CONNECTING POOR OCCUPANT RESPIRATORY HEALTH WITH THE PRESENCE OF FUNGI IN DAMP INDOOR ENVIRONMENTS THROUGH META-ANALYTIC INVESTIGATIONS AND PUBLIC HEALTH POLICY DEVELOPMENTS

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at George Mason University

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DEDICATION

This doctoral dissertation is dedicated to all those who have supported my dream of accomplishing it, especially my mother and mentor Dorothy Lee Hayes Dodson. Innovation is a tradition in our family, and Dot has encouraged me to follow my interests and apply myself to reach goals complementing those interests. This doctoral dissertation is one of those goals. I am blessed to have friends such as Fran, Sue and Annaliesa; my son Todd Alexander, and my committee members, all of whom have given their time, expertise, and patience to enable me to complete this dissertation.

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LIST OF ABBREVIATIONS

AIM	Allergen Integrated Management
AA of NC	Asthma Alliance of North Carolina
a_W	Water activity
CI	Confidence Interval
CRS	Chronic Rhinosinusitis
ECM	Extra Cellular Material
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
H_o	Null Hypothesis
Ig	Immunoglobulin
ICD	International Statistical Classification of Diseases and Related Health Problems
IL	Interleukin
IOM	Institute of Medicine
kD	Kilodalton
mVOCs	Microbial Volatile Organic Compounds
NCDHHS	North Carolina Department of Health and Human Services
NIOSH	National Institute for Occupational Safety and Health
PBAPs	Primary Biological Aerosol Particles- Particulate Matter less than 10 microns
ROQ	Rhinitis Outcomes Questionnaire
µg/ml	microgram per milliliter
USCDC	United States Centers for Disease Control
USEPA	United States Environmental Protection Agency

ABSTRACT

CONNECTING POOR OCCUPANT RESPIRATORY HEALTH WITH THE PRESENCE OF FUNGI IN INDOOR ENVIRONMENTS

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For the last 25 years, scientists and policy-makers have suspected that fungi colonizing indoors have a detrimental influence on the respiratory health of occupants. To address this public health concern, a meta- analysis was performed that examined the association between fungal agents and nasal hypersensitivities in indoor environments. The effect size estimates (odds ratios) from thirty individual statistics lend support to evidence that occupants exposed to fungal agents are more likely to exhibit symptoms of nasal hypersensitivities. The meta-analytic test statistic of $Z= 8.48$ ($p<0.00001$); summary $I^2 = 44\%$; and, summary odds ratio= 1.58 [Children (1.63); Adults (1.49); (95% C. I.)] links fungal agents and nasal hypersensitivity symptoms. This study's findings acknowledge the consistency of association between fungal agents and hypersensitivities and moreover support the need for public health policies addressing indoor fungal contamination in order to protect community respiratory health.

Key words: fungus, mold, spore, respiratory, rhinitis, exposure, indoor, public health, meta-analysis, Review Manager, allergy, allergen, environment, public policy.

Chapter 1: Introduction

In recent years, scientists and policy-makers have suspected that the presence of fungal colonization, commonly referred to as "mold," in indoor environments has a detrimental influence on occupant health. In order to better understand the connection between fungi in indoor environments and adverse health effects, the United States Centers for Disease Control and Prevention (USCDC) asked the Institute of Medicine (IOM), which was established in 1970 by the National Academy of Science and whose mission it is to examine policy matters pertaining to public health, to conduct a comprehensive review of scientific literature dealing with possible connections between fungal colonization and respiratory and allergic symptoms.

The Institute of Medicine conducted an evidence-based investigation and published Damp Indoor Spaces and Health in 2004 (Clark et al., 2004). The committee of experts representing the IOM stated therein that they found "sufficient evidence of an association" between health outcomes and the presence of mold or other agents in damp indoor environments for upper respiratory tract (nasal and throat) symptoms, cough, as well as wheeze and asthma symptoms in sensitized asthmatic persons. To a lesser degree of certainty, the committee also reported a "limited or suggestive evidence of an association" among health outcomes and the presence of mold or other agents in damp indoor environments for symptoms typical of lower respiratory illness, i.e. asthma, in

otherwise healthy children. The uncertainty of these associations is partially due to lack of understanding of the agent responsible for the health outcome. (Table 1).

Table 1. Categories of evidence referenced in the Institute of Medicine report. (Clark et al., 2004).

Summary of the categories of evidence used in this report

Sufficient Evidence of a Causal Relationship

Evidence is sufficient to conclude that a causal relationship exists between the agent and the outcome. That is, the evidence fulfills the criteria for "sufficient evidence of an association" and; in addition; satisfies the following criteria: strength of association, biological gradient, consistency of association, biologic plausibility and coherence, and temporally correct association.

