolize metals, and can produce metal-containing gasses and aerosols.” The report, prepared for the Office of Environmental Engineering and
Technology Demonstration, also recognized that the area of biomethylation of metals was “poorly studied”. However, the report did not consider the effect of microbial degradation of antimony-based flame retardants (US EPA, 1995). This is not surprising as the biomethylation of antimony under aerobic conditions was not reported in the scientific literature until 1998 (Andrewes et al., 1998).

**History of Scheele’s Green**

A well-documented historical phenomenon that caused numerous deaths in Europe in the late 19th century may have modern day relevance. Arsenic poisoning due to fungal volatilization of arsenic-containing pigments during the late 1800’s in Europe has been documented (EPA, 1992; Foster, 1949). During the intervening century research has revealed that biomethylation of arsenic and other metalloids can occur by many fungi and bacteria. Despite the well recognized phenomena, little consideration has been made for the potential impact that toxic metalloid volatiles may have on building occupants due to fungal growth on building materials treated with metalloids (EPA, 1983; EPA, 1992; EPA, 1995; NAS, 2000). While arsenic was extensively used in pressure treated lumber until a recent change to copper-based preservatives, the use of antimony as a fire retardant is pervasive in many building materials, textiles and adhesives (USGS, 2004).

Research into the biomethylation of metalloids can be traced back to numerous cases of arsenic poisoning in Europe in the 1800’s. In Germany cases of poisoning were ascribed to wallpaper and tapestries printed with arsenical pigments (EPA, 1992; Foster,
The predominant pigment used was known as Scheele’s Green (copper arsenate, CuHAsO$_3$), but other arsenic containing pigments were also implicated, Schweinfurth green, Paris green, Vienna green and emerald green. According to contemporaneous accounts, the arsenical papers were extensively used, especially in bedrooms, throughout Europe from “the palace down to the navvy’s hut”. In 1897 it was finally recognized by an Italian physician Bartolomeo Gosio that when wallpaper containing Scheele’s green pigment became damp and moldy, a volatile form of arsenic was released. The volatile gas, later determined to be trimethylarsine, was recognized by its “garlic-like” odor. Initially, the fungus *Scopulariopsis brevicaulis* was recognized as the microbial culprit of the pairing, but other species were later found to be able to biomethylete arsenic (Foster, 1949).

**Biomethylation of Metalloids (As, Sb, and Bi)**

Further research into the mechanism of microbial biomethylation of arsenic and other metalloids by fungi was carried out by Frederick Challenger in the 1930’s at the Leeds School in England. He proposed a mechanism for trimethylarsine formation by aerobic fungal metabolism. Further research by others has expanded this mechanism to other elements (i.e. antimony, bismuth and tin) and microorganisms (Bentley & Chasteen, 2002; Lovley, 2000; Michalke et al., 2000). The reduction and subsequent methylation of metals and metalloids is believed to essentially follow the same pathway described by Challenger.
Figure 3: Periodic table section showing metalloids

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- **Metals**
- **Metalloids**
- **Non-metals**
- **Liquid at 20°C**
The top line indicates mechanism for the reduction of As (V) to As (III). Structures are as follows: $R_1 = R_2 = OH$, arsenate; $R_1 = CH_3$, $R_2 = OH$, methylarsonate; $R_1 = R_2 = CH_3$, dimethylarsinate. For reduction of trimethylarsine oxide to trimethylarsine, the process is a little different. Following proton addition, the structure $H-O-As^+$ $(CH_3)_3$ reacts with hydride ion leading to elimination of $H_2O$. The bottom line indicates the methylation of an As (III) structure with $S$-adenosylmethionine (SAM) [shown in abbreviated form as $CH_3-S^+--(C)_2$]. A proton is released and SAM is converted to $S$-adenosylhomocysteine [abbreviated form, $S-(C)_2$] (Bentley & Chasteen, 2002).
Figure 5: Challenger mechanism for the conversion of arsenate to trimethylarsine

Arsenate to arsenite to methylarsonate to methylarsonite to dimethylarsinate to dimethylarsinite to trimethylarsine oxide to trimethylarsine. The top line of structures shows the As (V) intermediates. The vertical arrows indicate reduction reactions to the As (III) intermediates (bottom line), and the diagonal arrows indicate the methylation steps by SAM (see Fig 2 for details of the reduction and methylation processes) (Bentley & Chasteen, 2002).

Challenger observed that *Aspergillus versicolor* formed trimethylarsine to a limited extent in 1948. In 1984 Cullen et al. reported the yeast *Candida humiculus* converted chromated copper arsenate to trimethylarsine. This report has relevance in that chromated copper arsenate, used extensively as a preservative in pressure treated lumber, is very similar to the wallpaper pigments that prompted Gosio’s work. Additionally, the
wood rot fungus *P. schweinitzi* has been shown to produce trimethylarsine and trimethylantimony when grown in the presence of arsine and antimony (Bentley & Chasteen, 2002).

Prior to the mid-1990’s there was little evidence for microbial methylation of antimony. Challenger described a case of chronic antimony poisoning in Vienna, Austria at the beginning of the 20\(^{th}\) century. Despite his suspicion that a volatile antimony compound was being released from silk curtains used to fix colors to the fabric with an antimony compound, he could not identify any using the limited analytical methods available at the time. Finally biomethylation of antimony by the bacteria *P. fluorescens* was confirmed in 1996 at Sam Houston State University in Huntsville, Texas (Gürleyük et al., 1997). In 1998 two studies reported the biomethylation of antimony by the fungus *Scopulariopsis brevicaulis* (Andrewes et al., 1998; Jenkins et al., 1998a).

The environmental fate of this volatile antimony compound (trimethylantimony, trimethylstibine, or Sb(CH\(_3\))\(_3\)) in the atmosphere has been reported. The oxidative products of trimethylstibine were described as “a range of cyclic and linear oligomers” containing stibine oxide units. Understanding the atmospheric chemistry of trimethylstibine in the indoor and outdoor environments is important in understanding its mobilization and potential for human exposure (Bentley & Chasteen, 2002). The most difficult aspect of detecting trimethylstibine is its fast oxidation in gas phase. Studies have reported the gas phase rate constants for the oxidation of trimethylstibine and trimethylarsine to be 10\(^3\) and 10\(^6\) M\(^{-1}\) s\(^{-1}\) respectively (Parris and Brinckman, 1976).
According to estimates by Jenkins et al. the half-life of trimethylstibine in air with oxygen is around 50 milliseconds (Jenkins, 1998; Parris & Brinkman, 1976). Trimethylstibine is also much less volatile compared to trimethylarsine. The vapor pressure of trimethylstibine is 103 torr and trimethylarsine is 322 torr at 298 K. This means that trimethylstibine would have a higher tendency than trimethylarsine to remain in solution if they were to exist in the same solution. These two facts point to the major downfall of previous experiments in which the evolved gases were aspirated with sterile air into a solution to give a precipitate for further analysis. With such a fast oxidation rate, it is very likely that trimethylstibine is oxidized to trimethylstibine oxide \([(\text{CH}_3)_3\text{SbO}]\), before it reached the sample collection media. Attempts to reduce the atmospheric oxidation of trimethylstibine have included decreasing the amount of O\(_2\) in the aspiration air to 8% by adding N\(_2\). However, the low oxygen content obstructs the growth of the fungi, inhibiting the metabolic mechanism causing the release of the antimony (Gürleyük, 1997).

Until the introduction of the flame retardant, antimony trioxide after World War II, the potential for widespread public exposure to antimony was low. Antimony-based flame retardants are now commonly used for indoor building materials, textiles, and furnishings (ATSDR, 1992; USGS 2004). However, the US EPA currently does not believe the use of these flame retardants in plastics or textiles will result in significant exposure to consumers. The belief stated in the review was not based on any cited research, but rather a cursory understanding of the physical properties of antimony trioxide, namely its low volatility, low water solubility and assumption that the flame
retardants are tightly bound to the matrix (EPA, 1983). However, due to the findings of preliminary toxicity studies that chronic exposure to antimony compounds resulted in damage to the heart, kidneys, liver and lungs, the EPA recommended further studies to evaluate chronic toxicity be conducted (EPA, 1983). Despite recognition of the “biotransformation” that can occur to antimony in the natural environment, no consideration was made of this possible mechanism occurring within indoor environments (EPA, 1983).

Prevalence of Antimony in Building Products

Antimony trioxide is a flame retardant frequently used in building materials common to homes, commercial buildings and schools. Approximately 60% to 65% of all antimony used in the United States is in the form of antimony trioxide (Sb₂O₃) as a flame retardant (USGS Mineral Commodity Profiles: Antimony 2004). Antimony trioxide is also used in a wide variety of plastics which include flexible polyvinyl chloride (PVC), polyolefins, polystyrene, polyethylene terephthalate (PET), acrylonitrile-butadiene-styrene (ABS), and polyurethanes.

Antimony trioxide is also used as a stabilizer in plastics, as a pigment in enamels, paints, and rubber, as an antisolarant, decolorizer and a fining agent in glass. It is added, along with halogenated hydrocarbons, as a flame retardant synergist in adhesives, plastics, rubber and textiles (USGS, 2004).
Use of antimony trioxide in the U.S. has grown since the 1960’s. In 1999 nearly 23,000 metric tons of antimony-based flame retardants were used, accounting for 57% of primary antimony production (USGS, 2004). Antimony trioxide flame retardants are specified for use in adhesives & coatings, furniture, insulation, mattresses, roofing materials, textiles, wall and floor coverings, circuit boards, electrical connectors, relays and switches. Applications are also marketed and described for consumer products, including appliance housings, battery casings, business machines, consumer electronics, TV housings and all types of wire and cable sheathing (Great Lakes Chemical Corporation, 2003).

Antimony trioxide’s use as a pigment has been surpassed by titanium dioxide in the United States, with the exception of some exterior oil-based paints and enamels. The main advantage of antimony trioxide as a paint pigment is its resistance to “chalking” and UV degradation. These properties have lead to its use in yellow paints for school busses and yellow striping applied to road pavements. Pigments made from antimony trisulfide and antimony pentasulfide are used for coloring rubber black and shades of yellow, orange and red. However interior paint products containing Timonox® Flame Retardant were available in the United Kingdom as recently as 2000 (MSDS, 1999). Based on analysis of 400 paint samples, house paints with >2% antimony were reported to be rare, constituting less than 0.5% of old paint films (van Alphen, 1998).

Other uses for antimony compounds include:
• Fluid lubricants for increased chemical stability.
• A phosphor in fluorescent lamps.
• Antimony pentasulfide is used as a vulcanization agent for red rubber.
• Antimony trisulfide is used in primers for ammunition, high explosives and in fireworks.
• Tartar emetic (hydrated potassium antimonyl tartar) has been used as a pesticide, for stomach disorders, to treat the parasitic disease leishmaniasis, and a mordant for some acid textile dyes.

Consumption of primary antimony in the United States has grown at a rate of ~5.6% per year since the early 1980’s. The two driving uses for primary antimony appear to be as a flame retardant in plastics and as a catalyst for PET (USGS, 2004).

After recognizing the many types of products that contain antimony in the indoor environment a search was made for building materials, including HVAC related products, coatings and paints that are specified for indoor use. Table 1 shows products that were found to contain antimony oxides. Initial estimations of antimony trioxide content were based on the Material Safety Data Sheets (MSDS) provided by product manufacturers. However, the amount (i.e. mass) of antimony trioxide could not be determined from product literature because product density and coverage rates were not specified. Experimentally derived data was necessary to determine the potential contribution that various products could have to the amount of antimony in the indoor environment.
The materials initially evaluated for antimony content were chosen after a search of material safety data sheets (MSDS) identified a variety of materials and coatings that reportedly contain antimony. Most products did not specifically list the concentration of antimony, typically indicating that “less than” a certain amount by weight is present in the product. In order to assess the tentatively identified materials for antimony content those with the highest reported amounts were purchased and tested.

Reviewing the material safety data sheets and product literature revealed several brands of fiberglass ductboard that contain up to 3% antimony trioxide by weight. Because ductboard is coated with antimony trioxide in a factory setting, with presumably small tolerances, the variability of this manufactured material was expected to be low. The same may not be true with paintings and coatings applied to walls during or after construction. To determine the range of antimony levels on materials after painting with a flame retardant coating experimental data would be necessary. Fiberglass ductboard was chosen as the test material to evaluate the potential for mold growth to release antimony from building materials.

### Table 1: Products containing antimony trioxide fire retardant*

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Trade Name</th>
<th>Manufacturer</th>
<th>Antimony trioxide content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adhesive</td>
<td>Pyralux ®</td>
<td>DuPont</td>
<td>Not Disclosed</td>
</tr>
<tr>
<td>Paint Pigment</td>
<td>Sicotan ®</td>
<td>BASF</td>
<td>5% - 17%</td>
</tr>
<tr>
<td>PET Resins: Polyester Glass</td>
<td>115FR BK112</td>
<td>BASF</td>
<td>1% - 5% by wt</td>
</tr>
<tr>
<td>Reinforced Resin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interior Wall &amp; Ceiling Paint</td>
<td>Timonox Paint</td>
<td>Akzo Nobel</td>
<td>[2.5% - 10%]</td>
</tr>
<tr>
<td>Paint, Yellow Corn color</td>
<td>A-100 Exterior Satin</td>
<td>Sherwin-Williams</td>
<td>1% by wt</td>
</tr>
<tr>
<td>Latex</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 1: Products containing antimony trioxide fire retardant* (continued)

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Trade Name</th>
<th>Manufacturer</th>
<th>Antimony trioxide content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fire Retardant Varnish</td>
<td>Ultra-hide Insul-blaze coating</td>
<td>Glidden Paint, ICI Paint</td>
<td>1% - 5% by wt</td>
</tr>
<tr>
<td>Aerosol Lubricant</td>
<td>-G75, 27A Aerosol</td>
<td>Sandstrom Products</td>
<td>1% - 5% by wt</td>
</tr>
<tr>
<td>Fiberglass Insulation</td>
<td>CM-26 (coated) OEM Diffuser board, Tuf-Skin Rx ®</td>
<td>Johns Manville</td>
<td>&gt;0.1% by wt in tape, facing, adhesives and/or coating</td>
</tr>
<tr>
<td>All Service Jacketing Roofing Insulation Product</td>
<td>ASJ 3035; ASJ 4535</td>
<td>Johns Manville</td>
<td>1.6% - 4.0%</td>
</tr>
<tr>
<td>Kraft paper backing laminated w/ flame retardant adhesive</td>
<td>FSK-25</td>
<td>Johns Manville</td>
<td>&lt;2%</td>
</tr>
<tr>
<td>Flame retardant adhesive</td>
<td>LAWX-235D</td>
<td>Johns Manville</td>
<td>7% - 12%</td>
</tr>
<tr>
<td>Fiberglass thermal insulation w/ Kraft paper backing</td>
<td>AcoustaTherm Batts</td>
<td>CertainTeed</td>
<td>3% by wt</td>
</tr>
<tr>
<td>Fiberglass Ductboard</td>
<td>ToughGard ™</td>
<td>CertainTeed</td>
<td>0.9% to 3.0% by wt</td>
</tr>
</tbody>
</table>

*Information compiled from MSDS provided by product manufacturers. Products with less than 1% Antimony by weight do not have to list Antimony on the MSDS. Therefore this listing cannot be considered comprehensive.

