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Healthy Homes Issues: Mold

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Preface

In 1998, Congress appropriated funds and directed the U.S. Department of Housing and Urban Development (HUD) to “develop and implement a program of research and demonstration projects that would address multiple housing-related problems affecting the health of children.” In response, HUD solicited the advice of experts in several disciplines and developed a preliminary plan for the Healthy Homes Initiative (HHI). The primary goal of the HHI is to protect children from housing conditions that are responsible for multiple diseases and injuries. As part of this initiative, HUD has prepared a series of papers to provide background information to their current HHI grantees, as well as other programs considering adopting a healthy homes approach. This background paper focuses on molds and provides a brief overview of the current status of knowledge on:

- The extent and nature of mold hazards in the home;
- Assessment methods for mold hazards in the home;
- Mitigation methods for mold hazards in the home; and
- Information needs in the field of mold research.

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Healthy Homes Issues: Mold

1.0 OVERVIEW OF THE PROBLEM

There are over 200 species of fungi to which people are routinely exposed indoors and outdoors (NAS, 2000). These include mold-like fungi, as well as other fungi such as yeasts (unicellular fungi forming pasty colonies) and mushrooms, which are characterized by the familiar fruiting bodies people think of as “mushrooms.” The terms “mold” and “mildew” are non-technical names commonly used to refer to any fungus that is growing in the indoor environment (Burge and Otten, 1999). These names are used interchangeably, although mildew is often applied to growths on fabrics, window sills or bathroom tiles. Because molds and mildews may be any of several natural classes of fungi, these names are not interchangeable with the nomenclature used in biological classification systems (Burge and Otten, 1999).

In general, molds are characterized by a visible vegetative body, or colony, composed of a network (mycelium) of threadlike filaments (hyphae), which infiltrate the mold’s food or habitat. Mold colonies may appear cottony, velvety, granular, or leathery, and may be white, gray, black, brown, yellow, greenish, or other colors (Burge and Otten, 1999). Many reproduce via the production and dispersion of spores. They are usually saprophytes (i.e., they feed on dead organic matter) and, provided with sufficient moisture, can live off of many materials found in homes, such as wood, cellulose in the paper backing on drywall, insulation, wallpaper, glues used to bond carpet to its backing, and everyday dust and dirt.

Research indicates that certain molds can cause a variety of adverse human health effects, including allergic reactions and immune responses (e.g., asthma), infectious disease (e.g., histoplasmosis¹), and toxic effects (e.g., aflatoxin-induced liver cancer) (ACGIH, 1999). Molds are thought to play a role in asthma in several ways. They are known to produce a large number of proteins that are potentially allergenic, and there is sufficient evidence to support associations between fungal allergen exposure and asthma exacerbation and upper respiratory disease (NAS, 2000). In addition, molds may play a role in asthma via release of irritants that increase potential for sensitization, or release of toxins (mycotoxins) that affect immune response (NAS, 2000). Finally, mold toxins can cause direct lung damage leading to pulmonary diseases other than asthma (NAS, 2000).

2.0 EXTENT AND NATURE OF MOLD HAZARDS IN THE HOME

2.1 Environmental and Housing Factors Affecting Mold Growth

In indoor environments, mold originates from two sources: mold infiltrating from outdoors (e.g., through open windows), and mold colonization on the interior of the home. Molds can obtain nutrients and moisture sufficient for growth from water-affected building materials such

¹ A disease caused by the inhalation of spores of the fungus *Histoplasma capsulatum* (associated with bird or bat droppings); disease is most often asymptomatic but occasionally produces acute pneumonia or an influenzalike illness and spreading to other organs and systems in the body.

as wallboard and insulation materials, as well as carpets, furniture, and clothing. Using a score system based on material bioavailability, Gravesen et al. (1999) evaluated the susceptibility of various building materials to mold attack. They found that the products most vulnerable to mold attack were water damaged, aged organic materials containing cellulose, such as wooden materials, jute, wallpaper, and cardboard.

Different fungal species vary with regard to environmental conditions required for optimal growth, but all are influenced by moisture, temperature, light, and the substrate nutrient concentration and type (Burge and Otten, 1999). One of the most important factors affecting mold growth in homes, however, is moisture level. In general, most molds require fairly wet conditions (near saturation), lasting for many days, to extensively colonize an environment (NAS, 2000). However, the U.S. Environmental Protection Agency (USEPA) and the Centers for Disease Control and Prevention (CDC) recommend that it should be assumed that buildings or materials soaked for more than 48 hours are contaminated with mold unless proven otherwise by adequate environmental sampling or cleaned according to EPA recommendations (USEPA, 2001; CDC, 2005). In addition to affecting the extent of mold colonization, moisture availability can also affect the types of fungi present. For example, certain *Penicillium* species grow in relatively dry environments (e.g., in house dust with a high relative humidity), while others, such as *Basidiomycetes* and *Stachybotrys* species, require continuously wet substrates such as soaked wallboard, water reservoirs for humidifiers, or drip pans (Burge and Otten, 1999; Bush and Portnoy, 2001). Relative humidity also affects spore release for some molds (e.g., *Aspergillus* and *Penicillium*), with spore release occurring with lowering humidity after initial growth at high humidity levels (Foarde et al., 1997a). One reviewer concluded that “the worst-case scenario for the development of an indoor mold problem involves a series of water intrusion events that allow large quantities of biomass and mycotoxins to form, then a period of drying that promotes the dispersion of spores and colony fragments, followed by their deposition throughout the building” (Fog Nielsen, 2003).

As moisture availability changes, it has been observed that the species composition (i.e., the rank order of dominant species) will also often change. Some of the most abundant fungi genera found in homes without severe water damage include: *Alternaria*, *Cladosporium*, *Penicillium*, yeasts, and *Aspergillus* (Burge and Otten, 1999; American Academy of Pediatrics, 1998; Bush and Portnoy, 2001; Gravesen et al., 1999). Most of these molds do not typically produce mycotoxins (Etzel, 2000), but may be important as sources of mold allergens. In contrast, under certain very damp conditions (i.e., in the presence of water-soaked cellulosic materials), toxin producing *Stachybotrys chartarum* may be prominent (Flannigan, 1997). In general, whether or not a potentially toxigenic fungus produces toxins is dependent on environmental conditions and nutrient source (Burge and Ammann, 1999).

Housing features that can increase moisture levels and growth of mold include poor ventilation, excess production or condensation of water in the house (e.g., humidifiers, unvented clothes dryers), and water leakage or flooding (Lawton et al., 1998; Gravesen et al., 1999). Basements are likely to have higher mold concentrations than other indoor areas, especially in the winter (Ren et al., 1999). Li and Kendrick (1995) investigated 15 homes in Ontario and found that overall fungal levels (as assessed by counting spores in environmental samples) were highest in living rooms, followed by family rooms, kitchens, bathrooms, and bedrooms. Also in this

study, it was observed that fungal levels increased with the presence of damp conditions and carpets, and decreased where forced-air heating systems, dehumidifiers, air filters, and air conditioners were present. Douwes et al. (1999) also found that fungal levels, as assessed by measurement of extracellular polysaccharide (EPS) fungal cell wall components from *Aspergillus* and *Penicillium* species (EPS-*Asp/Pen*), were highest in living room floor dust. In addition, EPS-*Asp/Pen* levels were 2 to 3 times higher on carpeted floors than on smooth floors, and this was confirmed by another study that adjusted for repeated measures (Chew et al., 2001). However, Ren et al. (2001) found that fungal spores in indoor air could not be consistently predicted by housing characteristics. In Ren's study, surrogate measures of fungal presence in homes, such as damp spots, water leakage, or water damage, as reported by household questionnaires, were not significantly related to the presence of culturable fungi measured in indoor air. Of note, geographic differences in home furnishings and climate should be considered when evaluating home characteristics and concentrations of fungi in air or dust samples (Chew et al., 2003).

Several studies have characterized mold in homes without significant moisture problems or visual mold growth (Chew et al., 2003; Gots et al., 2003; Su et al., 2001; Solomon, 1975; Ren et al., 1999; and Horner et al., 2004). Horner et al. (2004) reported the results of one such HUD-funded study that was conducted in 50 post-1945 detached single family homes in metropolitan Atlanta, Georgia. Indoor and outdoor air and interior settled dust samples were collected in summer and winter and culturable fungi were counted and identified. Although higher airborne mold concentrations were found in the indoor and outdoor samples collected in the summer, the indoor samples collected did not differ by rankings of mold type prevalence or abundance with outdoor samples. Water indicator fungi (*Chaetomium*, *Ulocladium*, and *Stachybotrys*) were identified in only 3% of the settled dust samples plated out on two different types of media. The researchers also reported that "leaf surface fungi" (e.g., *Cladosporium*, *Alternaria*, *Epicoccum*, and *Curvularia*) represented > 20% of the total colonies in at least 85% of the settled dust samples (thus, replicate dust samples with < 20% of colonies from leaf surface fungi may be indicative of a mold/moisture problem).

2.2 Exposure and Health Effects

The Institute of Medicine of the U.S. National Academy of Sciences recently published a comprehensive review of the scientific literature regarding the relationship between damp or moldy indoor environments and the manifestation of adverse health effects, particularly respiratory and allergic symptoms (IOM, 2004). Table 1 summarizes the Institute's findings regarding the strength of the association between health outcomes and (a) exposure to damp indoor environments or (b) the presence of mold or other agents in damp indoor environments. (It was necessary to present findings regarding associations with the presence of mold or other agents separately from those regarding exposure to damp indoor environments in general, because much of the reviewed literature did not collect objective measurements of biogenic agents in damp indoor environments.)