Sufficient Evidence of an Association

Evidence is sufficient to conclude that there is an association. That is, an association between the agent and the outcome has been observed in studies in which chance, bias, and confounding can be ruled out with confidence.

Limited or Suggestive Evidence of an Association

Evidence is suggestive of an association between the agent and the outcome is limited because of chance and bias. Confounding cannot be ruled out with confidence.

Inadequate or Insufficient Evidence to Determine Whether an Association exists

The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence of an association. Alternatively, no studies exist that examine the relationship.

The ranking of "sufficient" evidence of an association followed by the "limited or suggestive" evidence of an association are the two categories that are bordered by the upper category- "sufficient" evidence of a "causal relationship" and the lower category- "inadequate or insufficient evidence to determine whether an association exists." The

committee defined the merits of each category and did not conclude that a causal relationship existed between the presence of fungal agents in damp, indoor spaces and a detrimental effect on respiratory health. One theory that the evidence-based review failed to support was the association of different gradients of fungal contamination with the onset of health outcomes or absence of health outcomes. (Table 2).

Table 2. The Institute of Medicine's summary of findings regarding association between health outcomes and presence of mold or other agents in damp indoor environments. (Clark et al., 2004).

Sufficient Evidence of a Causal Relationship

No outcomes met this definition

Sufficient Evidence of an Association

Upper respiratory (nasal and throat) tract symptoms
Asthma symptoms in sensitized asthmatic persons
Hypersensitivity pneumonitis in persons susceptible to mold or bacteria in damp indoor environments
Wheeze
Cough

Limited or Suggestive Evidence of an Association

Lower respiratory illness in otherwise-healthy children

Inadequate or Insufficient Evidence to Determine Whether an Association Exists

Dyspnea (shortness of breath)
Airflow obstruction in healthy persons
Mucous membrane irritation syndrome
Chronic obstructive pulmonary disease
Inhalation fevers
Lower respiratory illness in healthy adults
Rheumatologic and other immune diseases
Acute idiopathic pulmonary hemorrhage in infants
Skin symptoms
Asthma development
Gastrointestinal problems
Fatigue
Neuropsychiatric symptoms
Cancer
Reproductive effects

As a result of the ranking of evidence-based literature which links the presence of fungi to detrimental health outcomes in sensitized individuals in damp indoor environments, and which suggests a connection between the presence of fungi and detrimental health outcomes in otherwise healthy children in damp indoor environments, the committee encourages the development of public health mechanisms to reduce or prevent the incidence of damp indoor environments, and thus theoretically to reduce or prevent the onset of poor respiratory health outcomes exhibited by occupants.

One of the IOM committee goals focuses on collaborations of stakeholders to achieve healthier indoor environments. If stakeholders were offered evidence-based reasoning that strongly linked the presence of hydrophilic fungal colonization, such as *Aspergillus, Penicillium, Cladosporium*, and *Alternaria* as markers for exposures, and upper respiratory illnesses as health outcomes to those exposures, stakeholders may be able to frame policies directing timely efforts to remediate buildings after a flooding event to avoid negative health outcomes to the building's occupants.

The research contained in this dissertation was launched by the important work of the IOM committee investigation of associations between damp indoor environments, fungal colonization, and poor respiratory health of occupants. Specifically, the research included an evidence-based meta-analysis of the relationship between the presence of fungal agents detected within indoor environments and the occurrence of upper respiratory allergic responses exhibited by occupants. Furthermore, the research focused on how the findings of the meta-analysis may influence policy-making within local government municipalities with North Carolina.

Research statement and *Null* hypothesis

Hypothesis (null): tested. There is no association between fungal agents and nasal hypersensitivities in indoor environments.

In recent years, scientists and policy-makers have suspected that the presence of fungal colonization within indoor environments, commonly associated with the presence of airborne spores, had a detrimental influence on occupant respiratory health, specifically allergies and asthma. A meta-analytic quantitative review of current published literature may better describe the relationship between fungi in indoor environments and adverse upper respiratory symptoms of human occupants.

The mission of the Institute of Medicine in publishing Damp Indoor Spaces and Health was to conduct an evidence-based investigation to gain understanding of the connection between fungi in indoor environments and adverse health effects (Clark et al., 2004). The committee of experts representing the IOM stated therein that they found "sufficient evidence of an association" between health outcomes and the presence of mold or other agents in damp indoor environments for upper respiratory tract (nasal and throat) symptoms, cough, wheeze, and asthma symptoms in sensitized asthmatic persons. The committee clearly defined the parameters of each category and at the time of publication could not conclude that a causal relationship existed between the presence of fungi in damp, indoor spaces and poor respiratory health.