### Health Effects of Exposure to Antimony Compounds

Little is known about the toxicity of antimony in comparison with knowledge about other metals such as lead, cadmium, mercury, or arsenic. The chemical form of antimony contributes greatly to its toxicity and a general “Antimony Toxicity” is not known to exist. Each form of the metal must be considered as a separate toxicological entity. The mechanism of toxicity for antimony compounds is unclear; but is probably related to antimony’s high affinity for sulfhydryl (-SH) groups, which are essential for the structure and function of proteins.

Trivalent antimony concentrates in the red blood cells, while the pentavalent form is found in the plasma. Both forms are excreted in the urine and feces, but urine contains more of the Sb$^{3+}$ and feces contain more of the Sb$^{5+}$. Reported reference values of
antimony in the blood or serum range from 2.5 – 28.7 nmol/L (0.3 – 3.5 µg/L), but less than 80 nmol/L is also reported as a normal value. Reported reference values of antimony in the urine range from 1.7 – 18 nmol/L (0.2 – 2.2 µg/L), but less than 160 nmol/L is also reported as normal (Baldwin and Marshal, 1999; medtox.org, 2003). Organic trivalent antimony has a greater affinity for the liver and red blood cells and is excreted more slowly (Beliles, 1994). In general, trivalent antimony compounds exhibit 10 times the toxicity of pentavalent forms. Prolonged exposure to many antimony-containing compounds is known to cause respiratory irritation (Krachler et al., 2001).

Human poisoning due to chronic exposure to stibine or trimethylstibine has not been reported, although numerous cases of acute toxicity have been described (Beliles, 1994; Parish et al., 1979). Prior to its use as a flame retardant, human exposure to antimony was believed to be limited to foundries and manufacturing operations where the gas trimethylstibine is applied as a dopant to semiconductors (Trimethylstibine, 1991). From what is known about trimethylstibine, chronic exposure is unlikely due to its rapid oxidation rate to less volatile forms of antimony oxides (Paris & Brinkman, 1976).

Very little information is available on health effects from acute or chronic exposure to trimethylstibine. Much of the toxicity and health endpoints must be extrapolated from data available from studies on antimony (Sb), antimony trioxide (Sb₂O₃) and stibine (SbH₃). Exposure to the methylated form of stibine, trimethylstibine (Sb(CH₃)₃) by inhalation may cause bleeding gums, metallic taste, nausea, laryngitis, anemia, skin eruptions, liver and kidney damage (MSDS Trimethylstibine, 2003).
Stibine is reported to equal or surpass arsine in toxicity, and causes specific toxic effects that closely resemble those of arsine. Based on a 1903 German publication *On Stibine and Yellow Antimony*, one of the toxic mechanisms of stibine is reaction with hemoglobin in red blood cells, leading to their destruction. Antimony is reported to increase the activity of heme oxygenase (Beliles, 1994; Thayer, 1984; Thayer, 1995). Also described are direct effects on brain tissue cells leading to various degrees of degeneration. Workers exposed to stibine also demonstrated marked weakness, headache, nausea, severe abdominal and lower back pain, and blood in the urine. The authors of the article state that “further research on the chronic and acute effects of exposure to arsine and stibine is needed” (Blackwell & Robbins, 1979).

Acute exposure to stibine is reported to cause hemolytic anemia and acute renal failure (DeWolff, 1995). Pneumoconiosis, lung irritation and other respiratory effects have been reported in workers (ACGIH, 1986; Beliles, 1994; DeWolff, 1995). One of the early signs of overexposure to stibine in humans may be hemoglobinuria.

It remains unclear as to the mechanism that is responsible for antimony’s genotoxicity (Krachler et al., 2001). A recent report on plasmid DNA damage, caused by stibine and trimethylstibine, points to a possible mechanism for the carcinogenicity of antimony. Trimethylstibine and stibine were found to be equipotent with trimethylarsine when evaluated using a plasmid DNA-nicking assay. Growing evidence suggests that DNA damage by arsenic and antimony is due to the production of reactive oxygen
species (ROS) and resulting oxidative stress. However, this study was only able to demonstrate DNA damage at a concentration 400 times the OSHA PEL of 0.5 mg/m\(^3\) (Andrewes et al., 2004).

The decisions made by the US EPA and the CPSC to allow the use of antimony trioxide on indoor building products never considered the possibility of material decomposition due to fungal growth and the subsequent biomethylation and release of trimethylstibine (EPA, 1983; NAS, 2000).

The U.S. EPA listed antimony as a priority pollutant in 1986. In 2000 the U.S. Consumer Product Safety Commission (CPSC) released a report on the Toxicological Risks of Selected Flame Retardants. This risk assessment was performed because the CPSC was considering promulgating standards that would require most residential upholstery fabric to be treated with flame retardant chemicals. Because many flame retardant chemicals, including antimony trioxide, exhibit toxic properties an assessment was made of the risk to building occupants. Despite no consideration for the potential of microbial biomethylation and mobilization of antimony in a gaseous form, the report did conclude that “worst-case” exposure to particle phase degradation products via the inhalation route could pose a non-cancer risk of respiratory irritation, quantified by a Hazard Index of 1.2. Additionally, the report concluded the unit risk of lung cancer (cancer potency factor) is \(7.1 \times 10^{-4}/\mu\text{g antimony trioxide/m}^3\). This translated into a lifetime excess cancer risk estimate of \(1.7 \times 10^{-4}\) due to exposure to antimony trioxide.
particles, or 1.7 additional lung cancers per 10,000 people exposed for 70 years (NAS, 2000).

Based upon the findings of both an inhalation hazard index of greater than one and a potential cancer risk, the committee recommended that the potential for particle release from treated fabrics be investigated (NAS, 2000).

**Trends in Antimony Use and Asthma Prevalence**

One way of examining associations is to compare historical indicators of exposure with disease prevalence. Asthma prevalence data is available for a 15 year period from 1980 to 1995 (CDC, 1998). Because the primary use of antimony trioxide is fire retardants, and most of it has been imported to the United States, imports of $\text{Sb}_2\text{O}_3$ can serve as a useful surrogate indicator of the population’s exposure to this product additive.

Antimony oxide import data is available from 1950 through 1999. Examining overlapping data (1980 – 1995) for antimony oxide imports and asthma prevalence revealed a positive correlation ($r^2 = 0.9343; P<0.01$) (CDC, 1998; USGS, 2004). Figure 6 shows the available published data for both asthma prevalence and antimony oxide imports (CDC, 1998; USGS, 2004). Estimates of antimony oxide imports were interpolated from the charted data and compared with published asthma prevalence rates for 6 time periods. Figure 7 shows the scatter plot and linear regression of the data (USGS, 2004).
After 1995 antimony imports continued to rise, but peak in 1997. Falling levels may be a result of an overall shift in manufacturing of textiles and building materials to other countries. It is likely that fire retardant usage continues to increase, but that much of it is applied in the overseas factories where the products are manufactured. The reduction of antimony trioxide imports may also be effected by manufacturers shifting to other fire retardants.

For the time period between 1980 and 1995 asthma prevalence rose 75% and antimony oxide imports rose 82%. Asthma prevalence increased 5% per year while antimony oxide imports increased 5.4% per year. While this comparison and positive correlation does not offer conclusive data for a causal link, it does suggest a possible association between exposure to antimony fire retardants and an increased prevalence of asthma.
Figure 6: Antimony oxide imports and asthma prevalence in the U.S.

Figure 7: Antimony oxide imports vs. asthma prevalence in the U.S.
Using a common public health paradigm to evaluate causal links, the Bradford-Hill criteria, the following items should be examined before a causal link is presumed from any statistical association.

1. Consistent and unbiased findings. Relationships that are demonstrated across a number of studies, different populations, different circumstances, and different study designs.
2. Strength of association. Strong associations are less likely to be caused by bias.
3. Temporal sequence. Exposure must precede the disease, and the latency period.
4. Biological gradient (i.e. dose-response). Changes in exposure relate to a change in relative risk, quantitative relationships between the risk factor (exposure) and the outcome, intensity or duration of exposure may be measured.
5. Specificity and biological plausibility. If an exposure leads to a single or characteristic effect, or affects people with a specific susceptibility. Causal mechanism proposed must not contradict what is known about natural history and biology of disease, must fit with know facts. Proposed causal mechanism should be biologically plausible. When removed from the offending agent the health complaints disappear or resolve.
6. Experimental evidence. Controlled experiments should be able to demonstrate onset of the disease in animal models.

Most of the criteria set forth by Sir Austin Bradford-Hill have not been fully examined, much less met. However, two compelling points encourage further examination of this possible link between asthma and antimony trioxide fire retardant.
The first is an association between a surrogate indicator of exposure, namely antimony oxide imports, with the asthma prevalence in the U.S. Recognizing the multitude of indoor products and materials that are treated with antimony trioxide, should encourage researchers and federal regulatory agencies, such as the US EPA and CPSC, to conduct a comprehensive assessment of antimony exposures in all types of indoor environments.

The second point to consider is the mechanism of toxicity attributed to antimony trioxide particulate inhalation. The respiratory irritancy of these antimony compounds is well known when acute exposure occurs even at very low concentrations. For asthmatics, exposure to any respiratory irritants may result in exacerbation of symptoms. No studies have been identified that examined respiratory irritation and the development of chronic bronchial hyper-reactivity (i.e. asthma) with chronic exposure to any form of antimony. The potential for adverse health effects from chronic exposure has been recognized for years, but not yet studied in human populations.

Due to the widespread use of antimony-based fire retardants a large portion of the U.S. population is potentially exposed to antimony on a daily basis. When microbial degradation of treated building materials occurs, there is the possibility of antimony fire retardant mobilization and the generation of aerosols. Based on what is known about the toxicity of other antimony compounds, chronic exposure to moldy indoor environments may present a previously unrecognized public health hazard.
FOCUS OF RESEARCH

Fungal growth in buildings poses numerous challenges to researchers, building owners and occupants. Public health agencies promote prevention and remediation of mold and water damage, but limitations in current knowledge hamper meaningful research. The first problem is that a sensitive, low-cost tool that can detect hidden mold growth does not exist. Mold often begins to grow within wall cavities and spaces not accessible for inspection. By the time mold damage is visible in the occupied areas massive structural damage can take place. The second impediment to reducing public health risks associated with indoor mold growth is understanding the biological mechanisms that make occupying moldy buildings hazardous.

Carbon Dioxide as a Screening Test for Hidden Mold Growth

Despite over ten years of research and limited field use, measuring microbial VOCs has not proven to be a practical tool for detecting hidden mold growth. The high cost of laboratory sample analysis and the many well known interferences by building product emissions make interpretation of the data difficult at best. However, since the development of sensitive, hand-held, portable carbon dioxide sensors, real-time measurement of confined spaces can be performed. If fungal growth on common building materials could be determined to generate sufficient concentrations of CO₂ to elevate the concentration within a wall cavity or plumbing chase above the background
concentration found in occupied spaces, it may serve as a useful screening test for microbial metabolic activity. A small probe can be inserted to draw air from the wall cavity into a monitor. Concentrations within a wall cavity without any metabolically generated CO$_2$ should be the same or lower than indoor concentrations. By also measuring the pressure differential, the air flow direction can be determined and the air exchange rate may be estimated for the wall cavity. Monitoring of CO$_2$ levels in laboratory studies has been used as a test to indicate sterility of control samples (Borjesson et al., 1992). If it were found that fungi produced sufficient CO$_2$ to elevate interstitial concentrations, the absence of elevated CO$_2$ levels within wall cavities may be a useful indicator for the absence of mold growth, an especially useful test after water damage has occurred. Whether or not mold growth was truly prevented is a question that often remains after drying efforts have concluded.

**CO$_2$ Generation Rates During the Life Cycles of Two Mold Species**

In an attempt to derive carbon dioxide generation rates necessary to model CO$_2$ concentrations within wall cavities and static HVAC ducts, two types of fungi often found in water damaged buildings were chosen. *Stachybotrys chartarum* was chosen for growth on gypsum wallboard and *Aspergillus versicolor* was chosen for growth on fiberglass ductboard. Both of these fungi have been associated with occupant health complaints in water damaged buildings and both are capable of producing allergens, irritants, VOCs, and mycotoxins (Hodgson et al., 1998; Johanning et al., 1996). Because fungi are living, growing organisms, a single point measurement was not considered.
predictive. Therefore, a series of experiments were designed to measure CO$_2$ production throughout their life cycles, ranging from 24 hours after inoculation to 32 weeks.

**Potential for Antimony Release from Sb$_2$O$_3$ Flame Retardant as Trimethylstibine**

Speculation about antimony mobilization and release from environmental and indoor sources has existed since the early 1990s (EPA, 1992; EPA 1995; Gürleyük, 1996; Jenkins et al., 1998a). Laboratory experiments published in 1997 and 1998 demonstrated the ability of certain fungi and bacteria to liberate antimony as trimethylstibine. The first experimental evidence that antimony was converted to trimethylstibine by microorganisms was published in 1997 (Gürleyük et al., 1997). Aerobic biomethylation of soluble inorganic antimony by the fungus *Scopulariopsis brevicaulis* was first demonstrated in 1998 (Andrews et al., 1998; Jenkins et al., 1998a). Recognition of this mechanism prompted the question, could fungal growth on fire retardant treated building materials release antimony as trimethylstibine? In light of the widespread use of antimony-base fire retardants and the myriad of adverse health effects antimony exposure can cause, the impact of fungal growth on treated building materials should be examined.