The Institute did not find sufficient evidence of a *causal* relationship with any health outcomes. However, the Institute found sufficient evidence of an association between both categories of exposure and symptoms of the upper respiratory tract (nasal and throat), asthma symptoms in sensitized asthmatic persons, wheeze, and cough. They also found sufficient evidence of an

association between mold or bacteria in damp indoor environments and hypersensitivity pneumonitis in susceptible persons (i.e., persons with a family history sensitivity). In addition, the Institute found limited or suggestive evidence of an association between both categories of exposure and lower respiratory illness in otherwise healthy children. They also found suggestive evidence of an association between exposure to damp indoor environments (but not the presence of mold) and dyspnea (shortness of breath) and asthma development. Finally, the Institute concluded that evidence was inadequate or insufficient to determine an association with many other health effects, including but not limited to airflow obstruction (in otherwise healthy persons), mucous membrane irritation syndrome, and chronic obstructive pulmonary disease. “Inadequate or insufficient evidence” does not rule out the possibility of an association; it generally means that published studies did not have the quality, consistency or statistical power to permit a conclusion about an association. The Institute of Medicine also stated that “these conclusions are not applicable to immunocompromised persons, who are at increased risk for fungal colonization or opportunistic infections.”

Mold exposure in homes occurs primarily via inhalation of airborne spores and fungal fragments; some airborne fragments have very small particle size and may be far more numerous than airborne spores (Green et al., 2005; and Gorny et al., 2002). Molds are also present in household dust and on surfaces, with exposure occurring when particles are disturbed and become airborne or, less commonly in residential situations, through dermal contact or ingestion. Release of mold spores or fragments into indoor air from mold colonies is usually dependent on some sort of mechanical disturbance, although for some types of molds slight air movement may be sufficient (e.g., air movement by a fan), or spores may become airborne through natural spore discharge mechanisms. Most molds release spores ranging in size from 2 to 10 μm (although some genera, such as *Alternaria*, have conidia (a type of spore) ranging from 20-60 μm), but some may be released as chains or clumps of spores (NAS, 2000).

Allergens. Many molds produce numerous protein or glycoprotein allergens capable of causing allergic reactions in people. These allergens have been measured in spores, as well as other fungal fragments (Green et al., 2005; Sporik, 1993); however, most allergen seems to be located in germinating spores, in the hyphal tips, and in mycelia (Mitakakis et al., 2001; Green et al., 2003). Some of the major fungal allergens identified and isolated to date include those from *Aspergillus fumigatus*, *Aspergillus oryzae*, *Alternaria alternata*, *Cladosporium herbarum*, *Penicillium citrinum*, *Penicillium chrysogenum*, *Trichophyton tonsurans*, *Malassezia furfur*, and *Psilocybe cubensis* (NAS, 2000). An estimated 6-10% of the general population and 15-50% of those who are genetically susceptible (atopic) are sensitized to mold allergens (NAS, 2000). Research clearly indicates that exposure to mold plays a role in the exacerbation of asthma symptoms in sensitized individuals, although the association between mold exposure and asthma development remains undetermined (IOM, 2004; NAS, 2000). The clearest association between mold exposure and asthma is for sensitization to *Alternaria* (Halonen et al., 1997; Perzanowski et al., 1998), although this may be because the allergens of this genus (*Alt a 1* and *Alt a 2*) are well characterized relative to other mold species (Ibarrola et al., 2004; Asturias et al., 2005; NAS, 2000; Platts-Mills and Woodfolk, 2000).

Table 1. Summary of Institute of Medicine Findings Regarding the Association Between Health Outcomes and (1) Exposure to Damp Indoor Environments or (2) the Presence of Mold or Other Agents in Damp Indoor Environments

Sufficient Evidence of a Causal Relationship
(no outcomes met this definition)

Sufficient Evidence of an Association

Upper respiratory (nasal and throat) tract symptoms	Wheeze
Asthma symptoms in sensitized asthmatic persons	Cough
Hypersensitivity pneumonitis in susceptible persons ^a	

Limited or Suggestive Evidence of an Association

Lower respiratory illness in otherwise-healthy children	Asthma development ^b
Dyspnea (shortness of breath) ^b	

Inadequate or Insufficient Evidence to Determine Whether an Association Exists

Airflow obstruction (in otherwise-healthy persons)	Skin symptoms
Mucous membrane irritation syndrome	Gastrointestinal tract problems
Chronic obstructive pulmonary disease	Fatigue
Inhalation fevers (non-occupational exposures)	Neuropsychiatric symptoms
Lower respiratory illness in otherwise-healthy adults	Cancer
Rheumatologic and other immune diseases	Reproductive effects
Acute idiopathic pulmonary hemorrhage in infants	

Note: This table combines two tables from the IOM report: one that summarized findings regarding the association between health outcomes and exposure to damp indoor environments, the other that summarized findings regarding the association between health outcomes and the presence of mold or other agents in damp indoor environments. The Institute presented its findings in two tables because much of the reviewed literature did not identify or isolate the active agents in damp indoor environments. Differences between the two IOM tables are identified in notes a and b. Furthermore, these conclusions are not applicable to immunocompromised persons, who are at increased risk for fungal colonization or opportunistic infections.

^a For the presence of mold or bacteria in damp indoor environments.

^b For exposure to damp indoor environments. The Institute of Medicine found inadequate or insufficient evidence to determine whether an association exists between dyspnea and asthma development and the presence of mold or other agents in damp indoor environments.

Results of skin-prick testing² of 1,286 children with asthma in the National Cooperative Inner City Asthma Study's (NCICAS) showed that the most common positive allergen sensitivity in these children was to *Alternaria* (38%), followed by cockroach (36%) and the *Dermatophagoides pteronyssinus* house dust mite (31%) (Kattan et al., 1997).

While detecting allergic sensitization to molds is difficult in infants, some data suggest that infants at risk for developing allergic disease experience respiratory symptoms which may or may not be allergic in nature. In a study conducted by Belanger et al. (2003), a positive exposure-response was found between levels of mold (measured by a portable air sampler) in the home and wheeze/persistent cough in the first year of life among children whose mothers had asthma, and between mold levels and persistent cough among children of mothers without asthma. Gent et al. (2002) assessed the potential for increased incidence of respiratory symptoms after household exposure to particular fungal genera, namely *Cladosporium* (in 62% of homes) and *Penicillium* (in 41% of homes) in a population of infants 1-12 months of age at high risk for developing asthma. To the extent that the measured mold sampled represented longer-term exposure concentrations, the study results suggested that the infants studied who were exposed to high levels of *Penicillium* had higher rates of wheeze and persistent cough. The authors also suggested that because there are considerable seasonal variations in some molds, including *Cladosporium*, intermittent exposures may contribute only sporadically to respiratory symptoms. Other molds, such as *Penicillium*, seem to be present at more consistent levels year round. Previous studies note that relationships between exposure to mold and respiratory symptoms of children are complicated and may depend on a variety of potentially confounding factors, such as the season in which mold samples were collected and the presence of other moisture dependant biological hazards such as endotoxins (Gent et al., 2002; Thorne et al., 2005).

Toxics and Irritants. Many molds are also known to produce mycotoxins, which are toxic metabolites that can be a health hazard to birds and mammals upon natural exposure, i.e., ingestion, dermal contact, or inhalation. While common outdoor molds present in ambient air, such as *Cladosporium cladosporioides* and *Alternaria alternata*, do not usually produce toxins, many other different mold species do (Burge and Ammann, 1999). Genera producing fungi associated with wet buildings, such as *Aspergillus versicolor*, *Fusarium verticillioides*, *Penicillium aurantiogriseum*, and *Stachybotrys chartarum*, can produce potent toxins, measurable in mold mycelia, spores, and the matrix in which the mold is growing (Burge and Ammann, 1999). A single mold species may produce several different toxins, and a given mycotoxin may be produced by more than one species of fungi. Furthermore, toxin-producing fungi do not necessarily produce mycotoxins under all growth conditions, with production

² Of the tests used to determine whether an individual is sensitive to an allergen, the skin prick is the most common method. A small amount of allergen is introduced into the skin by making a small puncture through a drop of allergen extract. Swelling occurs if the patient is allergic to the specific allergen. If skin-prick tests are all negative, a physician may use a more sensitive intradermal test. Generally, intradermal testing is used to test for allergy to insect stings or penicillin (Li, 2002). A blood test, called a RAST (radioallergosorbent) test, may sometimes be used. This is a more expensive method, is generally less sensitive than skin testing, and requires more time for results to be available. It is generally used only when skin tests cannot be performed. Allergen extracts are produced commercially according to Food and Drug Administration (FDA) standards.

being dependent on the substrate it is metabolizing, temperature, water content and humidity (Burge and Ammann, 1999). Some toxin-producing molds have a higher water requirement than common household molds and tend to thrive only under conditions of chronic and severe water damage (American Academy of Pediatrics, 1998). For example, *Stachybotrys* typically only grows under continuously wet conditions (Burge and Otten, 1999). However, recent literature indicates that temperature is a stronger rate limiting factor in mycotoxin production than water (Llorens et al., 2004). An overview of some common molds, mycotoxins, and associated health effects is presented in the American Conference of Government of Industrial Hygienists' publication *Bioaerosols: Assessment and Control* (ACGIH, 1999) and the American Industrial Hygiene Association's *Field Guide for the Determination of Biological Contaminants in Environmental Samples* (Dillon et al., 2005).