Another evidence-based evaluation of the literature noted in the IOM report, as well as additional literature cited from an independent research review identifying

additional citations from 1980 to the present, may provide a heterogeneous collection of studies suitable for a meta-analytic examination. Selecting statistical models, specifying study variables, quantifying effect modifiers, adjusting for bias, utilizing confounders, and performing relative risk analysis were all important tasks of a meta-analysis attempting to further describe the relationship between the presence of fungal colonization in damp buildings and poor occupant respiratory health.

Many governmental policymakers rely on the expertise of national and state agencies as policies are framed. As of June 2010, national and state leaders are emphasizing the need to reduce allergens in indoor environments to improve the health of citizens diagnosed with respiratory allergies in communities throughout the United States. The month of May has been proclaimed by federal and some state governments as "Asthma Awareness Month." The U.S. Environmental Protection Agency's Asthma Forum 2007 called for communities nationwide to enroll in their "Communities-in-Action" program to better care for the asthmatic population of the United States.

Currently, the North Carolina State Department of Health and Human Services (NCDHHS) organizes and sponsors an annual Asthma Summit meeting, which includes health representatives from most of the state's 100 counties seeking to better assist local communities in addressing the health concerns associated with chronic allergies. The NCDHHS is an active partner in the Asthma Alliance of North Carolina (AA of NC) where policy development directed towards community respiratory health improvement is a shared goal. (Figure 1).

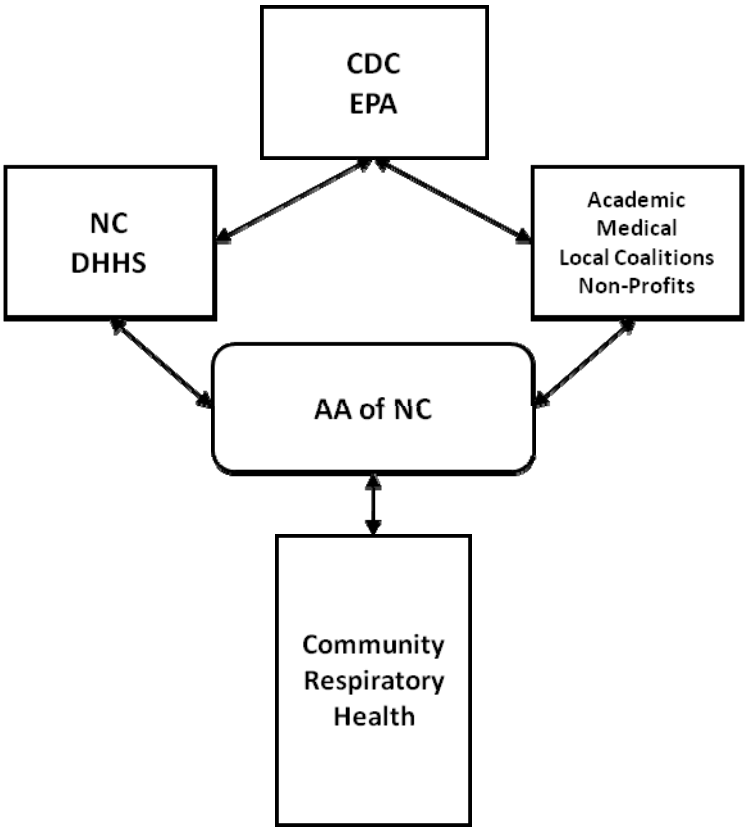

Figure 1. Community respiratory health improvement: A shared goal among federal, state, and local stakeholders.

Establishing a causal link between fungal components and poor respiratory health outcomes is not a simple policy issue. The identification of environmental fungal agents and corresponding respiratory illnesses is a challenging task. Allergens are proteinaceous fungal agents that elicit immunological hypersensitivity responses by an individual upon secondary exposure. This triggered response may be on a continuum somewhere between early-response symptoms to delayed-response symptoms. These symptoms may be clinically identified with upper respiratory illness. (Figure 2).