Previous studies have been carefully controlled laboratory tests using homogeneous substrates amended with antimony compounds. These studies were intended to examine the ability of specific fungal species to biomethylate antimony. Their results indicated the biological mechanism was possible and appeared to occur with many types of antimony additives, including antimony trioxide (Andrews et al., 1998; Jenkins et al., 1998a). Based on the positive results from earlier studies and the
speculation of the authors I chose to focus this research on actual building materials that contained antimony trioxide. Using manufactured building materials did not allow for precise control over the amount and form of antimony added, and introduced many possible interferences due to limited knowledge of the proprietary material content. Conversely, use of building materials enables me to directly address the question of antimony mobilization from fire retardant treated building materials that have been demonstrated to support fungal growth in buildings.

**Measuring Antimony Release from Mold Growth on Sb$_2$O$_3$ Flame Retardant-Treated Building Materials**

Using a commercial laboratory, samples of various materials reported to contain antimony were analyzed for antimony content. For varnish and paint, the products were applied to oak panels and gypsum wallboard respectively for analysis. Some pieces were wetted and inoculated with fungal spores to determine if they could grow on the material.

Table 2 shows the results of ICP analysis for total antimony in test materials of various sizes. These data show the comparable amount of antimony present in each type of material tested. Differences between new materials and materials with mold growth were observed, but variations in application rates of antimony-containing coatings and fungal biomass density could have accounted for them. Additionally, confidence in the data was not high, as the laboratory reported their calibration consisted of a blank and a single point standard. Inconsistencies in measurement results, reported units and the lack of quality assurance information reduced my confidence in the results from the
commercial laboratory. Due to low confidence in these results I decided to perform all further analyses for antimony in the research laboratory with available methods described later in the methods section.

Table 2: ICP analysis results from preliminary study to identify materials containing Sb$_2$O$_3$ fire retardant

<table>
<thead>
<tr>
<th>Material Description</th>
<th>Total Sb (µg) in Sample</th>
<th>µg (Sb) cm$^{-2}$</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiberglass Liner Coating (no mold growth)</td>
<td>286</td>
<td>185</td>
<td>3/12/2004</td>
</tr>
<tr>
<td>Fiberglass Liner Coating (Heavy mold growth)</td>
<td>323</td>
<td>210</td>
<td>3/12/2004</td>
</tr>
<tr>
<td>Fiberglass Liner Coating (moderate mold growth)</td>
<td>281</td>
<td>182</td>
<td>3/12/2004</td>
</tr>
<tr>
<td>Fiberglass Liner Coating New</td>
<td>334</td>
<td>220</td>
<td>3/12/2004</td>
</tr>
<tr>
<td>Painted Gypsum Paper with mold growth</td>
<td>4.40</td>
<td>0.870</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Painted Gypsum Paper with mold growth</td>
<td>3.80</td>
<td>0.864</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Painted Gypsum Paper with mold growth</td>
<td>2.40</td>
<td>0.784</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Painted Gypsum Paper with NO mold growth</td>
<td>3.85</td>
<td>0.802</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Un- Painted Gypsum Paper with mold growth</td>
<td>&lt;0.3 (BDL)</td>
<td>&lt;0.07</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Un- Painted Gypsum Paper with mold growth</td>
<td>2.45</td>
<td>1.17</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Varnished Oak Panel with mold growth on 1-side</td>
<td>17.8</td>
<td>4.12</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Varnished Oak Panel with mold growth on 1-side</td>
<td>10.6</td>
<td>3.08</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Varnished Oak Panel with mold growth on 1-side</td>
<td>11.6</td>
<td>5.55</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Fiberglass Liner Coating with mold growth</td>
<td>402</td>
<td>227</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Fiberglass Liner Coating with mold growth</td>
<td>364</td>
<td>205</td>
<td>1/13/2004</td>
</tr>
<tr>
<td>Fiberglass Liner Coating with mold growth</td>
<td>338</td>
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Initial results revealed the amount of antimony in the varnish and paint was 100-1,000 times less than the fiberglass ductboard. Mobilization of antimony from the ductboard material was assumed to be easier to detect if it occurred. Additionally, the consistency of the application rate for varnish and paint was expected to be greater than the coatings and adhesives applied to ductboard during its factory manufacturing process.

The fungal species chosen to inoculate the ductboard test materials was *Aspergillus versicolor*. A series of experiments were designed to measure volatile antimony emissions and the residual amount of antimony remaining in the test material beginning at ~24 hours after inoculation and continuing for 29 weeks.
MATERIALS AND METHODS

CO₂ Generation from Fungal Growth on Gypsum Wallboard and Fiberglass Ductboard

Carbon dioxide generation was studied from two types of fungi that are often found growing on water damaged building materials. *Stachybotrys chartarum* was inoculated onto gypsum wallboard and *Aspergillus versicolor* was inoculated onto fiberglass ductboard. The fiberglass ductboard was delaminated into two major components, the air-side “coated surface”, which normally faces the air stream of constructed ducts, and the foil faced scrim kraft paper (FSK) exterior wrapping with adhesive.

Pieces of each material type were inoculated and the carbon dioxide concentrations measured inside an environmental chamber at regular periods during fungal growth to calculate the CO₂ production rate. After carbon dioxide measurements were taken, the fungal biomass density was estimated by collecting core samples from the materials and measuring β-N-acetylhexosaminidase activity. This endogenous enzyme correlates with mold biomass and a conversion factor was reported for the strains of fungi chosen for this study (Reeslev et al., 2003).
By knowing the approximate surface area of inoculated building materials with mold growth, the biomass density of fungi and the carbon dioxide production rate at equilibrium in the chamber, the carbon dioxide production rate per surface area of mold and per biomass of mold were calculated using equation 1.

Gypsum wallboard (GWB) test pieces were prepared from a single 4 ft x 8 ft (1.22 m x 2.44 m) sheet of material purchased from The Home Depot in Tampa, Florida. Individual pieces were cut from the 1/2” (1.27 cm) thick sheet, each measuring 10 cm x 10 cm. The edges were sealed with commercial grade seam tape commonly used to finish interior walls (The Home Depot in Tampa, Florida). The edges were sealed to protect the interior gypsum core from damage and loss during handling. No paint or coating was applied to the finish or back side. Two holes were drilled through the upper edge of each piece to allow insertion of two 3 mm (1/8 inch) diameter brass rod. The brass rods were used to hang the test pieces in a vertical position. To help maintain a constant spacing between each test piece, a 1.27 cm (½ inch) length of polyethylene tubing was placed on the brass rod between each piece of GWB test material. Five GWB test pieces were used for the experiment and altogether constituted a “test rig”. See Figure 8.
Fiberglass ductboard test pieces were also prepared from a single sheet of 4 ft x 10 ft (1.22 m x 3.05 m) sheet of material (CertainTeed ToughGard Duct Board) purchased from Jim Air Distributors Supply in Tampa, Florida. Individual pieces were cut from the 1½” (3.81 cm) thick sheet, each measuring 10 cm x 10 cm. Each test piece was delaminated into two sections. The first section was the air-side surface coating with 1.27 cm (½ inch) of fiberglass insulation. The second section consisted of the foil-faced skim kraft (FSK) paper exterior wrapping with adhesive. Only 2 to 4 millimeters of fiberglass insulation was left on this material to ensure the adhesive was readily inoculated. Test pieces were placed onto brass rods in a similar manner to the gypsum wallboard pieces described earlier.
Both test organisms, *Stachybotrys chartarum* (IBT 9695) and *Aspergillus versicolor* (IBT 16000) were obtained from the culture collection of Biocentrum, Technical University of Denmark (CDC PHS Permit No. 2003-09-094). The cultures were revived and grown on malt extract agar (MEA) in covered sterile Petri Dishes for 15 weeks (Fisher Scientific, Catalog #B11403). *Stachybotrys chartarum* cultures were thin until inoculated onto MEA supplemented with rice nutrient (Uncle Bens Instant Rice) to obtain heavy growth. Culture plates were sealed and maintained in an incubator at 25°C.

Spore suspensions of test organisms were harvested from agar plates by adding 80 ml of distilled water with 0.01 ml of Tween 80 and stirring with a sterile loop. The spore suspensions were then diluted to a final volume of 100 ml. Each spore suspension was then placed into a 250 ml stainless steel pressurized spray mister and the hand pump was activated 20 times. The delivery rate of spore suspension was earlier determined to be at a rate of 1 ml/sec when filled with 100 ml of liquid and pressurized with 20 strokes.

Gypsum wallboard test pieces were soaked in a bath of distilled de-ionized water for ten minutes and allowed to equilibrate for 30 minutes. The test organism *Stachybotrys chartarum* (IBT 9695) was inoculated onto the entire front and back surfaces of 5 pieces of gypsum wallboard (GWB) within a biosafety cabinet. Each piece was inoculated with 3 ml of spore suspension on each side (30 ml total). The concentration of spores present in the suspension was not determined. The goal of this inoculation procedure was to ensure equal inoculation for all test pieces in an attempt to encourage homogeneous growth.
Five control test pieces of gypsum wallboard were prepared, but not inoculated with any spore suspension, or wetted with water. These control pieces were kept dry, but when tested for CO₂ production, a 30 ml beaker of distilled de-ionized water was set in the chamber in an attempt to account for possible carbon dioxide release from the water.

The test organism *Aspergillus versicolor* (IBT 16000) was inoculated onto 5 pieces of fiberglass ductboard surface coating, each measuring 10 cm x 10 cm x 1.5 cm. Surface coating test pieces were placed into a biosafety cabinet and each piece was coated with 3.45 mg/cm² of Malt Extract Agar (MEA) powder to serve as a nutrient source and then inoculated with 5 ml of spore suspension onto the entire coated surface side (25 ml total), but not the back surface. Previous studies have reported fungal growth on fiberglass material can be slow or inhibited in the absence of accumulated nutrients such as dust (Samimi & Ross, 2003). MEA powder was chosen because it should not have introduced other fungal colonies. Control test pieces of fiberglass ductborad were prepared in the same manner as the GWB.

*Aspergillus versicolor* (IBT 16000) was also inoculated onto the adhesive side of 5 pieces of fiberglass ductboard FSK exterior wrapping with adhesive. No MEA powder was applied to these pieces as the adhesive was expected to provide sufficient nutrient source.
All test and control pieces were placed onto brass rods in a similar manner as the GWB and a 1.27 cm (½ inch) space left between each piece to allow adequate chamber air mixing. The assembled test rigs were self-supporting and readily fit into the test chambers and growth chamber described below.

Test chambers were constructed of 5 liter stainless steel food-grade containers with clear acrylic lids (d = 19 cm, h = 18 cm) (OGCI 18/8 Stainless Steel Anaheim, CA) (See Figures 9 and 10). The lids were sealed using a silicone gasket, clamping ring closure and Teflon tape to reduce leakage around the gasket. Each test chamber was fitted with two stainless steel Swagelok® fittings in the acrylic lid. The exhaust valve was fitted with a ¼ inch stainless steel tube that extended to the bottom of the chamber, enabling air to flow into the chamber near the top and exit near the bottom. This air flow pattern was intended to prevent stratification of the dense carbon dioxide gas.

Figure 9: Photograph of test chamber, acrylic lid, inlet and exhaust fittings
During periods when the test materials were not being measured for emissions they were kept in growth chambers. Growth chambers were 5 liter stainless steel containers with acrylic lids, but without the Swagelok® valve fittings. The growth chambers were kept at room temperature 23-26°C (74-79°F) during the periods between laboratory experiments.

Supply air to the chamber was filtered through a 37 mm polycarbonate filter (0.8 µm pore size) as it entered and exited the chamber. Filtering the air into the chamber reduced the chance of inoculation by other fungal spores and cross contamination. Filtering the air being exhausted out of the chamber helped to stabilize the air flow and enabled easier control of the air flow rate using the in-line adjustment valve and rotameter. Air flow was supplied using the laboratory vacuum which provided long-term, steady air flow.

During chamber tests for carbon dioxide the 5 liter chamber with test rig was placed in the work area of a biosafety cabinet. Loading and unloading of the chamber was performed under the hood to prevent exposure to the fungal spores and cross contamination of other materials.
A typical chamber test of carbon dioxide generation proceeded as follows.

Inoculated test materials were removed from a sealed growth chamber and placed into a test chamber. Carbon dioxide monitors were placed in-line with each chamber exhaust and data logging began. A single carbon dioxide monitor was used to measure and record intake air conditions (Temp., RH, and CO₂). Air flow rates were set to 250 ml/min using an in-line rotameter that was periodically checked against a primary calibration device (BIOS Dry-Cal Model-H). The chambers were allowed to reach equilibrium before recorded concentrations were used to calculate generation rates. The carbon dioxide monitors had internal data logging capability and concentrations were recorded every 10 minutes for the ambient air entering the chambers and for the exhaust air exiting the chambers. Once the chambers reached equilibrium, after 2 to 3 hours (6 to
9 air changes), recorded carbon dioxide concentrations were used to calculate the average generation rate described in equation 3.

Carbon dioxide was measured using a multi-function, integrated monitor with a non-dispersive infrared sensor placed in-line with the chamber exhaust air flow (AQ-5001 Pro, Quest Technologies, Oconomowoc, WI). The non-dispersive infrared (NDIR) sensor was reported to have a range of 0 to 5,000 parts per million (ppm), an accuracy of ± 3%, and resolution of 1 part per million (ppm). The CO₂ sensor calibration was verified prior to use with nitrogen (>99.80%) for zero adjustment and 1,000 ppm carbon dioxide for span adjustment (Air Liquide America, Cambridge, MD). The monitor used a resistance temperature detection sensor for temperature measurement with a range of 0°C to 60°C (+32°F to +140°F) and an accuracy of ± 0.5°C (± 0.9°F) with a resolution of 0.06°C (0.1°F). The monitor used a capacitive sensor to measure relative humidity (RH). The accuracy is reported to be ± 3% at 25°C and the resolution is 0.1% RH. These monitors had internal data logging capability and simultaneously recorded temperature, dew point temperature, relative humidity, and carbon dioxide.