Although epidemiological studies that specifically examine exposure to mycotoxins in indoor residential environments are relatively limited, there is substantial evidence of a relationship between mycotoxin exposure (via ingestion and inhalation) and adverse health effects in occupational (agricultural and food processing) settings and animal studies (Rao et al., 1996; Miller, 1994; American Academy of Pediatrics, 1998; Burge and Ammann, 1999). The most frequently studied mycotoxins are produced by species of *Aspergillus* (e.g., aflatoxins), *Fusarium*, *Penicillium*, *Stachybotrys*, and *Myrothecium* (e.g., satratoxins, trichothecenes) (Burge and Ammann, 1999). Known health effects depend on the kind of mycotoxin and the nature of the exposure, but include mucous membrane irritation, skin rashes, dizziness, nausea, and immunosuppression (Burge and Ammann, 1999). Although evidence is very limited in residential environments, aflatoxins (produced by *Aspergillus flavus* and *parasiticus*) have also been linked to liver cancer in food processing settings (Burge and Ammann, 1999). Toxins from *Stachybotrys chartarum* have been most commonly associated with lung inflammation and hemorrhage in animal studies (Nikulin et al., 1996, 1997, as cited in Burge and Ammann, 1999) and non-specific symptoms (headaches, sore throats, flu symptoms, diarrhea, fatigue, and dermatitis) in case studies (Dill et al., 1997 and Croft et al., 1986, both as cited in Burge and Ammann, 1999).

In indoor environments, associations have also been reported for pulmonary hemorrhage deaths in infants and the presence of *Stachybotrys atra* (Etzel et al., 1998; Elidenir et al., 1999). Although this specific association has not been conclusive (CDC, 2000), some research supports the potential for general mycotoxin exposure in the indoor environment to result in adverse effects on respiratory health (NAS, 2000; Sorenson, 1999, American Academy of Pediatrics, 1998). It has also been suggested that very young children may be especially vulnerable to certain mycotoxins (American Academy of Pediatrics, 1998; Etzel, 2000). For example, Etzel (2000) suggests that exposure to the trichothecene mycotoxins, which are known to be potent protein synthesis inhibitors, may result in pulmonary capillary fragility in the rapidly growing lungs of infants.

The American College of Occupational and Environmental Medicine concluded that "delivery by the inhalation route of a toxic dose of mycotoxins in the indoor environment is highly unlikely at best, even for the hypothetically most vulnerable subpopulations" (ACOEM, 2002). Other reviewers of the literature have come to the same general conclusion (Robbins et al., 2000; Robbins et al., 2003; Hardin et al., 2003).

Other compounds produced by fungi, including (1→3) β-D-glucans and volatile organic compounds (often referred to as microbial volatile organic compounds or MVOCs), are also suspected to play a role in certain adverse reactions described as “sick building” or “building related symptoms” (Burge and Otten, 1999; Douwes, 2005; Rylander, 1992). Glucans are a major component of the cell walls of most molds, and have been observed to have irritant effects similar to (but less potent than) those of bacterial endotoxins. MVOCs, which are produced by molds as byproducts of growth or degradation of substrates and often have strong or unpleasant odors, may also be responsible for some non-specific building related symptoms such as headaches, nasal irritation, dizziness, fatigue, and nausea (EPA, 2001). Research on the role of MVOCs in specific disease is still in the early phase (Walinder et al., 2005; EPA, 2001). Schleibinger et al. (2005) concluded that MVOCs should not be used as predictors for mold damage in indoor environments, because the concentrations produced are generally too low to be detected in indoor air.

3.0 METHODS USED TO ASSESS MOLD HAZARDS IN THE HOME

In general, visual observation of active or past microbial growth, or measurement of mold in dust or samples of source material, can be used to establish potential for mold exposure. As inhalation is the primary exposure pathway for molds, air sampling for mold can also be used to estimate the likelihood of exposure (Dillon et al., 1999).

The following section provides the reader with an overview of the range of assessment methods and technologies that are available, from both a research and programmatic perspective. The level of rigor involved in assessing mold hazards in a research setting surpasses that which is practical or necessary for programmatic or public health use. From a housing or public health perspective, a home assessment is generally constrained by the need for cost effective methods that are sufficient to allow for the identification of mold problems in the home environment. Current guidance generally discourages collecting and analysis of environmental samples for mold analysis in most situations (USEPA, 2001; CDC 2005). This is based on factors such as cost, the high variability in sampling results (both spatial and temporal variability), and the fact that remediation decisions are generally not based on sampling results. Significant residential mold problems can usually be identified based on visual observation and/or the presence of odors. Situations where sampling might be conducted include those in which the source of the mold is unclear, litigation is involved, or to test a surface to document adequate cleaning or remediation. Note, however, that some experts recommend that sampling should not be used to verify adequacy of cleaning, because of the high risk of false negatives (Horner, 2006).

3.1 Visual Assessment and Occupant Questionnaires

High humidity levels and excess dampness have clearly been associated with mold growth, as well as increased levels of some environmental allergens, such as those produced by dust mites. Visual inspection for dampness, observable mold growth, and detection of musty odors, often obtained from occupant questionnaires, are the most frequently used methods to assess the potential for indoor mold exposure. Visual observation of mold growth, however, is limited by the fact that fungal elements such as spores are microscopic, and a mold problem may not be

apparent until growth is extensive. In some cases, destructive sampling (e.g., the removal of wallboard) is required to assess the extent of fungal contamination (Dillon et al., 1999). A device called a boroscope, which employs fiber optics technology to make observations in building cavities by inserting the instrument through a small hole drilled in materials such as wall board, can be used by home inspectors to facilitate assessment of hidden mold damage. Although direct observation of visible fungal growth is usually sufficient to warrant a recommendation for mitigation, further air or source sampling (discussed below) may be conducted for documentation purposes and to record the types of fungi that predominate (Burge and Otten, 1999).

Many moisture problems in homes are due to structural deficiencies. Common points of inspection for buildings with dampness problems include: rain leaks (e.g., on roofs and wall joints); surface and groundwater leaks (e.g., poorly designed or clogged rain gutters and footing drains, basement design problems); plumbing leaks; and stagnant water in appliances (e.g., dehumidifiers, dishwashers, refrigerator drip pans, and condensing coils and drip pans in HVAC systems). In addition, assessment is also conducted for water vapor migration and condensation problems, including: uneven indoor temperatures, poor air circulation, air conditioning systems, soil air entry into basements, contact of humid unconditioned air with cooled interior surfaces, and poor insulation on indoor chilled surfaces (e.g., chilled water lines). Portable, hand-held moisture meters, for the direct measurement of moisture levels in materials, may also be useful in qualitative home assessments to aid in pinpointing areas of potential biological growth that may not otherwise be obvious during a visual inspection (ACGIH, 1999; Dillon et al., 2005).

A variety of different protocols exist for assessing water damage in homes; for example, a visual assessment tool for inspecting homes for evidence of mold and moisture has been developed for Cleveland, Ohio, by the Cuyahoga County (Ohio) Board of Health for use in HUD-sponsored research (Dillon et al, 1999; EHW, 2004). An overview of additional techniques and issues of concern in conducting visual assessments of homes for mold contamination is presented in *Bioaerosols: Assessment and Control* (ACGIH, 1999; see Chapter 4, “The Building Walkthrough”). Chapter 3 of the Institute of Medicine report, *Damp Indoor Spaces and Health*, provides a list of questions used to define dampness used in 25 epidemiological studies (IOM, 2004). For large-scale assessments, e.g., in multifamily buildings, a sophisticated visual and olfactory inspection tool for moisture and mold developed by a NIOSH team may be useful (see Park et al., 2004).

3.2 Sample Collection

Quantitative assessment of indoor molds generally involves sampling of a representative environmental medium in the home and quantification of the measure of interest (e.g., allergen concentration, total fungal biomass, or spore count). Because preparation requirements for environmental samples vary with the analysis techniques to be used, investigators should plan a collection procedure accordingly. Standard methods for quantitative sampling of mold or models that would allow for estimates of inhalation or dermal exposure to molds from sampling results are not available (IOM, 2004; Dillon et al., 1999).

Air and dust sampling, as well as direct sampling of mold colonies where visible mold growth is present, are used to estimate environmental levels of fungi. Generally, indoor environments contain large reservoirs of mold spores and hyphal fragments in settled dust and contaminated building materials. Chew et al. (2003) reported that concentrations of fungi in settled dust generally correlate weakly with those in indoor air. Indoor air fungi levels were strongly associated with outdoor air levels, and the investigators speculated that the two different metrics (air and dust samples) represent different types of fungal exposure, indicating that it may be necessary to collect both air and dust samples. Recent evidence suggests that very fine airborne particles (<1 micrometer aerodynamic equivalent diameter (AED)) can carry fungal fragments and/or metabolites such as allergens (Green et al., 2005; Gorny et al., 2002). Size characterization is important to detect these particulates, which could be much larger in number than spores.