Recorded data from each monitor was immediately downloaded after each chamber test onto a laptop computer with Quest Suite Proprietary software supplied by the carbon dioxide monitor manufacturer (Quest Technologies Oconomowoc, WI). Data were then exported into Microsoft Excel for analysis, graphing and calculation.
For quality control, chamber tests were conducted on empty chambers and on chambers with un-inoculated test materials plus beakers of distilled, de-ionized water. These data are reported in Appendix 1.

After carbon dioxide measurements were complete the test rig was removed from its test chamber and one core sample was cut from each test piece, totaling 5 in all. Core samples were collected using a 1.4 cm diameter aluminum metal tube core cutter, providing samples with an area of 1.54 cm². Samples were placed into beakers for immediate analysis of fungal enzyme activity, to determine the mold biomass density (p. 76).

For each test material with mold growth carbon dioxide measurements were determined at different points in time after inoculation. For *Stachybotrys chartarum* inoculated onto gypsum wallboard CO₂ generation rates were determined at 24 hours after inoculation, and at weeks 1, 4, and 9.

Gypsum wallboard test material previously wetted and placed in a chamber was later tested to evaluate CO₂ production from older colonies of growth at 27, 29, and 32 weeks post wetting. These test pieces were not inoculated in the same manner as described above. Instead, they were saturated with tap water and placed in a sealed chamber. Growth occurred from spores that “naturally occurred” on these materials. Microscopic analysis of these pieces revealed the predominant colonization by *Stachybotrys sp.*, but colonies of other fungal species were also observed to be present.
Comparable tests for CO$_2$ production from *Aspergillus versicolor* were performed on test rigs containing fiberglass ductboard surface coating and FSK exterior wrap with adhesive. Prior to each test the test rig was transferred from its growth chamber to the test chamber. During the transfer moisture content of the test pieces was measured using a Delmhorst BD-2000 pin-type moisture meter. When the materials were found to be drying out distilled, deionized water was added to support continued microbial activity.

*Chamber Performance Parameters*

Test chambers were constructed, evaluated and operated in accordance with ASTM D 5116-97 Standard Guide for Small-Scale Environmental Chamber Determinations of Organic Emissions From Indoor Materials/Products (ASTM, 1999). The air change rate of the chamber was calculated using equation 4. The chamber was designed and operated to ensure good mixing and the tracer gas decay test for quantifying mixing found the mixing level to be 71% using equation 5.

**Equation 1: Emission Rate per Surface Area of Mold Growth**

Emission Rate $\text{ER}_{area} = C_s(N/L_A)$

Where $\text{ER}_{area}$ = emission rate $\mu$g (CO$_2$) cm$^{-2}$ h$^{-1}$

$C_s$ = steady state chamber concentration $\mu$g/L

$C_s = C_{out} - C_{in}$

$C_{out}$ = CO$_2$ concentration of exhaust air from chamber (ppm) x 1.8 = $\mu$g/L

$C_{in}$ = CO$_2$ concentration of ambient air into chamber (ppm) x 1.8 = $\mu$g/L

$N$ = air change rate, h$^{-1}$

$L_A$ = Loading factor, cm$^2$/L
Equation 2: Emission Rate per Mass of Mold Growth

Emission Rate $ER_{mass} = C_s(N/L_M)$

Where $ER_{mass} =$ emission rate $\mu g (CO_2) mg^{-1} h^{-1}$

$C_s =$ steady state chamber concentration $\mu g/L$

$C_s = C_{out} - C_{in}$

$C_{out} =$ CO$_2$ concentration of exhaust air from chamber (ppm) $\times 1.8 = \mu g/L$

$C_{in} =$ CO$_2$ concentration of ambient air into chamber (ppm) $\times 1.8 = \mu g/L$

$N =$ air change rate, $h^{-1}$

$L_M =$ Loading factor (mass of mold growth), mg/L

Equation 3: Average Emission Rate$_{(mass \ or \ area)}$

$$ER_{MA} = \frac{\sum_{i=1}^{n} ER_{hourly}}{n}$$

Where $ER_{MA} =$ Average emission rate mass: $\mu g (CO_2) mg^{-1} h^{-1}$ or area: $\mu g (CO_2) cm^{-2} h^{-1}$

$ER_{hourly} =$ Emission rate at each hour after chamber reaches equilibrium

$n =$ Number of discrete measurements taken hourly with chamber at equilibrium

Equation 4: Chamber Air Change Rate

$$N = \frac{Q}{V} \ (3 \ ACH)$$

Where $N =$ Air change rate ($hr^{-1}$)

$Q =$ volume of air in to the chamber ($L/hr$)

$V =$ Volume of chamber ($L$)

The chamber mixing level was calculated using equation 5.

Equation 5: Chamber Mixing Level

$$\eta = \left(1 - \frac{\sum_{i=1}^{n} \left[C_A(t_i) - C(t_i)(t_i - t_{i-1})\right]}{\sum_{i=1}^{n} [C(t_i)(t_i - t_{i-1})]}\right) \times 100\%$$
Mixing level = 71%

Where \( \eta \) = mixing level

\[ N = \text{Chamber air change rate in units of inverse time (hr}^{-1}\text{)} \]

\[ t_n = \text{time constant of chamber} = N^{-1} \]

\[ C_{a}(t_i) = \text{tracer gas concentration in chamber exhaust} \]

\[ C(t_i) = \text{concentration for perfectly mixed system, calculated by } C(t) = C_{oe}^{-Nt} \]

\[ n = \text{number of discrete concentration measurements} \]

\[ t_i = \text{time of the } i^{th} \text{ concentration measurement} \]

\[ C_o = \text{tracer gas concentration at } t=0 \]

Chamber product loading was 200 cm\(^2\) (mold growth) L\(^{-1}\) for gypsum wallboard, and 100 cm\(^2\) (mold growth) L\(^{-1}\) for fiberglass ductboard surface coating and FSK exterior wrap with adhesive. Air exchange rate for the test chambers was 3 air changes per hour (ACH) with a flow rate Q of 250 ml/min.

Mobilization of Antimony Trioxide Due to Fungal Growth on Fiberglass Ductboard

The amount of antimony trioxide present on fiberglass ductboard (CertainTeed ToughGard), was reported on an MSDS dated June 21, 1999 to be 0.9%, but reported on a later MSDS from August 1, 2003 to contain up to 3.0% antimony trioxide by weight. Preliminary samples were collected and sent to First Environmental Laboratories, Inc. in Naperville, Illinois for ICP analysis (results reported in Table 2). The total concentration of antimony found in fiberglass ductboard surface coating plus the FSK exterior wrap was 451 \( \mu \text{g/cm}^2 \) and 417 \( \mu \text{g/cm}^2 \) when measured using the ICP and Spectrophotometer methods respectively. This equated to only 0.23% to 0.25% antimony by weight when the reported product density of 48 kg/m\(^3\) is used. This discrepancy indicated that either
the manufacturer was greatly over-reporting the amount of Antimony Trioxide present in the product or the acid recovery method used to analyze the product was inefficient. If the ductboard contained 0.9% to 3% as described, the mass of antimony trioxide would have been 1,641 to 5,472 µg Sb/cm². A possible explanation of this discrepancy is that antimony concentrations of individual components used to produce the final ductboard product, such as the adhesive, foil-paper backing and surface coating contained greater percentages of antimony. Once combined with the fiberglass and resin which constitute the majority of the final product’s mass, the percentage of antimony trioxide falls well below the amounts reported.

The effect of mold growth on antimony mobilization was assessed in two ways. The first method measured stibine, as total antimony, in the exhaust air of each chamber during a 4 hour period after the chambers reached equilibrium. NIOSH Method 6008 was used to collect and measure stibine as total antimony (NIOSH, 1994). The method calls for collection of air samples onto mercuric-chloride (HgCl₂)-coated silica gel sorbent tubes (SKC Cat No. 226-10-02).

Samples were collected at a flow rate of 250 ml/min. The total volume collected was 60 L. Samples were immediately extracted for analysis after the end of the collection period. The sorbent was placed into an acid washed 50-ml beaker along with 25 ml of concentrated HCl (Fisher Scientific, Trace Metals Grade Cat # A508-4). After an extraction time of 30 minutes 15 ml of the extract was transferred by pipette to a 125 ml separatory funnel. Ceric sulfate was added (15 mg) and allowed to dissolve for 1 min.
Isopropyl ether (Fisher Scientific CAS 108-20-3) was then added (15 ml) and the separatory funnel shaken for 30 sec. The two phases were allowed to separate for 1 min and the aqueous layer discarded. A working solution of Rhodamine B (20 ml) was added and shaken for 60 sec. The two phases were again allowed to separate for 1 min and the lower aqueous layer discarded. The remaining solution was drained into a 15 ml centrifuge tube, capped tightly and centrifuged for 2 minutes at 2,000 rpm. The supernatant was transferred to an absorption cell and capped for measurement.

Samples were placed into a matched silica absorbance cell. Isoprolyl ether was used in the reference cell. Sample absorbance was read at 552 nm using a Cary UV/Visible Spectrophotometer.

Before each day’s analysis a calibration curve was determined using an isopropyl ether blank and 6 different concentrations of antimony in concentrated HCl, ranging from 0.05 µg/ml to 1.50 µg/ml. NIOSH Method 6008 was evaluated at a relative humidity of 85% and over a wide range of air concentrations, from 0.119 to 1.01 mg/m³ using 20 L air samples. The reported limit of detection (LOD) for the method is 0.4 µg SbH₃ per sample (NIOSH, 1994). This equates to a LOD for antimony emissions of 0.1 µg Sb/hr from the 500 cm² test rig, or 0.0002 µg Sb cm⁻² hr⁻¹.

Calibration standards for the sample analysis were created using a Certified Reference Standard Solution of 1,000 ppm ±1% Antimony in dilute HNO₃ (VWR Scientific-Ultra Scientific, Catalog Number ULICM-214). Calibration standards were
placed into polypropylene bottles. A sufficient volume of calibration standard solutions were made to ensure that all tests could be performed using the same initial solutions. A positive control was not used in the chamber test to verify the collection and recovery efficiency of the complete test system because trimethylstibine standards are not available for such uses (Krachler et al., 2001). A supplier of trimethylstibine for semiconductor manufacturing was contacted, but the purity of their product was uncertain and handling of the liquid was a hazardous operation. The high concentration trimethylstibine was reported to be a pyrophoric gas that spontaneously combusts when mixed with air (MSDS Trimethylstibine, 2003). Generation of stibine (SbH$_3$) using an electrolytic metallic hydride generator was considered, but due to the explosive hazard it posed, attempts to generate stibine were not carried forward (Saltzman, 1961).

The second assessment for antimony mobilization was analysis of bulk samples from the test materials for antimony content. Core samples ($n=5$) were collected after each carbon dioxide and stibine generation test. After analysis for $\beta$-N-acetylhexosaminidase activity, described later, 1.4 cm diameter core samples were extracted using concentrated HCl and analyzed using a modified NIOSH 6008 method (NIOSH, 1977). Extracts were diluted to a greater degree so the final solution was within the measurement range of the method, 0.05 to 1.5 $\mu$g Sb/ml. Results using this method agreed with measurements of the same materials analyzed using the ICP/MS method 3050B/6010B. See Table 2 for ICP/MS preliminary data.
Measurement of Fungal Biomass on Gypsum Wallboard and Fiberglass Ductboard

Fungal biomass was estimated by measuring $\beta$-N-acetylhexosaminidase enzyme activity and applying a conversion factor previously reported for the specific fungal cultures that were used (Reeslev et al., 2003). Core samples cut from test materials (1.4 cm diameter) were analyzed using a fluorometric method (MycoMeter, Copenhagen, Denmark) that provided rapid results within an hour. Five samples were taken from the test material after each chamber test for carbon dioxide and stibine. Each sample was individually incubated for 30 minutes at 23°C, or adjusted to compensate for temperature variations. After extraction, 100 µl of the enzyme substrate solution was transferred to a developer solution and read using a Picofluor fluorometer (Turner Designs, Sunnyvale, CA) according to the manufacturer’s instructions (MycoMeter, 2002). Prior to analysis the fluorometer was calibrated using a supplied standard and all reagent blanks were measured. All sample fluorescent values were corrected using reagent blank measurements.

Fungal biomass density was estimated using the conversion factors reported for Stachybotrys chartarum (IBT 9695) and Aspergillus versicolor (IBT 16000) (Reeslev et al., 2003). The fungal growth area for each sample was 1.54 cm$^2$. The limit of quantification (LOQ) for fungal biomass for the method, using the conversion factors reported by Reeslev et al., were 0.8 µg mold/cm$^2$ and 0.5 µg mold/cm$^2$ for Stachybotrys chartarum (IBT 9695) and Aspergillus versicolor (IBT 16000) respectively (2003). The sample mass was not measured because of the high moisture content that could not be dried without affecting the fungal colonies metabolic activity.
Equation 6: Calculating fungal biomass density

\[
BMD = \left( \frac{F}{A} \right) \frac{1}{CF}
\]

Where BMD = Biomass density of mold growth (mg/cm\(^2\))

\[
F = \text{Fluorescent units formed by sample in 30 min at standard temperature}
\]

\[
A = \text{Area of sample (cm}^2\text{)}
\]

\[
CF = \text{Conversion Factor of Fluorescent units formed per milligram mold biomass dry weight per 30 min at standard temperature}
\]

\[
CF_{SC} = 8,275 \text{ F/mg for Stachybotrys chartarum (IBT 9695)}
\]
\[
CF_{AV} = 12,370 \text{ F/mg for Aspergillus versicolor (IBT 16000)}
\]

Statistical Analysis of Measurements

Correlation coefficients were used to evaluate relationships between fungal biomass density and carbon dioxide emission rates, and between fungal biomass density and antimony concentrations in the test materials. To determine if measurements of antimony in test materials changed over time the Student-t test was used (Microsoft Excel Statistical Package). Antimony measurements taken at each period after mold growth had been established were compared with initial antimony concentration measurements taken at the time of inoculation, before mold growth had established. Two-tailed t-tests were performed to determine if the sample means for each data set were statistically different at the alpha level of 0.05, with an assumption of unequal variance.
RESULTS

**CO₂ Production Rates on Gypsum Wallboard**

Carbon dioxide production from building materials varied with time, mold biomass density and the material surface area with mold growth. With exception of the chamber test performed ~24 hours after inoculation, the measurements of CO₂ were made after the mold growth was assumed to have reached the stationary phase. Measures of biomass density suggest the colony reached stationary phase by week four, but did not reach its maximum biomass density until week 29. Gypsum drywall inoculated with *Stachybotrys chartarum* produced measurable amounts of CO₂ in all experiments, from 24 hours to 32 weeks post inoculation. Table 3 shows the carbon dioxide production rates measured during chamber experiments of gypsum wallboard inoculated with *Stachybotrys chartarum* over the course of 32 weeks. The amount of CO₂ produced per cm² of mold growth was calculated using equation 1, and the amount of CO₂ produced per mg of mold was calculated using equation 2. The average rate of CO₂ produced per hour was calculated using equation 3 for both units. Table 3 also shows the estimated biomass density of mold at the time of each test. The biomass was calculated using equation 6.
### TABLE 3: Carbon dioxide production rates for *S. chartarum* on gypsum wallboard

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<th>Week (24 hours)</th>
<th>µg (CO$_2$) cm$^{-2}$ hr$^{-1}$</th>
<th>µg (CO$_2$) mg$^{-1}$ (Mold) hr$^{-1}$</th>
<th>Biomass density (µg/cm$^2$) (S. chartarum)*</th>
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* Biomass Density (mg mold/cm$^2$) = (MV/1.54 cm$^2$)/8,275 Conversion Factor (Reeslev, 2003)

** Replicate test on a separate piece of GWB with higher biomass density of mold growth.

### Figure 11: CO$_2$ generation and biomass density on gypsum wallboard

Carbon dioxide production rates for *S. chartarum* grown on gypsum wallboard

rang from 0.30 to 1.30 µg (CO$_2$) cm$^{-2}$ hr$^{-1}$ and 1.58 to 135 µg (CO$_2$) mg$^{-1}$ (mold biomass) hr$^{-1}$ throughout its growth cycle. Compared to other studies where this strain of *Stachybotrys chartarum* was grown on gypsum wallboard, the maximum biomass density
achieved was low and did not reach its peak until much later (Reeslev, 2003). In light of these results, the values obtained may be reflective of slow mold growth.

**CO₂ Production Rates on Fiberglass Ductboard**

Fiberglass ductboard (surface coating) inoculated with *Aspergillus versicolor* produced measurable amounts of CO₂ in all experiments, from 24 hours to 29 weeks post inoculation. Table 4 shows the carbon dioxide production rates measured during two separate chamber experiments of fiberglass ductboard inoculated with *Aspergillus versicolor* over the course of 29 weeks and 9 weeks. The amount of CO₂ produced per cm² of mold growth was calculated using equation 1, and the amount of CO₂ produced per mg of mold was calculated using equation 2. The average rate of CO₂ produced per hour was calculated using equation 3 for both units. Table 4 also shows the estimated biomass density of mold at the time of each test. The biomass was calculated using equation 6.

**TABLE 4: Carbon dioxide production rates for *A. versicolor* on ductboard surface coating**

<table>
<thead>
<tr>
<th>Week</th>
<th>μg (CO₂) cm⁻² hr⁻¹</th>
<th>μg (CO₂) mg⁻¹ (mold) hr⁻¹</th>
<th>Biomass density (μg/cm²) (<em>A. Versicolor)</em></th>
<th>μg (CO₂) cm⁻² hr⁻¹</th>
<th>μg (CO₂) mg⁻¹ (mold) hr⁻¹</th>
<th>Biomass density (μg/cm²) (<em>A. Versicolor)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (24 hrs)</td>
<td>1.66</td>
<td>176</td>
<td>9</td>
<td>3.08</td>
<td>598</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>0.82</td>
<td>10.5</td>
<td>78</td>
<td>2.65</td>
<td>115</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>2.02</td>
<td>8.83</td>
<td>228</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.15</td>
<td>3.56</td>
<td>324</td>
<td>1.18</td>
<td>3.18</td>
<td>371</td>
</tr>
<tr>
<td>7</td>
<td>1.91</td>
<td>12.86</td>
<td>148</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>1.60</td>
<td>15.65</td>
<td>103</td>
<td>1.17</td>
<td>2.48</td>
<td>470</td>
</tr>
<tr>
<td>29</td>
<td>1.13</td>
<td>2.96</td>
<td>381</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Biomass Density (mg mold/cm²) = (MV/1.54 cm²)/12,370 Conversion Factor (Reeslev, 2003)
** Values represent a second experiment on the same material.
-: No Data Collected
Figure 12: CO₂ generation and biomass density on ductboard surface coating test #1

![Graph showing CO₂ generation and biomass density on ductboard surface coating test #1.]

Figure 13: CO₂ generation and biomass density on ductboard surface coating test #2

![Graph showing CO₂ generation and biomass density on ductboard surface coating test #2.]

82
Carbon dioxide production rates for *A. versicolor* grown on fiberglass ductboard surface coating ranged from 0.82 to 2.02 µg (CO₂) cm⁻² hr⁻¹ and 2.96 to 176 µg (CO₂) mg⁻¹ (mold biomass) hr⁻¹ in Test #1, and 1.17 to 3.08 µg (CO₂) cm⁻² hr⁻¹ and 2.48 to 598 µg (CO₂) mg⁻¹ (mold biomass) hr⁻¹ in Test #2. Measures of biomass density suggest the colony reached stationary phase by week four in Test #1, but may not have reached stationary phase in Test #2 by week nine.

Fiberglass ductboard FSK exterior wrap inoculated with *Aspergillus versicolor* produced measurable amounts of CO₂ in all experiments, from 24 hours to 29 weeks post inoculation. Measures of biomass density suggest the colony did not reach stationary phase. Table 5 shows the carbon dioxide production rates measured during chamber experiments of fiberglass ductboard FSK exterior wrap inoculated with *A. versicolor* over the course of 29 weeks. The amount of CO₂ produced per cm² of mold growth was calculated using equation 1, and the amount of CO₂ produced per mg of mold was calculated using equation 2. The average rate of CO₂ produced per hour was calculated using equation 3 for both units. Table 5 also shows the estimated biomass density of mold at the time of each test. The biomass was calculated using equation 6.
TABLE 5: Carbon dioxide production rates for *A. versicolor* on ductboard FSK exterior wrapping

<table>
<thead>
<tr>
<th>Week</th>
<th>µg (CO$_2$) cm$^{-2}$ hr$^{-1}$</th>
<th>µg (CO$_2$) mg$^{-1}$ (mold) hr$^{-1}$</th>
<th>Biomass density (µg/cm$^2$)</th>
<th>(A. Versicolor)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.75</td>
<td>392</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.93</td>
<td>218</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.50</td>
<td>94.6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.02</td>
<td>4.92</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0.68</td>
<td>18.6</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>1.76</td>
<td>3.95</td>
<td>445</td>
<td></td>
</tr>
</tbody>
</table>

*Biomass Density (mg mold/cm$^2$) = (MV/1.54 cm$^2$)/12,370 Conversion Factor (Reeslev, 2003)

Figure 14: CO$_2$ generation and biomass density on FSK exterior wrapping

Carbon dioxide production rates for *Aspergillus versicolor* grown on fiberglass ductboard FSK exterior wrapping ranged from 0.02 to 1.93 µg (CO$_2$) cm$^{-2}$ h$^{-1}$ and 3.95 to 392 µg (CO$_2$) mg$^{-1}$ (mold biomass) h$^{-1}$ throughout its growth cycle.
Modeled Concentration of CO$_2$ in Wall Cavities

To model CO$_2$ concentrations within an enclosed cavity such as a wall cavity or HVAC duct, the model described in equation 7 was used. This equation was solved for the following assumptions, and presented as equation 8, air exchange rate, $I = (Q/V)$, generation rate ($G$), and the outdoor concentrations ($C_o$) remain constant.

**Equation 7: Model for CO$_2$ concentration inside a wall cavity and HVAC Duct**

$$\frac{dC_t}{dt} = \frac{-Q}{V} (C_o - C_t) + G$$

**Equation 8: Used to calculate equilibrium concentration of wall cavity and HVAC duct at equilibrium**

$$C_t = C_o + \frac{G}{Q} \left(1 - e^{-It}\right)$$

where $C_t$ = Concentration of CO$_2$ at time t,

$C_o$ = outdoor CO$_2$ concentration,

$G$ = generation rate of CO$_2$ in the space,

$Q$ = volumetric airflow rate into and out of the space,

$V$ = volume of cavity,

$I$ = air exchange rate ($Q/V$)

The concentration of carbon dioxide at equilibrium in a wall cavity could only become elevated above ambient concentrations by 25 ppm if over $\frac{1}{2}$ of the surface area on one side of the cavity, or $\frac{1}{4}$ of the total internal surface area, was supporting mold.
growth (*S. chartarum*). In a single wall stud bay, with a volume of 88 L, it would take 
~0.45 m$^2$ of mold growth to elevate CO$_2$ concentrations by ~25 ppm. However, this rise in CO$_2$ could only be expected during the first 9 weeks of mold growth. In later stages of growth, measured between 27 and 32 weeks, the generation of CO$_2$ could not be expected to cause an elevation of more than 25 ppm. When the situation of a larger wall cavity was modeled the same ratio was necessary to cause an elevation of CO$_2$ by 25 ppm or more.

An elevation of 25 ppm in CO$_2$ was chosen as a significant rise based on the reported accuracy of the hand-held CO$_2$ monitor used. At 375 ppm the accuracy of the monitor is reported to be ± 3%, or 12 ppm. In order for the measurements taken from inside the cavity and in the ambient air to be distinctly different they must differ by at least 25 ppm from ambient concentrations. At a higher ambient concentration of 1,000 ppm, the difference necessary to demonstrate elevated CO$_2$ is 60 ppm.

Table 6 shows the modeled CO$_2$ concentrations within a wall cavity given the described parameters and assumptions.

**Table 6: Modeled concentrations of CO$_2$ inside a wall cavity colonized with (*S. chartarum*)**

<table>
<thead>
<tr>
<th>Age of mold growth</th>
<th>Single Stud Bay 16 in x 8 ft $^{(1)}$</th>
<th>Single Stud Bay 16 in x 8 ft $^{(2)}$</th>
<th>Single Wall 10 ft x 8 ft $^{(3)}$</th>
<th>Single Wall 10 ft x 8 ft $^{(4)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>413 ppm</td>
<td>394 ppm</td>
<td>414 ppm</td>
<td>395 ppm</td>
</tr>
<tr>
<td>4 weeks</td>
<td>451 ppm</td>
<td>413 ppm</td>
<td>453 ppm</td>
<td>414 ppm</td>
</tr>
<tr>
<td>31 weeks</td>
<td>392 ppm</td>
<td>384 ppm</td>
<td>392 ppm</td>
<td>384 ppm</td>
</tr>
</tbody>
</table>
(1) 9,000 cm$^2$ of mold growth (~entire wall surface)
(2) 4,500 cm$^2$ of mold growth (~1/2 wall surface)
(3) 74,322 cm$^2$ of mold growth (~entire wall surface)
(4) 37,161 cm$^2$ of mold growth (~1/2 wall surface)

16” Stud Bay Volume = 88 L
10’ Wall Volume = 708 L
Air Change per hour from outdoors = 1
Outdoor concentration of CO$_2$ = 375 ppm

**Modeled Concentration of CO$_2$ in HVAC Systems**

The amount of mold growth (*A. versicolor*), in a static HVAC duct or air handling unit (AHU) cabinet, necessary to cause an elevation of CO$_2$ by more than 25 ppm was ~1/2 of the total internal surface area. Approximately 8 m$^2$ of mold growth would be necessary to elevate CO$_2$ by 25 ppm in a single duct measuring 40.6 cm x 43.2 cm x 0.975 m (16 in x 17 in x 32 ft) with an internal surface area of 16.35 m$^2$. Approximately 1.1 m$^2$ of mold growth on fiberglass lining within an AHU cabinet would be necessary to elevate CO$_2$ by 25 ppm, or ~ ½ of the total internal surface area.

**Table 7: Modeled concentrations of CO$_2$ inside a static supply duct (*A. versicolor*)**

<table>
<thead>
<tr>
<th>Age of mold growth</th>
<th>Single Supply Duct 16 in x 17 in x 32 ft (1)</th>
<th>Single Supply Duct 16 in x 17 in x 32 ft (2)</th>
<th>AHU Cabinet 2 ft x 2 ft x 3 ft (3)</th>
<th>AHU Cabinet 2 ft x 2 ft x 3 ft (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours</td>
<td>463 ppm</td>
<td>419 ppm</td>
<td>430 ppm</td>
<td>403 ppm</td>
</tr>
<tr>
<td>4 weeks</td>
<td>436 ppm</td>
<td>405 ppm</td>
<td>413 ppm</td>
<td>394 ppm</td>
</tr>
<tr>
<td>29 weeks</td>
<td>435 ppm</td>
<td>405 ppm</td>
<td>413 ppm</td>
<td>394 ppm</td>
</tr>
</tbody>
</table>
(1) 163,509 cm² of mold growth (~entire duct surface)
(2) 81,755 cm² of mold growth (~1/2 duct surface)
(3) 22,311 cm² of mold growth (~entire AHU cabinet surface)
(4) 11,156 cm² of mold growth (~1/2 AHU cabinet surface)

32 ft long duct Volume = 1,716 L
AHU Cabinet Volume = 372 L
Air Change per hour from outdoors = 1
Outdoor concentration of CO₂ = 375 ppm

**Discussion**

Chamber tests for carbon dioxide generation from growth of *Stachybotrys chartarum* on gypsum wallboard reflected the different stages of growth commonly recognized. Early log-phase growth occurs once conditions allowed germination of spores, and was characterized by an exponential increase in biomass density. This phase of growth appeared to coincide with a disproportionately large CO₂ generation rate with respect to the biomass of mold present. Because the experimental design limited the area of mold growth to the surface area of test materials, only the biomass density could increase. Variations in carbon dioxide generation rates appear to be a function of metabolic activity and biomass density.