Before the decision is made to sample, there should be a clear justification for the sampling. Sampling is most beneficial when used to augment a visual inspection or survey information, and to help address particular questions that derive from the inspection (e.g., the extent of contamination within a building). Table 2 summarizes several sampling strategies for molds.

Table 2. Selected Mold Sampling Strategies¹

Type of Environmental Sample	Sampling Techniques	Advantages/ Disadvantages	Possible/Example Results
Surface	<ul style="list-style-type: none"> - Press collection material (e.g., a contact plate or adhesive tape) against a surface - Wipe small area with a wetted swab, cloth, or filter - Vacuum sample of settled dust. 	<ul style="list-style-type: none"> - Non-destructive - Spatially and temporally variable - Settled dust samples expected to be less temporally variable and be a better indicator of exposure over time. 	<ul style="list-style-type: none"> - Detection of past mold colonization or active growth - Identification of surfaces/areas where previously airborne mold spores and fragments have settled and accumulated
Bulk	<ul style="list-style-type: none"> - Remove section of building material (e.g., wallboard) 	<ul style="list-style-type: none"> - Destructive technique 	<ul style="list-style-type: none"> - Detection of past mold colonization or active growth
	<ul style="list-style-type: none"> - Vacuum or wipe larger area to collect loose bulk material (e.g., settled dust) 	<ul style="list-style-type: none"> - Non-destructive - Dust can have inhibitors like salt which can limit growth of some fungal taxa - Spatially and temporally variable - Efficiency of bulk dust collection may be affected by environmental factors (see discussion below) 	<ul style="list-style-type: none"> - Identification of surfaces/areas where previously airborne mold spores and fragments have settled and accumulated
Air	<ul style="list-style-type: none"> - Static sampler - Personal sampler - With HVAC off and on 	<ul style="list-style-type: none"> - Useful if it is suspected that the ventilation systems are contaminated - Air levels are variable, especially with disturbance - Short-term air samples limit sensitivity 	<ul style="list-style-type: none"> - Detection of mold contamination where the presence of mold is suspected but cannot be identified by a visual inspection or bulk sampling

¹ See text for references.

Source Sampling. Source sampling methods used in investigations of mold contamination in homes includes bulk and surface sampling.

In bulk sampling techniques, portions of environmental materials (e.g., settled dust, sections of wallboard, pieces of duct lining, carpet segments, or return air filters) are collected and tested to determine if molds have colonized a material and are actively growing and to identify surfaces areas where previously airborne mold spores and fragments have settled and accumulated (Martyny et al., 1999). For fixed materials, bulk samples are cut or otherwise removed from the source and thus this technique may be somewhat destructive. For loose materials, such as floor dust, bulk samples are typically collected using wipe sampling or a hand-held vacuum with a special filter. Various factors, including design of the vacuum collector, surface characteristics (e.g., carpet vs. smooth floor), and other environmental characteristics have all been shown to affect the efficiency of dust collection (Wang et al., 1995; NAS, 2000). For example, Wang et al. (1995) observed that when collecting dust with a vacuum sampler from a shag carpet surface, lower relative humidity (e.g., around 20 percent, as would be encountered during a dry, cold season) increased the intensity of the electrostatic field on the carpet and thus significantly decreased the collection efficiency of the vacuum.

HUD has developed a recommended “Vacuum Dust Sample Collection Protocol for Allergens” for use by HUD Healthy Homes Initiative grantees (HUD, 2004a). The protocol is adapted from sampling methods used in the National Survey of Lead in Allergens in Housing and the Inner-City Asthma Study, and it is supported by a companion HUD document, “Background and Justification for a Vacuum Sampling Protocol for Allergens in Household Dust” (HUD, 2004b). A hand-held portable vacuum cleaner -- electric powered, not battery operated -- is recommended, with a filter, sleeve or thimble dust collection device. Most electric powered canister vacuum cleaners are essentially equivalent in their measurement of indoor allergens, but it is necessary to choose a model that can accommodate the dust collection device that will be used (HUD, 2004a). Sampling locations vary with the objectives and resources of the study.

Surface sampling in mold contamination investigations may also be used when a less destructive technique than bulk sampling is desired. For example, non-destructive samples of mold may be collected using a simple swab or adhesive tape. In general, surface sampling is typically accomplished by either pressing a collection material (e.g., a contact plate or adhesive tape) against a surface, or by wiping an area with a wetted swab, cloth, or filter (Martyny et al., 1999). The size of a collected surface sample is generally much smaller than that of a bulk sample. An overview of procedures and advantages of various contact sampling techniques, including agar plate methods, adhesive tape sampling, and surface-wash sampling, is presented in *Bioaerosols: Assessment and Control* (ACGIH, 1999; see Chapter 12, “Source Sampling” by Martyny et al., 1999).

Air Sampling. Air sampling is more technically challenging and has greater opportunity for error than source sampling (Horner, 2006). For routine assessments in which the goal is to identify possible mold contamination problems prior to remediation, it is usually unnecessary to conduct air sampling because decisions about appropriate remediation strategies can typically be made on the basis of a visual inspection (NYC, 2000). Air monitoring may, however, be necessary in certain situations, including: 1) if an individual has been diagnosed

with a disease associated with fungal exposure through inhalation, 2) if it is suspected that the ventilation systems are contaminated, or 3) if the presence of mold is suspected but cannot be identified by a visual inspection or bulk sampling (NYC, 2000).

Airborne mold particulates may include spores, fungal fragments, aggregates of spores or fragments, or materials contaminated with fungal product. The most commonly used methods available today for volumetric air sampling (i.e., when a known volume of air is collected) are based on one of the following principles: inertial impaction (e.g., multiple-hole impactors, slit samplers), centrifugal collection (e.g., agar-strip impactors, cyclone samplers), filtration (e.g., cassette filters attached to portable pumps), and liquid impingement (e.g., three-stage impingers) (Martyny et al., 1999). Gravitation or settling techniques (e.g., longer-term collection of settled spores onto a culture plate or microscope slide) can also be used, but they are non-volumetric and, due to large temporal and spatial variations, samples cannot readily be compared to one another or to volumetric samples (O'Meara and Tovey, 2000; Martyny et al., 1999).

Samplers may be either static or reflective of a personal breathing zone. Static filter samplers used to collect airborne substances are normally placed in a fixed position in a room and do not measure personal exposure (O'Meara and Tovey, 2000). Sampler design and flow rate have been shown to affect the quantity and size of particles sampled and thus can affect the apparent measured levels of a given airborne substance (O'Meara and Tovey, 2000). Both high-volume (60 to 1100 L/min) and lower-volume (6 to 20 L/min) filter samplers have been used, although it has been suggested that the lower-volume samplers may collect a more meaningful sample in relation to exposure because they better approximate breathing volumes of humans (O'Meara and Tovey, 2000). Breathing zone samplers often show much higher levels of collected mold particles than static samplers, likely due to the varying levels of dust that are resuspended in the personal breathing zone as a result of human activity; however, only minor differences in airborne mold levels between personal and static samplers are observed during high levels of dust disturbance (O'Meara and Tovey, 2000). It is generally recommended in the literature that outdoor air samples are collected concurrent with indoor samples for comparison purposes, both for measurement of baseline ambient air conditions (remote from obvious mold sources), and for baseline measurement of air entering a building (samples near outdoor air intakes) (ACGIH, 1999).

In selecting a type of air sampler for fungal collection, it is recommended that consideration be given to such factors as: the compatibility of the sampler with the analysis method to be used, what type of information is needed (e.g., concentration or identification of species), the concentration (e.g., very high or very low) of the mold at the test site, temperature extremes, the nature of the air stream where the sample will be collected, and possible collection constraints due to the presence of occupants (ACGIH, 1999). Comparative assessments of the performance of the different samplers (e.g., filter samplers, Andersen samplers, rotorod samplers, liquid impingers, and cyclone samplers) have been inconclusive, although certain samplers have been observed to perform better for specific purposes (e.g., the Andersen six-stage sampler for viable spore counts and the Burkard 24-hour samplers for total spore counts) (Flannigan, 1997). Many factors introduce significant variability into air sampling results and complicate interpretation, as discussed in Section 3.4.

3.3 Sample Analysis

Current methods available to analyze environmental samples from the home for mold hazards include:

- Counting colonies cultured for specific species.
- Identifying and/or counting spores.
- Chemical analysis of fungal components and biochemical/immunochemical markers to quantify total fungal loads (biomass).
- Immunoassays (ELISAs) to measure fungal-specific antigen levels.
- Genetic probe technologies to identify fungal species.

An overview of selected mold analysis methods and their applicability is presented in Table 3.