As an indicator of mold growth, carbon dioxide clearly increased as fungal biomass increased during the first 24 hours when compared to un-inoculated controls for all test materials. Chamber tests of clean, dry test materials without inoculation
demonstrated no measurable generation of carbon dioxide. The primary focus of this experiment was carbon dioxide generation during the stationary phase of mold growth, not during the log-phase. Only a single chamber test was performed during the log-phase within 24 hours after inoculation. Early stationary phase, weeks 1 through 9, demonstrated less carbon dioxide generation per area of mold growth or per mass of mold growth than during the log phase. Late stationary phase, weeks 27 through 32, demonstrated declining biomass density and CO$_2$ generation. This is not surprising as nutrient availability was probably the controlling factor that leads to reduced fungal metabolic activity. However, it was observed that throughout the various growth phases of both species, on both types of test materials, carbon dioxide generation rates were greater than 0.61 µg (CO$_2$) cm$^{-2}$ hr$^{-1}$ of mold growth during early stationary growth at 1 week and 0.30 µg (CO$_2$) cm$^{-2}$ hr$^{-1}$ of mold growth during late stage growth at 32 weeks.

Dispersion of carbon dioxide throughout the wall cavity could impact the measured CO$_2$ concentration if not evenly dispersed. A study on the dispersion of carbon dioxide inside closed spaces demonstrated that regardless of the source location within an enclosed space, such as a wall cavity, the gas is evenly dispersed throughout the entire cavity after 90 minutes (Rahamani, 2004). The time scale being evaluated for CO$_2$ generation by fungi is on the order of days or weeks, making the issue of stratification and uneven mixing irrelevant.

The modeling results predict that detectable elevations in carbon dioxide concentrations could be detected only from an area of mold growth that is greater than $\frac{1}{4}$
the internal surface area inside of a wall cavity. With larger areas of mold growth, or in cavities with lower ventilation rates, the concentration of carbon dioxide could become more elevated within a wall cavity or supply duct. However, the usefulness of CO$_2$ monitoring as an indicator of hidden mold growth is impractical and could not be used to demonstrate the absence of hidden mold growth.

In addition to the concentration of carbon dioxide, a monitoring relative humidity could be used as an indicator of high moisture conditions. Elevated relative humidity levels within a wall cavity may serve as an indicator of either a water source, infiltration of moisture-laden outside air, or microbial respiration. Measurement of both elevated carbon dioxide levels and high relative humidity could provide greater evidence that mold growth, or other metabolically active microorganisms, may be present within the building cavity.

**Measured Emission Rates of Stibine as Total Antimony**

All air samples collected for stibine analysis were below the detection limit of the method (0.4 µg SbH$_3$ per sample or 0.0002 µg Sb cm$^2$ hr$^{-1}$). Relative humidity measurements and observed condensation within the chambers indicated extremely high humidity, surpassing 95%. When the sorbent media was transferred from the collection tubes for extraction agglomeration of the silica beads was noted. It was hypothesized that high moisture levels in the chamber exhaust possibly interfered with collection of any stibine that may have been generated. The NIOSH method 6008 had only been evaluated at relative humidity as high as 85%.
Measured Antimony Levels in Test Materials with Fungal Growth

Bulk samples collected from fiberglass containing antimony trioxide (Sb₂O₃) were inoculated with *Aspergillus versicolor*. The mean (n = 5) concentration of antimony in the surface coating of ductboard materials before fungal growth was 255 µg (Sb) cm⁻² in materials used for Test #1 and 221 µg (Sb) cm⁻² in Test #2. Nine weeks after inoculation Test #1 materials had a mean antimony level of 191 µg (Sb) cm⁻² and Test #2 materials had reduced to 166 µg (Sb) cm⁻². These results show a reduction in antimony concentration of 25% for each test after 9 weeks of mold growth. The concentration of antimony after 9 weeks of fungal degradation was significantly reduced in both tests by 64 and 55 µg (Sb) cm⁻² or 25% (P-value <0.05). Table 8 shows the results of antimony measurements and biomass density of mold.

### Table 8: Antimony in fiberglass ductboard surface coating test material

<table>
<thead>
<tr>
<th>Week</th>
<th><em>Mold Density</em> µg (mold) cm⁻²</th>
<th>Antimony µg (Sb) cm⁻²</th>
<th>Std Dev</th>
<th><em>Mold Density</em> µg (mold) cm⁻²</th>
<th>Antimony µg (Sb) cm⁻²</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
<td>255</td>
<td>40.43</td>
<td>5</td>
<td>221</td>
<td>41.32</td>
</tr>
<tr>
<td>1</td>
<td>78</td>
<td>192</td>
<td>12.61</td>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>228</td>
<td>208</td>
<td>26.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>324</td>
<td>209</td>
<td>19.8</td>
<td>371</td>
<td>192</td>
<td>39.34</td>
</tr>
<tr>
<td>7</td>
<td>148</td>
<td>202</td>
<td>16.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>103</td>
<td>191</td>
<td>22.28</td>
<td>470</td>
<td>166</td>
<td>20.42</td>
</tr>
<tr>
<td>29</td>
<td>381</td>
<td>197</td>
<td>7.68</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*: No Data Collected

* The limit of quantification (LOQ) for fungal biomass for the method, using the conversion factors reported by Reeslev et al., were 0.8 µg mold/cm² and 0.5 µg mold/cm² for *Stachybotrys chartarum* (IBT 9695) and *Aspergillus versicolor* (IBT 16000) respectively.
Figure 15: Sb concentration and biomass density on fiberglass ductboard test #1

* Asterisks indicates mean antimony sample measurement was significantly different from initial measurement taken of test material $\alpha=0.05$.

Figure 16: Sb concentration and biomass density on fiberglass ductboard test #2

* Asterisks indicates mean antimony sample measurement was significantly different from initial measurement taken of test material $\alpha=0.05$. 
The mean concentration of antimony in new FSK Exterior Wrapping of ductboard materials at one week after inoculation, before fungal growth had become established, was 179 µg (Sb) cm\(^{-2}\). Nine weeks after inoculation these materials had a mean antimony level of 148 µg (Sb) cm\(^{-2}\) and after 29 weeks the mean antimony level had reduced to 101 µg (Sb) cm\(^{-2}\). These results show a reduction in antimony concentration of 17% after 9 weeks of mold growth and 43% after 29 weeks. The concentration of antimony after 29 weeks of fungal growth was significantly reduced by 78 µg (Sb) cm\(^{-2}\) or 43% (P<0.01).

Table 9: Antimony in fiberglass ductboard FSK exterior wrapping test material

<table>
<thead>
<tr>
<th>Week</th>
<th>Mold Density (µg (mold) cm(^{-2}))</th>
<th>Antimony (µg (Sb) cm(^{-2}))</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>179</td>
<td>35.19</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>167</td>
<td>18.97</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>188</td>
<td>22.16</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>148</td>
<td>7.35</td>
</tr>
<tr>
<td>14</td>
<td>36</td>
<td>150</td>
<td>22.64</td>
</tr>
<tr>
<td>29</td>
<td>445</td>
<td>101</td>
<td>25.71</td>
</tr>
</tbody>
</table>

Figure 17: Sb concentration and biomass density on FSK exterior wrapping

Ductboard Foil Backing
29 Weeks

* Asterisks indicates mean antimony sample measurement was significantly different from initial measurement taken of test material α=0.05.
Estimated Antimony Emission Rates

Test #1 on fiberglass ductboard surface coating suggests the mobilization took place over the first week of exponential mold growth, but Test #2 suggests the mobilization rate was relatively constant over the entire nine week period. Each scenario was examined. Loss of antimony from test materials was assumed to be from the antimony trioxide fire retardant reportedly applied during manufacturing. Because of limitations in the analytical method used, the molecular form of antimony present in the test material could not be directly determined. The available method only allowed for determination of total antimony in the solid substrate. Manufacturer documents indicated that antimony was applied to the product in the form of antimony trioxide as a fire retardant.

In Test #1 the substrate lost 25% of its initial mass of antimony per cm\(^2\) in the first week. This equates to 63 \(\mu g\) (Sb) cm\(^2\) over 1 week, or 168 hours, resulting in a theoretical generation rate of 0.38 \(\mu g\) (Sb) cm\(^2\) hr\(^{-1}\) (3.8 mg (Sb) m\(^2\) hr\(^{-1}\)). When mold growth slowed and biomass density began to subside, presumably due to nutrient limitations, antimony concentrations in the material stabilized. This accelerated release of antimony appears to occur during the rapid increase in mold biomass as indicated by the simultaneous increase in biomass density measurements.

The material in Test #2 lost 25% of its initial mass of antimony per cm\(^2\) at a relatively constant rate over the course of 9 weeks. This equates to 55 \(\mu g\) (Sb) cm\(^2\) over a 9 week, or 1,512 hours, resulting in a theoretical generation rate of 0.036 \(\mu g\) (Sb) cm\(^2\) hr\(^{-1}\).
hr\(^{-1}\) (0.36 mg (Sb) m\(^{-2}\) hr\(^{-1}\)). However, in this experiment mold growth did not subside during the testing. This pattern of biomass density suggests the fungal growth on this material had not reached its maximum and thus had not reached the stationary phase by 9 weeks of growth. This slower release of antimony appears to reflect the slower growth rate of this colony, as evidenced by the biomass density measurements.

The FSK exterior wrapping test material #3 lost 43% of its initial mass of antimony per cm\(^2\) over the course of 29 weeks. The trend of measurements appeared to be similar to Test #2 where the antimony was mobilized at a slow rate throughout the growth of the mold. This equates to 78 µg of (Sb) cm\(^2\) over the course of 29 weeks, or 4,872 hours, resulting in a theoretical generation rate of 0.016 µg (Sb) cm\(^2\) hr\(^{-1}\) (0.16 mg (Sb) m\(^{-2}\) hr\(^{-1}\)). The slow growth rate observed over the first 9 weeks of this experiment possibly reflects a slow downward trend in antimony concentration, but once exponential growth began at 29 weeks, a significant reduction in the antimony level in the test material was observed.

Initial antimony measurements were compared to antimony measurements taken at each subsequent period after mold growth had been established. Two-tailed t-tests of the sample means for each data set were performed to determine if they were statistically different at the alpha level of 0.05, with an assumption of unequal variance. Statistically different sample means are annotated with an asterisk on figures 15, 16 and 17.
Using the reduction of antimony concentrations from test materials, antimony aerosol generation rates were estimated. Generation rates for total antimony were calculated and used to model indoor air concentrations over the course of 1 week, 9 weeks and 29 weeks. A simple, single room mass balance model was used to estimate indoor air concentrations of antimony as the oxidative products of trimethylstibine or trimethylstibine oxides.

The generation rate of antimony estimated as an emission factor was 0.357 µg Sb cm\(^{-2}\) hr\(^{-1}\) for the condition of 25% reduction in 1 week; 0.0398 µg Sb cm\(^{-2}\) hr\(^{-1}\) for the condition of 25% reduction in 9 weeks; and 0.022 µg Sb cm\(^{-2}\) hr\(^{-1}\) for the condition of 43% reduction in 29 weeks. All of these theoretical emission factors are above the estimated limit of detection (0.0002 µg (Sb) cm\(^{-2}\) hr\(^{-1}\)) for the chamber test method used in the study.

Modeled Concentration of Trimethylstibine Oxides as Antimony

Equation 10: Used to calculate equilibrium concentration of antimony compounds in a 3,600 ft\(^2\) (334 m\(^2\)) home

\[ C_t = C_o + \frac{G}{Q} \left(1 - e^{-\frac{t}{\tau}}\right) \]

where  
- \( C_t \) = Concentration of Sb at time \( t \),  
- \( C_o \) = outdoor Sb concentration = 0,  
- \( G \) = generation rate of Sb in the space,
\[ Q = \text{volumetric airflow rate into and out of the space,} \]
\[ V = \text{volume of home,} \]
\[ I = \text{air exchange rate } (Q/V) \]

**Model assumptions:**

Volume = 815 m\(^3\) (28,800 ft\(^3\)) of a 3,600 ft\(^2\) home

Air Exchange Rate: 1 ACH

 Entire Duct System contained 61 m\(^2\) of mold growth on fire retardant treated duct.

 Containing 261.65 g of Sb\(_2\)O\(_3\)

 Single 32 ft (9.75 m) supply duct contained 8.18 m\(^2\) of mold growth on fire retardant treated duct. Containing 20.44 g of Sb\(_2\)O\(_3\)

All antimony was assumed to be released as the gaseous trimethylstibine as a result of fungal decomposition and rapidly oxidized to a particulate-phase trimethylstibine oxide.
Table 10: Modeled concentration of antimony as trimethylstibine oxides

<table>
<thead>
<tr>
<th>Source Description</th>
<th>Generation Rate (mg/hr)</th>
<th>Mass of Sb Mobilized</th>
<th>Resulting Concentration (mg/m$^3$)</th>
<th>Depicted in Figures 18 &amp; 19 as...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire Duct System</td>
<td>261.65 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25% in 1 week</td>
<td>373.81</td>
<td></td>
<td>0.4587</td>
<td>FIG 16: A</td>
</tr>
<tr>
<td>25% in 9 weeks</td>
<td>41.53</td>
<td></td>
<td>0.0510</td>
<td>FIG 16: B</td>
</tr>
<tr>
<td>43% in 29 weeks</td>
<td>23.1</td>
<td></td>
<td>0.0283</td>
<td>FIG 16: C</td>
</tr>
<tr>
<td>Single 32 ft duct</td>
<td>20.44 g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25% in 1 week</td>
<td>29.20</td>
<td></td>
<td>0.0358</td>
<td>FIG 17: D</td>
</tr>
<tr>
<td>25% in 9 weeks</td>
<td>3.25</td>
<td></td>
<td>0.0040</td>
<td>FIG 17: E</td>
</tr>
<tr>
<td>43% in 29 weeks</td>
<td>1.8</td>
<td></td>
<td>0.0022</td>
<td>FIG 17: F</td>
</tr>
</tbody>
</table>

Figure 18: Sb concentration in a home with all ducts supporting mold growth

![Graph showing estimated Sb concentration over time](image-url)
Discussion

Using *Scopulariopsis brevicaulis*, grown aerobically as a submerged culture in 500 ml flasks, volatile antimony compounds were measured. Using the reported data I estimated the generation rates from fungal growth in media amended with various sources of antimony. When antimony trioxide (Sb$_2$O$_3$) was added as the source of antimony, 0.004 µg Sb/hr was estimated to be mobilized over the 8 day experiment.
When potassium antimony tartrate (PAT) was added as the antimony source, 0.04 µg Sb/hr was estimated to be mobilized over the 8 day experiment. Because the experiment was performed on cultures in a liquid medium comparison with surface areas of growth cannot be made. When the initial amount of antimony added to the cultures is compared, the experiments by Jenkins et al. suggest that only 0.002% of the initial amount of antimony was mobilized in 8 days. However, the researchers concluded that increases in antimony concentration effected mobilized amounts in a disproportional manner. The amount of antimony mobilized only rose 2-fold when there was a 20-fold increase in the amount added to the cultures (Jenkins et al, 1998a).

The antimony emission rates calculated from measured reductions in antimony concentrations on ductboard materials were on the order of 10 to 100 times higher than those calculated from mobilization amounts reported by Jenkins et al. However, this was one of the few studies that reported measuring volatile antimony compounds from adding antimony trioxide to fungal cultures. Estimates of indoor antimony concentrations are maximum levels calculated using worst case conditions.

Without data identifying antimony in a gaseous form (stibine or trimethylstibine) the pathway of antimony mobilization from the antimony trioxide-treated building materials cannot be concluded. There are two possible mobilization pathways that may have occurred. The first is that fungal growth mobilized the antimony trioxide by uptake and biomethylation via the Challenger Mechanism. The trimethylstibine may have quickly oxidized and deposited onto the surfaces of the chamber or captured on the
exhaust filter before reaching the sorbent tube. The environmental fate of 
trimethylstibine in the environment is reported in the literature to be a multi-step process 
that results in formation of a mixture of antimony oxides and insoluble polymers. The 
residence time of trimethylstibine in the air is not expected to be long, requiring a 
constant source to result in a measurable amount.

The second possibility is that antimony trioxide was mobilized via mechanical 
means enhanced by fungal decomposition of the test materials. The antimony trioxide 
would have been in particulate form, and may have been shed along with fungal 
fragments, spores or degraded test material.

The two possible scenarios described above could result in occupant exposure to 
trimethylstibine oxides, antimony trioxide, or a combination of both compounds. The 
inhalation reference concentration (RfC) of antimony trioxide is 0.0002 mg/m³ (NAS, 
2000). An inhalton RfC has not been determined for trimethylstibine oxide or total 
antimony. Using the inhalation RfC for antimony trioxide a hazard index was estimated 
to range from 11, at the lowest emission rate, to 2,283 for the highest emission rate. Since 
the estimated concentrations do not represent lifetime exposures, calculation of a cancer 
risk was not performed.

When water damage occurs to building materials treated with antimony trioxide 
fire retardant, fungal growth can occur. This has been demonstrated on both components 
of fiberglass ductboard that contain antimony, specifically the surface coating and the
adhesive-FSK exterior wrap. Studies have also found fungal growth to occur on insulation materials, with and without added biocides (Ezeonu et al., 1994; Price et al., 1994; Samimi et al., 2003; Van Loo et al., 2004). Laboratory studies have demonstrated that at least some fungal species can biomethylate antimony, mobilizing it initially as the highly toxic volatile compound trimethylstibine, but rapidly oxidizing it to non-volatile precipitates of antimony. The purpose of this study was to determine if antimony trioxide, present on commonly used building materials, could mobilize antimony aerosols when fungal growth occurred. The volatile form of antimony was not successfully collected and identified, but material tests demonstrated significantly lower concentrations of antimony (P <0.05) remaining after fungal growth had occurred. Concentrations of antimony in test materials after fungal growth had occurred demonstrated a significant reduction from the original amount in three separate experiments. The major difference between each experiment was the rate at which the antimony concentrations reduced. The mechanisms causing variations in the mobilization rate of antimony from the test material could not be determined using this experimental design.
CONCLUDING REMARKS

Public Health Significance of Research

The etiologic agent of building occupant complaints, adverse health effects and disease outbreaks are often not determined specifically determined for any one case (O’Reilly, 1998). Rather, researchers and public health professionals often identify situations, conditions and contaminants that should not be present or are in excessively high concentrations. Guidance is then given to remove the contaminant sources and remedy the conditions as part of a trial and error approach. If the complaints cease or illnesses resolve, the recommendations are viewed as correct. Quite often the specific cause of an adverse health effect is not determined because no biomarkers are known to exist that indicate cessation of exposure. This has been the situation with most cases of fungal growth in indoor environments. Fungal growth in indoor environments has been associated with adverse health effects and when found, its removal is recommended (EPA, 2001; IOM, 2004; Macher, 1999). Research continues to identify fungal agents possibly responsible for occupant illnesses, but many symptoms and illnesses that are commonly attributed to fungal exposure have not been linked to measured fungal aerosols (IOM, 2004).
Some physicians and public health professionals in the United States posed a possible association between sudden infant death syndrome (SIDS) and exposure to moldy indoor environments. This proposed association resulted from the investigation of a cluster of infant deaths in the Cleveland area due to acute idiopathic pulmonary hemorrhage (AIPH) in 1993 (CDC, 1994; CDC, 1997; Etzel et al., 1998). The data collected during the initial investigation was reviewed by a CDC scientific task force and a very different conclusion was published in a March 2000 revision report. The post-hoc reviewers concluded that this study was not of sufficient quality to support an association between *S. chartarum* and AIPH. In addition, the reviewers noted that evidence from other sources supporting a causal role of *S. chartarum* in AIPH is limited. The task force concluded that *S. chartarum* was not clearly associated with AIPH (CDC, 2000).

Since the initial CDC report in 1994 two additional case reports of AIPH in infants have examined the link between pulmonary hemorrhage and exposure to *S. chartarum*. One case study published in 1999 identified likely exposure to *S. chartarum* and other fungi due to water damage in the home (Flappan et al., 1999). The second case report published in 2002 focused on isolation of *S. chartarum* from the lung of a child diagnosed with AIPH and identification of a new serine proteinase described as “satachyrase A” (Kordula et al., 2002).

Similarly in 1994 claims were made that fire retardants in crib mattresses were, in part, responsible for SIDS (Blair et al., 1995; Cook, 1994; Thompson & Faull, 1995). Researchers claimed that antimony and arsenic were released as volatile toxic forms into
the lungs of sleeping infants, resulting in death. Independent study conducted in England concluded that transformation of the trace concentrations of arsenic by bacteria into arsine gas was not possible under the aerobic conditions present in the mattress environment. However, possible inhalation exposure to antimony could not be dismissed. The authors of the study eventually concluded the emission of trimethylstibine would be too low to cause death and that SIDS had been occurring long before infants began sleeping on fire-retardant treated mattresses. The speculative identification of toxic gas formation in mattresses was eventually dismissed after study by the government appointed panel (DeWolff, 1995; Jenkins et al., 1998b; Limerick, 1998).

The available evidence clearly indicates that neither toxigenic fungi nor antimony fire retardants caused SIDS. However, the CDC investigators did not consider the potential for microbial release of antimony when evaluating the association between fungal growth on fire retardant-treated materials and AIPH. Antimony trioxide is strongly irritating to tissues and membranes. Of greater public health concern is the potential for chronic respiratory irritation resulting from exposure to antimony oxides including antimony trioxide released by fungal degradation of fire retardants.

In light of the documented mechanism and potential for antimony transformation by fungi from an immobile, non-volatile surface application to a volatile form that rapidly oxidizes and precipitates, the impacts on exposed occupant should be examined. The results of this limited study, while not conclusive, do support the speculation by previous
authors that fungal growth on antimony-containing building materials can mobilize the applied fire retardant. Further research on occupant exposures in environments where fungal growth on fire retardant-treated materials exists is justified. Populations with the greatest susceptibility such as infants, asthmatics and others with compromised respiratory systems could be adversely affected by exposure to antimony compounds in addition to allergens and irritants released from microbial sources. The potential for a synergistic effect should also be considered in both susceptible and healthy populations.

**Practical Applications of This Research**

Carbon Dioxide does not appear to be a sensitive indicator of hidden fungal growth in wall cavities and static HVAC Systems. Available sampling and analysis methods are impractical, expensive, or insensitive a new approach should be examined. Fluctuations in CO₂ generation rates that can be influenced by colony age, moisture deprivation and limited nutrient availability, make carbon dioxide a poor indicator of hidden fungal growth outside of laboratory conditions. Carbon dioxide could not be used to demonstrate the absence of fungal growth in a hidden cavity, as transient environmental conditions can inhibit CO₂ production of even well established colonies.

**Study Limitations**

Carbon dioxide generation rates were only examined during the initial log phase, and stationary phase of growth extending to 32 weeks. Trends in CO₂ generation rates for later stages of growth indicated that detectable carbon dioxide generation eventually ceased. Based upon the model used to predict concentrations within enclosed cavities at
least ¼ to ½ of the internal surface area must have fungal growth in order to detect a significantly elevated CO₂ concentration. While metabolism may be an important factor in the mobilization of antimony fire retardants, fungi also produce allergens and other irritants that may have an adverse impact on building occupants and remediation personnel. Until a full understanding of all the hazards that indoor mold growth presents, care must be taken to prevent human exposure and to effectively remediate conditions that allow fungal growth in indoor environments.

Measurement methods for trimethylstibine were based on detection of total volatile antimony, and were not capable of speciating the valence forms or molecular forms present. The rapid oxidation rate of trimethylstibine, on the order of 10⁶ M⁻¹ s⁻¹ most likely caused the precipitation of any trimethylstibine that was generated to particulate-phase oxides (Parris & Brinckman, 1976). The presence of an in-line filter may have captured antimony oxides before they reached the sorbent tube, but no analysis of the filter was ever performed. Additionally, high humidity of the test chambers may have interfered with collection or recovery of any trimethylstibine that may have been generated. Because a positive control test was not performed, due to the unavailability of a safe gas-phase standard and the explosive nature of stibine generators, full confidence in the ability to detect trimethylstibine or trimethylstibine oxides was not achieved.

Estimates of biomass present in samples were based upon conversion factors calculated from experiments performed in Denmark by Reeslev et al (2003). While the same strains of fungal species used to calculate the conversion factors were used in this
experiment, differences between growth substrates and laboratory conditions could have biased the results in an undetermined manner. As the repeated experiments with fiberglass ductboard surface coating demonstrated, growth rates can be different due to unaccounted for variables. Despite recognized limitations in the calculated biomass, based on the fluorometric assay of $\beta$-$N$-acetylhexosaminidase activity, these results were considered more reflective of biomass than traditional mycological methods that rely upon serial dilution and colony formation on nutrient agar. The important component of fungal growth considered here is hyphae formation, which typically accounts for 95-97% of fungal colony biomass. Culture based methods rely upon quantifying viable spores which account for only 3-5% of fungal biomass. The use of mold colony counts (CFU) is, to a large extent, a measure of sporulation, not fungal biomass (Schnurer, 1993).

**Recommendations for Future Research**

Examination of antimony trioxide fire retardant application to indoor products and building materials should be performed. A broad-based assessment of fire retardant treated materials currently in buildings should be performed to evaluate the impact of age and microbial degradation. Better indicators of exposure to antimony should be researched. Measurement of antimony in urine and feces only evaluates the portion that is absorbed into the body. Damage to the respiratory system due to surface irritation in the lungs, may not reflect the damage caused by unabsorbed antimony compounds.

An assessment of antimony exposures in homes could provide a better understanding of population risks. Beginning in 2005, CDC's National Health and
Nutrition Examination Survey (NHANES) will collect dust samples from approximately 7,000 participants' homes each year. Analysis of dust for antimony could help establish what levels Americans are exposed to. When researchers perform studies that attempt to assess mold exposure, they should also document the type of material that is supporting mold growth within a building. Air samples for antimony in moldy indoor environments could be compared to air concentrations and personal exposure samples for antimony in environments without mold contamination.

Field portable X-Ray Fluorescence devices, using X-ray tube technology or an Americium-source, can directly measure Sb on surfaces. If a survey determines that mold growth is occurring on antimony-containing materials, the difference between surfaces with mold growth and surfaces not supporting mold growth could indicate if mobilization of antimony into the indoor environment may be taking place.

Future research on the public health impacts of biological contamination of indoor environments should include assessment of aerosols released from the microbial degradation of building materials such as synthetic floorings, wall coverings, coatings, paints, adhesives and insulation. The potential for toxic and irritating aerosol emissions from the microbial break down or biomethylation of additives should be examined in depth. Understanding these seldom evaluated aerosols may help to explain the inconsistency of study findings contributing to the paradox of mold exposures and human health effects.
REFERENCES


ACGIH. (1986). Documentation of the Threshold Limit Values and Biological Exposure Indices. 5th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.