Table 3. Selected Methods for Analyzing Home Environmental Samples for Mold ¹

Analysis		Test Applicability	
Method (units)	Advantages/Limitations	Important Species	Data Obtained ²
Allergen immunoassays, ELISA ³ (µg/g or pg/m ³)	Not currently reliable for fungi (e.g., Alternaria counts must be very high or germinating)	Aspergillus, Alternaria, Cladosporium	Allergen levels (Asp f 1 and Alt a 1)
Spore Count	Intact spores may not account for total allergen load	All (Aspergillus, Penicillium, Trichoderma and yeasts difficult to identify)	Concentration of spores; spore identification
Culture	Viable fungi may not account for total allergen load	All	Species identification; Estimates of fungal concentrations
Chemical biomarkers (ergosterol, extracellular polysaccharides (EPS), B-glucan, VOCs, mycotoxins)	Ergosterol and EPS are good indicators of total biomass (components in all fungal hyphae and spores, cannot identify species)	Not species specific; Non-fungal sources can affect B-glucan and VOC results; Methods not well developed for fungal VOCs or mycotoxins in indoor environments.	Concentration of chemical biomarker; Estimates of fungal biomass
Polymerase chain reaction (PCR) based technologies (i.e., genetic probes)	Accurate: Based on targeting species-specific sequences of DNA. Identifies both viable and nonviable fungal elements, but is prone to amplifying sample contaminants.	Species specific, including but not limited to Alternaria, Aspergillus, Cladosporium and Penicillium	Mold identification to the species level

¹ See text for references.

² Allergens listed in this column are those for which monoclonal antibodies are typically commercially available for immunoassay purposes (see INDOOR Biotechnologies website, <http://www.inbio.com/index.html>)

³ Quantitative differences between allergen standards are currently an important source of assay (ELISA) variability.

No single method provides a complete assessment of the exposure hazard associated with an environmental sample, as discussed in Section 3.4 below. The quality of environmental microbiology laboratories performing analyses on samples for molds and other microbiological agents is monitored under an external peer review program sponsored by the American Industrial Hygiene Association (AIHA). This program, which includes the Environmental

Microbiology Proficiency Analytical Testing (EMPAT) Program and the Environmental Microbiology Laboratory Accreditation Program (EMLAP), is specifically for labs identifying microorganisms commonly detected in air, fluids, and bulk samples during indoor air quality studies. EMPAT is a performance evaluation program that uses proficiency testing to score participating laboratories. Proficiency in EMPAT is mandatory for labs seeking EMLAP accreditation. In the absence of standard methods, using laboratories with an accreditation, such as from the EMLAP, is particularly important (Horner, 2003). When a laboratory is accredited by AIHA, the laboratory and its clients have the assurance that the laboratory has met defined standards for performance based on examination of a variety of criteria. As of this writing, AIHA's website lists 87 accredited laboratories nationwide, including five in Canada, that are accredited under AIHA's EMLAP. Additional information on the EMPAT and EMLAP programs is available online at <http://www.aiha.org/SplashPages/html/topic-mold.htm>.

Culture Methods and Spore Examination. The growth of fungal colonies on specially prepared nutrient media (culture) from spores contained in air or dust samples is a common method used to assess mold populations. Following culture, identification of fungal species can often be accomplished with a dissecting or light microscope via examination of colony morphology or spore bearing structures. Culture results can also be reported in terms of colony forming units (CFU) per m³, g, or cm². The type of isolation media used to culture the fungal spores, however, can introduce substantial variability into the types and relative magnitudes of mold species that are cultured (Burge and Otten, 1999; Flannigan, 1997). Bias in culture measurements may be introduced because a highly nutritionally rich substrate favors the growth of fast-growing species, or because one species present in the sample may not compete well with another on the culture plate (Flannigan, 1997). For example, some genera such as *Penicillium* grow well and quickly on most media and thus may be over-represented in a culture sample, while others such as *Stachybotrys* grow slowly or not at all on commonly used substrates (Bush and Portnoy, 2001).

Many types of fungi are identifiable to the genus or species level, depending on the type of fungi sampled, via microscopic examination of spores in collected air and source samples (Burge and Otten, 1999; Pinchin Environmental, 2002). Spore counts can also be reported, typically in units of spores per m³, g, or cm². This method is relatively inexpensive, but time-consuming, and can give a general indication of atypical indoor mold growth.

Chemical Analyses. Methods using chemical analysis can be used to quantify total fungal loads (biomass), although, generally, these methods do not allow for identification of species. These methods can be based on chemical components (biomarkers) common to a particular group of organisms (e.g., ergosterol in the membranes of fungal hyphae and spores) (Flannigan, 1997). These markers can indicate the relative extent and presence of fungal growth, but do not measure fungal allergen exposure. Furthermore, their use in quantitating fungal exposure and relationship to human allergic disease is highly investigational (Bush and Portnoy, 2001). Results of dust analysis are typically expressed as concentration in units of weight of analyte per weight of settled dust. Results of air sample analysis are usually expressed volumetrically (e.g., µg/m³).

Mycotoxins. Methods currently available for detecting mycotoxins in environmental samples were designed for testing agricultural products and generally do not translate well to residential testing requirements (e.g., air samples with very low mycotoxin concentrations) (Burge and Ammann, 1999). Thin layer chromatography has been used to measure mycotoxins in some studies, although the usefulness of this technique is limited due to lack of sensitivity and susceptibility to interference (Burge and Ammann, 1999). High performance liquid chromatography (HPLC) and gas chromatography with mass spectrometric detection (GC-MS) have also been used for mycotoxin quantification, although these techniques are also limited due to specialized laboratory requirements and associated expense (Burge and Ammann, 1999). Various researchers have measured cell toxicity of particulate air samples and inferred the presence of mycotoxins. For example, Vesper et al. (2000) used a protein synthesis inhibition assay to evaluate the toxicity of air particulate samples during a *Stachybotrys chartarum* remediation study. Protein synthesis inhibition is an activity characteristic of trichothecene mycotoxins typically produced by *Stachybotrys*. Field sample extracts were assayed for trichothecene toxicity by comparison to a known sample, with the results expressed as toxin equivalents per cubic meter of air. Mycotoxin analysis can be used to detect the presence of certain fungi in the environment, but, more commonly, mycotoxin levels are only measured after the fungal species has been identified (Bush and Portnoy, 2001).

Other Chemical Components of Fungi. Ergosterol, which is a component of fungal cell membranes, has been used as an index of fungal mass in house dust and air samples, and can be analyzed using gas chromatography with mass spectrometric detection (Flannigan, 1997; Dillon et al., 1999). Ergosterol is not present in vascular plants, and therefore, in most indoor environments can be used as a specific measure of total fungal biomass (Dillon et al., 1999). Ergosterol measurement has been applied in assessments of house dust and air (Dillon et al., 1999), although, as with mycotoxin analysis, this highly specialized technique may have resource limitations for home assessments.

There are about 15 volatile organic compounds (VOCs) produced by fungi that may also be used as markers of fungal growth, although some are also emitted by bacteria (Dillon et al., 1999). VOCs can be collected on solid sorbents, extracted, and quantified using gas chromatography with mass spectrometric detection. Measurement of fungal VOCs may be particularly useful in some home assessments for detection of hidden mold growth because the compounds can permeate porous walls in buildings (Dillon et al., 1999). However, the uncertainties currently associated with accuracy of these methods preclude using this approach for routine investigations. For example, significant questions remain regarding reliable “signature” VOCs for a particular fungus, and how to deal with the variability in VOCs produced under different conditions (Ammann, 1999).

Immunoassays. To measure mold allergen levels in collected dust and air samples, enzyme-linked immunosorbent assays (ELISAs, a specific type of immunoassay) have been developed for some indoor mold allergens and some mold components such as (1→3) β-D-glucan and extracellular polysaccharides. An immunoassay is a laboratory technique that makes use of the

specific binding between the antigen associated with an allergen and its homologous antibody in order to identify and quantify a substance in a sample. However, although immunoassays have been developed for some major fungal allergens to date, this technology is not as highly developed or well standardized as that for house dust mite, cat, or cockroach allergens (Bush and Portnoy, 2001). Currently, monoclonal antibody ELISAs for *Alternaria* (Alt a 1) and *Aspergillus* (Asp f 1) are available from commercial laboratories (e.g., see Indoor Biotechnologies website at <http://www.inbio.com/services.html>) (Vailes et al., 2001).

(1→3) β-D-glucan, a component of some fungal, bacteria and plant cell walls has also been used as a surrogate measure of total fungal biomass in house dust and air (Douwes, 2005; Dillon et al., 1999; Flannigan, 1997). To date, only a few laboratories test environmental samples for β-glucan, and its utility in large-scale epidemiologic studies is hampered by its large “within-home variation” (Chew et al., 2001; Douwes, 2005). A new assay has been developed for (1→3, 1→6) β-D glucans which is more specific for fungi, but the glucan levels were not associated with decrements in lung function (Blanc et al., 2005). Further research into the role of (1→3, 1→6) β-D glucans in respiratory disease is warranted.

Mold extracellular polysaccharides (EPS) have potential usefulness as fungal measures, as they are produced in mycelia cell walls under almost all growth conditions (Dillon et al., 2005). Douwes et al. (1999) examined the relationship between measured EPS from *Aspergillus* and *Penicillium* species (EPS-*Asp/Pen*) and culturable fungi, reported home dampness, and respiratory symptoms. EPS-*Asp/Pen* levels were significantly correlated with total culturable fungi, and levels in living room floor dust were positively associated with home dampness and respiratory symptoms. EPS can be measured using a specific enzyme inhibition assay (EIA), and has been studied in residential and occupation environments (Chew et al., 2001; Wouters et al., 2000; Douwes et al., 1999). The within-home variability appears to be smaller than that of (1→3) β-D glucans (Chew et al., 2001).

Genetic probes. Polymerase chain reaction (PCR) based technologies (i.e., genetic probes), unlike other non-culture methods, can be used to identify certain biological particles such as fungi to the species level (Flannigan, 1997). The technology is based on targeting short, species-specific sequences of DNA, and allows for the rapid identification and quantification of molds in a matter of hours, eliminating the need for plating and culturing or identifying and counting. Genetic probes could prove particularly useful in situations where fungi are not otherwise easily differentiated on the basis of morphology (e.g., *Aspergillus* and *Penicillium*) or where culture methods are not useful because spores have lost their viability (O'Meara and Tovey, 2000).

Beneficial attributes of PCR are: (1) it is species specific, which may allow assessment for certain mold species suspected to be associated with health effects or environmental conditions; (2) unlike live culture analysis, it reports non-viable as well as viable molds, which is important because non-viable molds are potentially allergenic; (3) it results in fewer “non-detects” than live culture analysis; (4) it is apparently more reliable than live culture analysis because not all species may grow on the media used and because fast-growing species may overtake the slow-growing species; (5) it finds higher concentrations than culture analysis, sometimes by orders of magnitude; and (6) it is quicker and easier (Vesper et al., 2005; Vesper et al., 2004; Meklin

et al., 2004). In recent studies, the cited investigators found that results of PCR-analyzed settled-dust samples did not correlate with PCR-analyzed short-term air samples (five minutes or less). Also, PCR results did not correlate with culture-analysis results. Perhaps the main limitation of PCR is that it does not measure whether the mold is growing. The best established health effect of mold relates to the presence of mold growth (Horner, 2006).

In May of 2002, EPA's Office of Research and Development was granted a patent for this technology as applied to describing mold DNA sequences. The technology is available for licensing on a non-exclusive basis by laboratories, indoor air quality specialists, or other environmental professionals. As a result, the technique is becoming more widely available as several commercial labs have begun offering analysis of indoor samples (preferably dust, but can be applied to any medium) using genetic probes. As of October 2005, 13 companies have a license to utilize the EPA technology (available online: <http://www.epa.gov/nerlcwww/moldtech.htm>).

3.4 Interpretation of Results

Methods for assessing human exposure to fungal allergens and mycotoxins are relatively poorly developed (NAS, 2000) and interpretation of results is difficult. This is due, in part, to the fact that fungal allergens and toxins vary widely across mold species and because the traditional methods of mold population assessment (e.g., spore counts) do not have consistent relationships with levels of mold allergens or toxins. Furthermore, because viable mold measures do not include particles that are not culturable (non-viable spores or non-reproducing vegetative fragments) but that may have toxic or allergenic properties, investigations of mold-affected houses that focus only on assessing the number of culturable organisms may underestimate actual allergenic or toxic potential (Flannigan and Miller, 1994; Flannigan, 1997). Conversely, total measures of a fungal component (e.g., ergosterol or glucan) in a sample do not allow for identification of mold species, or provide information about the biologically active portion of the sample. Therefore, neither measure provides a complete assessment of the potential allergen or mycotoxin exposure hazard associated with an environmental sample. The accuracy of substituting measures of exposure to fungi for exposure to fungal allergens or toxins has not been determined (ACGIH, 1999), and direct measurement of allergens and toxins is limited by the current development and standardization of immunoassays for specific allergens and reliable, affordable techniques for mycotoxin analysis.

Further complicating the exposure assessment is variability associated with the collection of samples. The accuracy of quantifying air samples is complicated by large variations in airborne concentrations from room to room and temporally over relatively short periods of time, as well as outdoor concentrations with season (O'Meara and Tovey, 2000; Flannigan, 1997; Flannigan and Miller, 1994). The release of molds from carpets and walls or other surfaces has also been cited as an important factor in introducing variability into the magnitude and nature of indoor air spora collected (Flannigan, 1997). In addition, due to the ubiquitous presence of mold spores in the outdoor environment (often in concentrations far higher than indoors), it can be difficult to establish the presence of indoor mold growth using air sampling.

Dust sampling for molds is sometimes used to circumvent this temporal variability, although dust samples sometimes show differences in the relative abundance and types of mold in comparison to air samples (Flannigan, 1997; Dillon et al., 1999).

Professional inspectors frequently compare the types and levels of fungal organisms detected in various environments, e.g., outdoors vs. indoors, as a way of interpreting microbiological results. The qualitative diversity of airborne fungi outdoors should be similar to that measured indoors in the absence of mold contamination. Conversely, if one or more types of fungi dominates the indoor environment but is not detected outdoors, the sampled building may have a moisture problem and fungal contamination. However, that may not always be true. Spores of some outdoor fungi may infiltrate a house and persist under normal conditions long after outdoor sources are no longer present (Horner, 2006). In addition, levels of spore counts can vary by region and season (Gots et al., 2003; Ren et al., 1999). Another common indicator of indoor moisture problems is the consistent presence of fungi such as *Stachybotrys chartarum*, *Aspergillus versicolor*, or various *Penicillium* species at levels well above background concentrations (AIHA, 2003). See also the discussions of Horner et al. (2004) in section 2.1, above.

Using mold specific quantitative polymerase chain reaction (MSQPCR), Vesper et al. (2004) and Meklin et al. (2004) found that certain molds, which they labeled Group I molds, are found in higher concentrations in water-damaged homes than in other homes, while other molds (labeled Group II molds) are found in all homes. Group I molds included, but were not limited to: *Apergillus restrictus*, *Penicillium brevicompactum*, *Aspergillus niger*, *Paecilomyces variotii*, *Aspergillus ochraceus*, and *Trichoderma viride*. One way this information may be useful is in identifying homes that have suffered water damage but do not display easily identifiable signs of it. Another may be in narrowing the list of molds for which PCR analysis is necessary. Also, the investigators compared PCR-analyzed dust sample results from water-damaged homes of asthmatic children with those from control-group homes and found (1) that only Group I molds had higher concentrations in the water-damaged, asthmatic-occupied homes compared to the control homes, and (2) that certain Group I mold species had significantly higher concentrations (Vesper et al., 2005). The authors concluded that “if Group I molds are discovered, water-damaged remediation and mold removal might be considered as part of the total prevention plan in an asthmatic child’s home.”

Finally, there is the issue of comparison of results to standards that indicate potential hazard. The major limitations with existing quantitative guidelines for fungi are the lack of human dose/response data, reliance on short term grab samples analyzed only by culture methods, and the lack of standardized protocols for data collection, analysis, and interpretation (Rao et al., 1996). For example, Verhoeff and Burge (1997) conducted a review of peer-reviewed literature through 1995, and identified nine population based studies that examined the relationship between allergy and the presence of fungi in the home environment. All of the studies included quantitative measures of fungal presence in either air or dust. Evaluation of the studies indicated that although the existence of positive associations between fungal levels and health outcomes was supported in the literature at that time, inconsistency and inadequate validation of the measures used to evaluate exposure and health effects made determination of

guidelines for fungi in home environments based on health risk assessment impossible (Verhoeff and Burge, 1997).

The Institute of Medicine recommends that the evaluation of testing results “should, whenever possible, be based on:

- √ Comparison of exposure data with background concentrations or, better, a comparison of exposures between symptomatic and non-symptomatic subjects.
- √ Multiple samples, because space-time variability in the environment is high.
- √ Detailed information about sampling and analytic procedures (including quality control) and knowledge of the potential problems associated with those procedures.” (IOM, 2004)

In general, types of molds found inside buildings without mold problems should be similar to those found outdoors, and concentrations should also be similar inside and out (CDC, 2005).

Currently, there are no standard numerical guidelines for assessing whether there is a mold contamination problem in an area. In the U.S., there are no EPA regulations or standards for airborne mold contaminants (USEPA, 2001). Various governmental and private organizations have, however, proposed guidance on the interpretation of fungal measures of environmental media in indoor environments (quantitative limits for fungal concentrations).

Legislators in more than a dozen states and one federal legislator have introduced bills directed at the indoor mold problem. Legislation has been enacted in Arizona and California to study and review mold contamination of indoor environments. States, such as Texas, Louisiana, and California, have enacted legislation requiring the licensing of contractors conducting mold abatement activities.

Organizations that have produced guidelines on mold prevention and/or remediation include the ACGIH, the U.S. Occupational Safety & Health Organization (OSHA), the American Industrial Hygiene Association (AIHA), the Canada Mortgage and Housing Corporation (CMHC), the Commission of the European Communities (CEC), and the World Health Organization (WHO), as well as numerous smaller and/or local organizations like the New York Department of Health. Reviews of guidance offered by various groups to assist investigators in the interpretation are available in *Bioaerosols: Assessment and Control* (ACGIH, 1999) and in Rao et al. (1996).

Recommendations reported in Rao et al. (1996) vary widely, with quantitative standards/guidelines ranging from less than 100 CFU per m³ to greater than 1000 CFU per m³ as the upper limit for airborne fungi in non-contaminated indoor environments (Rao et al., 1996). Bush and Portnoy (2001) suggest that indoor spore counts equal to or greater than 1000/m³ and colony counts on the order of 1000 to 10,000 CFU per m³ likely represent indoor fungal contamination. In a review article, Portnoy et al. (2005) concluded that, “it seems reasonable to expect that total airborne spore counts attributable to indoor sources greater than 1,000 spores/m³ indicate a concern and those greater than 10,000 spores/m³ indicate a definite problem.”