Appendix 1: Carbon Dioxide Quality Control Data

Table 11: Empty chamber #4 on 5/29/2004

<table>
<thead>
<tr>
<th>Hr</th>
<th>µg/hr</th>
<th>µg hr⁻¹ cm⁻²</th>
<th>Ambient (ppm)</th>
<th>Chamber (ppm)</th>
<th>Area (cm²)</th>
<th>Diff CO₂</th>
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n=4  Mean  -0.07 µg hr⁻¹ cm⁻²  -1.25

Figure 20: Monitoring data from an empty chamber on 5/29/2004
Appendix 1 (Continued)

Table 12: Gypsum wallboard control CO$_2$ test on 5/22/2004

<table>
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<th>Hr</th>
<th>µg/hr</th>
<th>µg hr$^{-1}$ cm$^2$</th>
<th>Ambient (ppm)</th>
<th>Chamber (ppm)</th>
<th>Area (cm$^2$)</th>
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n=13  Mean  -0.01 µg hr$^{-1}$ cm$^2$  -0.08

Table 13: Ductboard surface coating control CO$_2$ test on 3/29/2004

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<th>µg hr$^{-1}$ cm$^2$</th>
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n=16  Mean  0.11 µg hr$^{-1}$ cm$^2$  2.13
Table 14: Ductboard surface coating control CO\(_2\) test on 4/49/2004

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<th>µg hr(^{-1}) cm(^2)</th>
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<th>Chamber (ppm)</th>
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</tbody>
</table>

n=5  
Mean  
0.14 µg hr\(^{-1}\) cm\(^2\)  
2.51

Figure 21: Monitoring data from control chamber on 4/49/2004
Appendix 1 (Continued)

Table 15: Ductboard surface coating control CO$_2$ test on 4/11/2004

<table>
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<th>Hr</th>
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</table>

n=6  Mean  -0.21  µg hr$^{-1}$ cm$^2$  -3.83

Table 16: Ductboard surface coating control CO$_2$ test on 4/25/2004

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</tr>
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<td>10</td>
<td>-108</td>
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<td>438</td>
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<td>-4</td>
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<td>11</td>
<td>135</td>
<td>0.27</td>
<td>427</td>
<td>432</td>
<td>492.3</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>-54</td>
<td>-0.11</td>
<td>426</td>
<td>424</td>
<td>492.3</td>
<td>-2</td>
</tr>
<tr>
<td>13</td>
<td>108</td>
<td>0.22</td>
<td>411</td>
<td>415</td>
<td>492.3</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>-54</td>
<td>-0.11</td>
<td>407</td>
<td>405</td>
<td>492.3</td>
<td>-2</td>
</tr>
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<td>15</td>
<td>-54</td>
<td>-0.11</td>
<td>403</td>
<td>401</td>
<td>492.3</td>
<td>-2</td>
</tr>
<tr>
<td>16</td>
<td>-81</td>
<td>-0.16</td>
<td>400</td>
<td>397</td>
<td>492.3</td>
<td>-3</td>
</tr>
</tbody>
</table>

n=8  Mean  0.00  µg hr$^{-1}$ cm$^2$  0.00
Table 17: Ductboard FSK exterior wrap control CO$_2$ test on 5/2/2004

<table>
<thead>
<tr>
<th>Hr</th>
<th>µg/hr</th>
<th>µg hr$^{-1}$ cm$^{-2}$</th>
<th>Ambient (ppm)</th>
<th>Chamber (ppm)</th>
<th>Area (cm$^2$)</th>
<th>Diff CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0</td>
<td>0.00</td>
<td>419</td>
<td>419</td>
<td>492.3</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>81</td>
<td>0.16</td>
<td>412</td>
<td>415</td>
<td>492.3</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>27</td>
<td>0.05</td>
<td>416</td>
<td>417</td>
<td>492.3</td>
<td>1</td>
</tr>
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<td>12</td>
<td>27</td>
<td>0.05</td>
<td>413</td>
<td>414</td>
<td>492.3</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>0.05</td>
<td>413</td>
<td>414</td>
<td>492.3</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>-54</td>
<td>-0.11</td>
<td>417</td>
<td>415</td>
<td>492.3</td>
<td>-2</td>
</tr>
<tr>
<td>15</td>
<td>27</td>
<td>0.05</td>
<td>414</td>
<td>415</td>
<td>492.3</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>135</td>
<td>0.27</td>
<td>405</td>
<td>410</td>
<td>492.3</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>-135</td>
<td>-0.27</td>
<td>424</td>
<td>419</td>
<td>492.3</td>
<td>-5</td>
</tr>
<tr>
<td>18</td>
<td>27</td>
<td>0.05</td>
<td>405</td>
<td>406</td>
<td>492.3</td>
<td>1</td>
</tr>
<tr>
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<td>0.27</td>
<td>400</td>
<td>405</td>
<td>492.3</td>
<td>5</td>
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<td>81</td>
<td>0.16</td>
<td>394</td>
<td>397</td>
<td>492.3</td>
<td>3</td>
</tr>
</tbody>
</table>

n=12
Mean 0.06 µg hr$^{-1}$ cm$^{-2}$ 1.17

Figure 22: Monitoring data from a control chamber on 5/2/2004
Appendix 2: Antimony Quality Control Data

To evaluate the potential for antimony mobilization from fiberglass ductboard surface coating a control set of test materials were initially measured on 3/28/2004. The results are provided in Table 11 below.

Table 18: Antimony analysis results for control test pieces on 3/28/2004

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Measured Absorbance</th>
<th>µg Sb/15 mL</th>
<th>µg Sb/mL (Analyte)</th>
<th>µg Sb/25 mL Extract</th>
<th>µg Sb/cm²</th>
<th>Mean</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.9381</td>
<td>12.2385</td>
<td>0.8159</td>
<td>305.9615</td>
<td>199</td>
<td>220.69</td>
<td>41.32</td>
</tr>
<tr>
<td>7</td>
<td>0.9465</td>
<td>12.3462</td>
<td>0.8231</td>
<td>308.6538</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.8286</td>
<td>10.8346</td>
<td>0.7223</td>
<td>270.8654</td>
<td>176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.2136</td>
<td>15.7705</td>
<td>1.0514</td>
<td>394.2628</td>
<td>256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.2926</td>
<td>16.7833</td>
<td>1.1189</td>
<td>419.5833</td>
<td>272</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These same materials were later inoculated on 5/30 and measured for Sb content, and fungal enzyme activity, but not CO₂ production. The results are provided in Table 12 below.

Table 19: Antimony analysis results for control test pieces on 5/30/2004

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Measured Absorbance</th>
<th>µg Sb/15 mL</th>
<th>µg Sb/mL (Analyte)</th>
<th>µg Sb/25 mL Extract</th>
<th>µg Sb/cm²</th>
<th>Mean</th>
<th>Std Dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>134</td>
<td>0.9182</td>
<td>10.4923</td>
<td>0.6995</td>
<td>262.3081</td>
<td>170</td>
<td>221.40</td>
<td>45.29</td>
</tr>
<tr>
<td>135</td>
<td>1.5038</td>
<td>17.4061</td>
<td>1.1604</td>
<td>435.1535</td>
<td>283</td>
<td></td>
<td></td>
</tr>
<tr>
<td>136</td>
<td>1.0551</td>
<td>12.1086</td>
<td>0.8072</td>
<td>302.7155</td>
<td>197</td>
<td></td>
<td></td>
</tr>
<tr>
<td>137</td>
<td>1.099</td>
<td>12.6269</td>
<td>0.8418</td>
<td>315.6730</td>
<td>205</td>
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<tr>
<td>138</td>
<td>1.3472</td>
<td>15.5573</td>
<td>1.0372</td>
<td>388.9315</td>
<td>253</td>
<td></td>
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</tr>
</tbody>
</table>
Appendix 2 (Continued)

The measurements were taken 10 weeks apart (March 28 until May 30). The mean sample concentration measured on March 28 was 220.69 µg (Sb)cm$^2$ and 221.40 µg (Sb)cm$^2$ on May 30. When these values are rounded to three significant digits they are identical. The sample results do not demonstrate a change in mean antimony concentration. All antimony analyses were performed using the same calibration standards prepared on 3/28/2004. Calibration curves were determined before analysis of each sample set. The calibration curves are provided below in Table 13 and Figures 23 through 31.
### Table 20: Antimony calibration data

<table>
<thead>
<tr>
<th>Calibration Data 3-28-04</th>
<th>Calibration Data 4-4-04</th>
<th>Calibration Data 4-11-04</th>
</tr>
</thead>
<tbody>
<tr>
<td>ug Sb/15 ml</td>
<td>ug Sb/15 ml</td>
<td>ug Sb/15 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>Measured Absorbance</td>
<td>Predicted Absorbance</td>
</tr>
<tr>
<td>0</td>
<td>0.0049</td>
<td>-0.0165</td>
</tr>
<tr>
<td>0.75</td>
<td>0.0491</td>
<td>0.0420</td>
</tr>
<tr>
<td>1.5</td>
<td>0.0888</td>
<td>0.1005</td>
</tr>
<tr>
<td>3.75</td>
<td>0.2715</td>
<td>0.2760</td>
</tr>
<tr>
<td>7.5</td>
<td>0.5451</td>
<td>0.5685</td>
</tr>
<tr>
<td>15</td>
<td>1.1607</td>
<td>1.1535</td>
</tr>
<tr>
<td>22.5</td>
<td>1.7417</td>
<td>1.7385</td>
</tr>
</tbody>
</table>

\[
y = 0.078x - 0.0165 \\
R^2 = 0.9995 \\
x = \frac{(\text{Abs} + 0.0165)}{0.078}
\]

<table>
<thead>
<tr>
<th>Calibration Data 4-24-04</th>
<th>Calibration Data 5-2-04</th>
<th>Calibration Data 5-16-04</th>
</tr>
</thead>
<tbody>
<tr>
<td>ug Sb/15 ml</td>
<td>ug Sb/15 ml</td>
<td>ug Sb/15 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>Measured Absorbance</td>
<td>Predicted Absorbance</td>
</tr>
<tr>
<td>0</td>
<td>0.0058</td>
<td>0.0157</td>
</tr>
<tr>
<td>0.75</td>
<td>0.0881</td>
<td>0.0747</td>
</tr>
<tr>
<td>1.5</td>
<td>0.1486</td>
<td>0.1338</td>
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<td>3.75</td>
<td>0.3052</td>
<td>0.3108</td>
</tr>
<tr>
<td>7.5</td>
<td>0.5666</td>
<td>0.6060</td>
</tr>
<tr>
<td>15</td>
<td>1.2398</td>
<td>1.1962</td>
</tr>
<tr>
<td>22.5</td>
<td>1.7715</td>
<td>1.7865</td>
</tr>
</tbody>
</table>

\[
y = 0.0787x + 0.0157 \\
R^2 = 0.9984 \\
x = \frac{(\text{Abs} - 0.0157)}{0.0787}
\]

<table>
<thead>
<tr>
<th>Calibration Data 5-30-04</th>
<th>Calibration Data 6-27-04</th>
<th>Calibration Data 7-31-04</th>
</tr>
</thead>
<tbody>
<tr>
<td>ug Sb/15 ml</td>
<td>ug Sb/15 ml</td>
<td>ug Sb/15 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>Measured Absorbance</td>
<td>Predicted Absorbance</td>
</tr>
<tr>
<td>0</td>
<td>0.0077</td>
<td>0.0295</td>
</tr>
<tr>
<td>0.75</td>
<td>0.1044</td>
<td>0.0930</td>
</tr>
<tr>
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<td>0.1684</td>
<td>0.1566</td>
</tr>
<tr>
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<td>0.3350</td>
<td>0.3471</td>
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<td>7.5</td>
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<td>0.6648</td>
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<tr>
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<td>1.3000</td>
</tr>
<tr>
<td>22.5</td>
<td>1.9217</td>
<td>1.9353</td>
</tr>
</tbody>
</table>

\[
y = 0.0847x + 0.0295 \\
R^2 = 0.9995 \\
x = \frac{(\text{Abs} - 0.0295)}{0.0847}
\]
Appendix 2 (Continued)

Figure 23: Antimony calibration curve 3-28-04

\[ y = 0.078x - 0.0165 \]
\[ R^2 = 0.9995 \]
\[ x = (\text{Abs} + 0.0165) / 0.078 \]
Appendix 2 (Continued)

Figure 24: Antimony calibration curve 4-4-04

\[ y = 0.0788x - 0.0026 \]

\[ R^2 = 0.9994 \]

\[ x = (\text{Abs} + 0.0026) / 0.0788 \]

Absorbance @ 552 nm vs. ug Sb/15 mL
Figure 25: Antimony calibration curve 4-11-04

\[ y = 0.0831x + 0.0113 \]
\[ R^2 = 0.9995 \]
\[ x = (\text{Abs} - 0.0113)/0.0831 \]

y = 0.0831x + 0.0113
R² = 0.9995
x = (Abs - 0.0113)/0.0831
Appendix 2 (Continued)

Figure 26: Antimony calibration curve 4-24-04

\[ y = 0.0787x + 0.0157 \]
\[ R^2 = 0.9984 \]
\[ x = \frac{\text{Abs} - 0.0157}{0.0787} \]

Absorbance @ 552 nm vs. ug Sb/15 mL
Appendix 2 (Continued)

Figure 27: Antimony calibration curve 5-2-04

\[
y = 0.083x + 0.0292 \\
R^2 = 0.9976 \\
x = (\text{Abs} - 0.0292) / 0.083
\]
Figure 28: Antimony calibration curve 5-16-04

\[ y = 0.0859x + 0.0218 \]
\[ R^2 = 0.9991 \]
\[ x = (\text{Abs} - 0.0218)/0.0859 \]
Appendix 2 (Continued)

Figure 29: Antimony calibration curve 5-30-04

y = 0.0847x + 0.0295
R² = 0.9995
x=(Abs-0.0295)/0.0847
Appendix 2 (Continued)

Figure 30: Antimony calibration curve 6-27-04

\[ y = 0.0882x + 0.0208 \]
\[ R^2 = 0.9995 \]
\[ x = (\text{Abs-0.0208}) / 0.0882 \]
Appendix 2 (Continued)

Figure 31: Antimony calibration curve 7-31-04

\[ y = 0.087x + 0.0122 \]
\[ R^2 = 0.9989 \]
\[ x = \frac{(Abs - 0.0122)}{0.087} \]

\begin{align*}
0.0051 & \quad 0.0914 & \quad 0.1593 & \quad 0.3237 & \quad 0.6308 & \quad 1.9558 \\
1.3546 & \quad & & & & \\
\end{align*}
About the Author

John David Krause received a Bachelor of Science Degree in Biological Sciences from Florida State University in 1989. While at Florida State he received an ROTC scholarship and the Distinguished Military Graduate Award. He served in the U.S. Army from 1989 until 1993 in Germany. He completed active duty in 1993 and was honorably discharged with the rank of Captain.

Mr. Krause served as the Florida Department of Health’s Industrial Hygienist and Indoor Air Quality Programs Coordinator from 1993 to 1996. In 1997 he founded Indoor Air Solutions, an indoor air quality consulting firm. While continuing to work, Mr. Krause completed a Masters of Science in Public Health, in Toxicology at the University of South Florida, College of Public Health in 1999.

While in the USF Ph.D. program he co-authored a paper in *Applied Occupational and Environmental Hygiene*, and presented at international conferences in Helsinki, Finland and Monterey, California.