Such guidelines based on total spore counts are only rough indicators, however. Other factors in addition to indoor spore counts should be considered. For example, the University of Minnesota Department of Environmental Health and Safety recommends consideration of several factors in addition to total spore counts when attempting to assess the severity of a mold contamination problem, including: the number of fungi indoors relative to outdoors, whether the fungi are allergenic or toxic, if the area is likely to be disturbed, whether there is or was a source of water or high relative humidity, if people are occupying the contaminated area or have contact with air from the location, and, whether there are immune compromised individuals or individuals with elevated sensitivity to molds in the area (University of Minnesota, 1996).

Given evidence that young children may be especially vulnerable to certain mycotoxins (American Academy of Pediatrics, 1998) and in view of the potential severity of diseases associated with mycotoxin exposure, some organizations support a more precautionary approach to limiting mold exposure (Burge and Otten, 1999). For example, the American Academy of Pediatrics recommends that infants under 1 year of age are not exposed at all to chronically moldy, water-damaged environments (American Academy of Pediatrics, 1998).

4.0 METHODS USED TO MITIGATE MOLD HAZARDS IN THE HOME

4.1 Guidelines for Mitigation and Personal Protection

Common intervention methods reported in the literature for residential mitigation of mold hazards include:

- Location and removal of sources of moisture (control of dampness and humidity and repair of water leakage problems).
- Increasing ventilation.
- Use of high-efficiency particulate air (HEPA) filters.
- Maintenance of heating, ventilation, and air conditioning systems.
- Cleaning of mold contaminated materials.
- Physical removal of materials with severe mold growth and porous materials that cannot be cleaned.
- Prevention of spore infiltration from outdoors by closing doors and windows and by using air conditioning.

The literature also consistently emphasizes the importance of worker protection when conducting mold assessment and mitigation projects. Activities such as cleaning or removal of mold-contaminated materials in homes, as well as investigations of mold contamination extent, have the potential to disturb areas of mold growth and release fungal spores and fragments into the air. For example, Vesper et al. (2000) measured a very high number of *Stachybotrys* spores in personal breathing zone samples of a worker during the implementation of a mold remediation program to remove *Stachybotrys* contaminated materials (i.e., wallboard, paneling and carpeting) from water damaged areas of a home. This suggested that residents should not

attempt greater than minor remediation without the proper protection, or preferably should employ a contractor trained in environmental remediation (Vesper et al., 2000). Recommended measures to protect workers during mold remediation efforts depend on the severity and nature of the mold contamination being addressed, but include the use of well fitted particulate masks or respirators that retain particles as small as 1 µm or less, disposable gloves and coveralls, and protective eyewear (ACGIH, 1999).

Various guidance documents for remediation of mold contamination have been developed.

- The New York City Department of Health has a set of guidelines, “Assessment and Remediation of Fungi in Indoor Environments,” that are widely recognized. The document, originally developed for *Stachybotrys* but expanded to be inclusive of all molds, addresses health effects, environmental assessment, remediation techniques, and hazard communication (available at <http://www.nyc.gov/html/doh/html/epi/moldrpt1.html>).
- The Institute of Inspection Cleaning and Restoration Certification produced guideline S500: Standard and Reference Guide for Professional Water Damage Restoration (available by contacting the IICRC headquarters at (360) 693-5675 or through e-mail at supplies@iicrc.org).
- The American Conference of Governmental Industrial Hygienists (ACGIH) bioaerosols committee published in 1999, “Biosaerosols: Assessment and Control,” a compilation of information on investigation strategies, sampling and analysis, and control of indoor bioaerosols, including molds (can be ordered from ACGIH at <http://www.acgih.org/home.htm>).
- The American Industrial Hygiene Association (AIHA) has recently prepared the “AIHA Mold Guideline on Assessment, Remediation, and Post-Remediation.” It describes a range of methodologies and techniques currently available to conduct assessments of mold growth in residential and commercial buildings. To order, call 703-849-8888.
- U.S. Environmental Protection Agency published guidance for “Mold Remediation in Schools and Commercial Buildings,” which includes many general principles also applicable to residential mold mitigation efforts (available from EPA online at http://www.epa.gov/iaq/molds/mold_remediation.html).
- U.S. Environmental Protection Agency published guidance, “A Brief Guide to Mold, Moisture, and Your Home,” for homeowners and renters on how to clean up residential mold problems and how to prevent mold growth (available from EPA online at <http://www.epa.gov/iaq/molds/images/moldguide.pdf>).

- The Canada Mortgage and Housing Corporation published, “Clean-up Procedures for Mold in Houses,” which provides qualitative guidance for mold mitigation (can be ordered from CMHC at <https://www.cmhc-schl.gc.ca:50104/b2c/b2c/init.do?language=en>).
- Health Canada published its “Fungal Contamination in Public Buildings” guide to assist investigators in recognizing and managing fungal contamination (available through Health Canada at http://www.hc-sc.gc.ca/hecs-sesc/air_quality/pdf/fungal.pdf).
- The Institute of Medicine of the National Academies published in 2004 a comprehensive review of the literature on causes, effects, assessment, remediation and prevention of interior dampness and mold. Entitled “Damp Indoor Spaces and Health,” it is available from the National Academies Press at www.nap.edu.
- The Centers for Disease Control and Prevention prepared guidance in 2005 on mold for cleanup and reconstruction efforts following hurricanes, “Mold: Prevention Strategies and Possible Health Effects in the Aftermath of Hurricanes Katrina and Rita.” See chapter 3 on cleanup and prevention and chapter 4 on personal protective equipment (available at <http://www.bt.cdc.gov/disasters/mold/report/>).

Although these and other mold remediation guidance documents share many of the same approaches for conducting residential mitigation of mold hazards, such as correction of moisture problems and removal of severely contaminated materials, specific criteria cited in the guidelines may vary. For example, ACGIH (1999) guidance regarding remediation techniques and personal protective equipment (PPE) is based on qualitative professional judgment of the extent of fungal contamination (defined as minimal, moderate, or extensive), while USEPA (2001) guidance for mold remediation in schools is based on quantitative estimates of the total surface area affected (defined as small (less than 10 ft²), medium (between 10 and 100 ft²), or large (greater than 100 ft²)). The New York City guidelines (NYC, 2000) differentiate between large isolated areas of contamination (30 to 100 ft²) and extensive contamination (greater than 100 contiguous ft² in an area).

In general, however, the literature agrees on the point that a particular strategy or combination of strategies recommended for a given mold abatement effort (including the degree of worker protection needed) will depend on site-specific factors, such as the contaminating agent, the type of substrate that is contaminated (e.g., whether porous or non-porous), the extent of the contamination, the location of the site requiring remediation, and the presence of highly susceptible occupants (ACGIH, 1999; Morey, 2000). For example, slight fungal contamination of a semi-porous concrete floor may only require cleaning, while extensive mold growth in a carpet will require complete removal. Appropriate PPE and containment measures for situations of minimal colonization (small isolated surface area contamination) might include contaminated source containment to minimize dust or spore dispersion (e.g., dust suppression methods such as misting, covering material with sticky sheeting or an encapsulant prior to

removal) and the use of a N-95 disposable respirator and gloves for PPE (ACGIH, 1999; NYC, 2000). However, use of a N-95 respirator does not necessarily result in decreased exposure if there is not a proper fit (Lee et al., 2005). For remediation of moderate or large areas of mold growth, or where sensitive individuals are present in the home, containment of the source by enclosing the work area with a plastic sheet and sealing with tape and negative pressurization may be warranted (NYC, 2000; ACGIH, 1999). In many cases, the protection and mitigation methods most appropriate must be determined using professional judgment, and it is often recommended that investigators seek additional advice, when needed, from occupational physicians, respiratory protection experts, or health and safety professionals to select appropriate PPE (USEPA, 2001; ACGIH, 1999).

4.2 Moisture Control

Because one of the most important factors affecting mold growth in homes is moisture level, controlling this factor is crucial in mold abatement strategies. Many simple measures can significantly control moisture, for example: maintaining indoor relative humidity at no greater than 50%-60% through the use of dehumidifiers, fixing water leakage problems, increasing ventilation in kitchens and bathrooms by using exhaust fans, venting clothes dryers to the outside, reducing the number of indoor plants, using air conditioning at times of high outdoor humidity, heating all rooms in the winter and adding heating to outside wall closets, sloping surrounding soil away from building foundations, fixing gutters and downspouts, and using a sump pump in basements prone to flooding (Bush and Portnoy, 2001; ACGIH, 1999; NYC, 2000). Vapor barriers, sump pumps, and above-ground vents can also be installed in crawlspaces to prevent moisture problems (Office of Native American Programs, 2001). A good description of the many issues in controlling moisture in buildings can be found in the Institute of Medicine's report, *Damp Indoor Spaces and Health* (IOM, 2004), particularly on how buildings get wet, in chapter 2, and barriers to dampness prevention and reduction and related public health approaches, in chapter 7. Numerous recommendations on managing moisture in and around a house are available in the HUD report, *Durability by Design* (HUD, 2002).

4.3 Removal and Cleaning of Mold Contaminated Materials

Non-porous (e.g., metals, glass, and hard plastics) and semi-porous (e.g., wood and concrete) materials contaminated with mold and that are still structurally sound can often be cleaned with detergent or bleach solutions or by using quaternary amine preparations; however, in some cases, the material may not be easily cleaned or may be so severely contaminated that it may have to be removed. Do not mix detergents and bleach. Some detergents have ammonia, which produces phosgene gas, a poisonous, suffocating gas, when mixed with bleach. It is recommended that porous materials (e.g., ceiling tiles, wallboards, and fabrics) that cannot be cleaned be removed and discarded (NYC, 2000; USEPA, 2001). In severe cases, clean-up and repair of mold-contaminated buildings may be conducted using methods similar to those used for abatement of other hazardous substances such as asbestos (Shaughnessy and Morey, 1999). For example, in situations of extensive colonization (large surface areas greater than 100 ft² or where the material is severely degraded), extreme precautions may be required, including: full containment (complete isolation of work area) with critical barriers (airlock and decontamination room) and negative pressurization, personnel trained to handle hazardous

wastes, and the use of full-face respirators with HEPA filters, eye protection, and disposable full-body covering (NYC, 2000; ACGIH, 1999).

Physical removal interventions have proven effective, although additional research is needed regarding the containment of mold spores during the renovation process (NAS, 2000). In addition to strategies presented in the specific guidance documents listed above, an overview of the various recommended practices for the remediation of mold-contaminated materials, including porous, semi-porous and non-porous material removal, HVAC system remediation, containment strategies, and judging remediation effectiveness is presented in *Bioaerosols: Assessment and Control* (ACGIH, 1999; see Chapter 15, “Remediation of Microbial Contamination” by Shaughnessy and Morey).

The effect of biocides (to kill existing growth) and antimicrobials (to suppress or prevent growth) on mold varies according to mold species, and more research is needed to fully assess efficacy (NAS, 2000; Foarde, 1998; Cole and Foarde, 1999). The different chemical classes of biocides include alcohols, aldehydes, halogens, hydrogen peroxide, phenolics, and quaternary ammonium compounds (Foarde, 1998). The use of biocides is discouraged by some experts, because little research has been conducted on their effectiveness for this use and because of the potential human health hazards associated with this use (USEPA, 1997; Foarde, 1998; Cole and Foarde, 1999). In addition, research indicates that dead mold material often still retains the allergenic or toxic properties of the mold (Foarde, 1998; NAS, 2000), and thus replacement is often cited as the best mitigation option.

Because of their potential to rapidly spread molds throughout a building, ventilation systems are of particular concern as mold contamination sources (Foarde et al., 1997b). It is possible to clean ducts made of bare sheet metal, and EPA recommends considering cleaning such ducts if substantial visible mold growth is present (USEPA, 1997). Cleaning porous, insulated ducts is difficult, however. There are no registered biocides for treatment of such duct materials (Foarde, 1998). Mechanical cleaning of such ducts has also been shown to be relatively ineffective. For example, a study investigating the effectiveness of mechanical cleaning of fibrous duct material contaminated with mold growth by vacuuming concluded that mechanical cleaning was only able to temporarily (for 6 weeks) reduce the surface mold load (Foarde et al., 1997b). EPA, the National Air Duct Cleaners Association, and the North American Insulation Manufacturers Association all recommend the replacement of wet or moldy fiberglass duct material (USEPA, 1997).

5.0 CURRENT RESEARCH AND INFORMATION GAPS

Possible areas of consideration for future research include:

Methodological Issues

- Standard methods for mold sampling.
- Standard methods for analysis of mold toxins.
- Standardized methods for analysis of mold allergens.
- Determination of performance criteria for analytic methods (accuracy, detection limits, etc.).

- Information on factors that affect exposure and methods to quantify exposure from environmental samples (e.g., relationship between vacuum dust, etc. samples and actual exposure).
- Further research on fungal measurement using indicators of fungal growth (e.g., microbial VOCs).

Health Issues

- Health-based exposure standards or guidelines for mold.
- Identification of threshold levels for sensitization to major residential mold allergens and for asthma exacerbation.
- Additional data on standard amounts and types of molds (airborne and surface) in residential environments (with and without moisture problems) for comparison studies.
- Appropriate levels of protection for mitigation workers.

Building and Structural Issues

- Health impacts of building design and management.
- Data to quantify which aspects of household water damage are related to respiratory illness.
- Standard criteria for assessing water damage.
- Standard, cost effective remediation procedures and criteria.
- Effective and standard preventive measures.

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Appendix A. Additional Internet Resources

In addition to the references and links appearing in the reference list above, the following table provides selected links with additional information on mold and mold contamination issues in homes.

Sponsoring Organization/Topic	Internet Web Site Address
Aerotech Laboratories, Inc. -- Indoor air quality testing information	http://www.aerotechlabs.com/
Affordable Comfort, Inc.	http://www.affordablecomfort.org
Allergy, Asthma & Immunology Online	http://www.allergy.mcg.edu/
American Academy of Allergy, Asthma and Immunology	http://www.aaaai.org/
American Conference of Governmental Industrial Hygienists (ACGIH)	http://www.acgih.org/home.htm
American Indoor Air Quality Council	http://www.iaqcouncil.org/
American Industrial Hygiene Association (AIHA) -- Environmental Microbiology Testing and Proficiency external peer review programs (EMPAT and EMLAP)	http://www.aiha.org/SplashPages/html/topic-mold.htm
American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc.	http://www.ashrae.org/
Assessment Guide for Building Owners (EPA and NIOSH)	http://www.cdc.gov/niosh/bagtoc.html
Asthma and Allergy Foundation of America	http://www.aafa.org/
Building Science Corporation - Publications and papers dealing with and preventing mold problems	http://www.buildingscience.com/resources/mold/default.htm
Canada Mortgage and Housing Corporation -- Healthy Housing & Sustainability Projects	http://www.cmhc-schl.gc.ca/en/imquaf/hehosu/index.cfm
Canada Mortgage and Housing Corporation -- Publications on moisture and mold	http://www.cmhc-schl.gc.ca/en/burema/gesein/Momo/index.cfm
Case Western Reserve University / General Clinical Research Center -- Pulmonary Hemorrhage and Hemosiderosis In Infants	http://gcrc.cwru.edu/stachy/default.htm
Centers for Disease Control and Prevention (CDC)	http://www.cdc.gov/
CDC -- Air Pollution and Respiratory Health Branch	http://www.cdc.gov/nceh/airpollution/default.htm
CDC -- Questions and Answers on Stachybotrys chartarum and other molds	http://www.cdc.gov/nceh/airpollution/mold/stachy.htm
CDC -- Report on Cleveland Pulmonary Hemosiderosis and Stachybotrys	http://www.cdc.gov/epo/mmwr/preview/mmwrhtml/mm4909a3.htm
Environmental Health Watch	http://www.ehw.org/
P&K Microbiology Services Inc.	http://www.stl-inc.com/About/News/P&K_Acquisition4-4-03.htm
Environmental Microbiology Laboratory, Inc.	http://www.emlab.com/
Health House Project of the American Lung Association	http://www.healthhouse.org/
Healthy Homes Partnership - USDA and HUD	http://www.uwex.edu/healthyhome/
HUD's Healthy Homes for Healthy Children	http://www.hud.gov/consumer/hhhchild.cfm
HUD's Office of Healthy Homes and Lead Hazard Control	http://www.hud.gov/offices/lead/
Indoor Air Pollution: An Introduction for Health Professionals (USEPA)	http://www.epa.gov/iedweb00/pubs/hpguide.html
Indoor Biotechnologies, ltd.	http://www.inbio.com/
Institute of Inspection Cleaning & Restoration -- fire and flood restoration	http://www.iicrc.org/
Johns Hopkins Asthma & Allergy	http://www.hopkins-allergy.org/
Master Home Environmentalist	http://www.alaw.org/air_quality/master_home_environmentalist/
Minnesota Department of Public Health - Mold in Homes	http://www.health.state.mn.us/divs/eh/indoorair/mold/
National Safety Council Indoor Air Program	http://www.nsc.org/ehc/indoor/iaq.htm
New York City Department of Health -- Guidelines on Assessment and Remediation of Fungi in Indoor Environments	http://www.ci.nyc.ny.us/html/doh/html/epi/moldrpt1.html
NIH National Institute of Environmental Health Sciences -- Asthma Homepage	http://www.niehs.nih.gov/airborne/home.htm
North Carolina State University Extension Service -- Mold, dust mites, fungi, spores, and pollen: Bioaerosols in the human environment	http://www.ces.ncsu.edu/depts/fcs/housing/pubs/fcs3605.html
Safer Child, Inc. -- Indoor Air Pollution	http://www.saferchild.org/indoor.htm
University of California -- Indoor Air Quality Tools: Education, Prevention	http://ehs.ucsc.edu/th/ehs.asp?page=indoor_air_quality

Sponsoring Organization/Topic	Internet Web Site Address
and Investigation	
University of Minnesota, Department of Environmental Health and Safety -- Fungi in Buildings	http://www.dehs.umn.edu/iaq/fungus/
University of Montana -- Healthy Indoor Air	http://www.montana.edu/wwwcxair/
USEPA -- Indoor Air Quality Homepage	http://www.epa.gov/iaq/
USEPA -- Mold Resources	http://www.epa.gov/iaq/molds/moldresources.html
USEPA -- Office of Children's Health Protection	http://yosemite.epa.gov/ochp/ochpweb.nsf/homepage
USEPA -- Mold Publications	http://www.epa.gov/iaq/molds/index.html