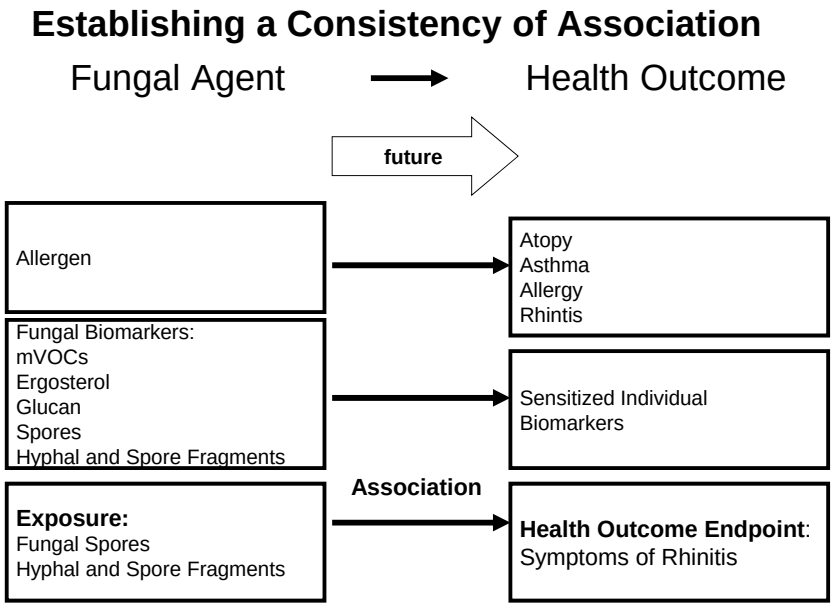

Figure 2. Defining the consistency of association between fungal agents and sensitized respiratory responses.

Several other organisms, including arthropods, are known producers of allergens within indoor environments. For example, house dust mites produce allergens that may trigger asthma exacerbations. This causal relationship between the presence of allergens produced by house dust mites in indoor environments and the disease expression of asthma is accepted by respected medical authorities and their corresponding guidelines (Chapman, 2010) (US Department of Health and Human Services, National Institutes of Health, National Heart, Lung, and Blood Institute, & National Asthma Education and Prevention Program, 2007) (Quoix, Mao, Hoyet, & Pauli, 1993) (Arlian, 1991) (Arlian,

Geis, Vyszenski-Moher, Bernstein, & Gallagher, 1984). This is an example of an evidence-based epidemiological association with evidence-based disease causation.

Fungi, on the other hand, are a composite of multiple species and it is difficult-to characterize specific fungal causative agents eliciting human sensitization responses (Rao, Burge, & Chang, 1996). (Figure 3).

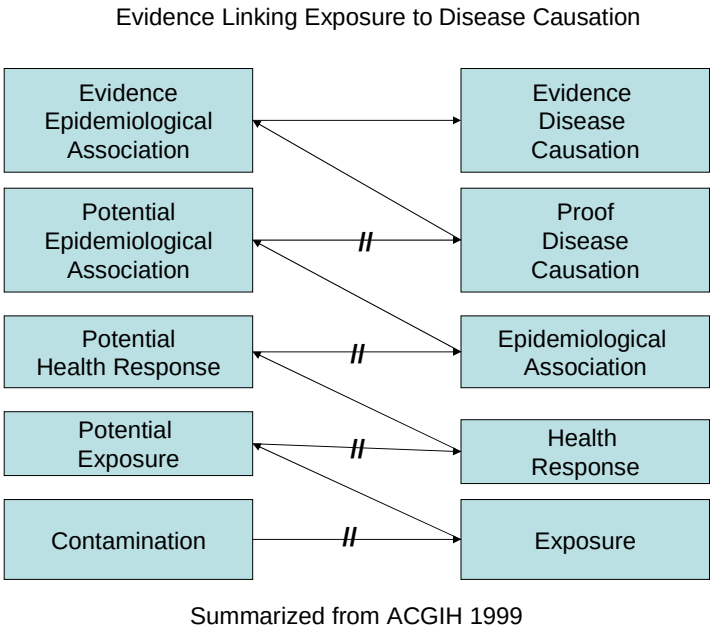

Figure 3. Multi-step process linking fungal agent exposure to disease causation. (summarized from Macher, 1999).

Historically, aerobiologists have sampled indoor environments to characterize the fungal component of the indoor air environment. In the case of wetted building materials, sampling may include surface sampling in addition to air sampling. Several authors have concluded that a combination of sampling techniques and assays may better characterize the identification and distribution of fungal agents during indoor

environment assessments (Ciaccio et al., 2008) (Peters, Muilenberg, Rogers, Burge, & Spengler, 2008) (Pitkaranta et al., 2008) (Baxter, Perkins, McGhee, & Seltzer, 2005) (Horner, Worthan, & Morey, 2004).

Aerobiologists have also published numerous studies investigating the fungal colonization of wetted building materials composed of cellulosic material, one type of material that provides nutritional and structural support for the fast-colonizing mitosporic fungi commonly found in indoor environments (Cooley, Wong, Jumper, & Straus, 2004) (Levetin & Govert, 2003) (Meklin et al., 2003) (Haverinen, Husman, Pekkanen, et al., 2001) (Tuomi et al., 2000) (Gravesen, Nielsen, Iversen, & Nielsen, 1999).

Fungal agents that trigger an allergenic response through inhalation were initially described as intact spores (Hopkins, Benham, & Kesten, 1930). Large mitospores such as *Alternaria* spp. maybe associated with seasonal allergies as dead leaves fall from trees in the autumn and become wet as they lay on the ground. Turbulent air then can carry the fungal spores from their source into a building where children may inhale spores, triggering an allergic response. (Figure 4).

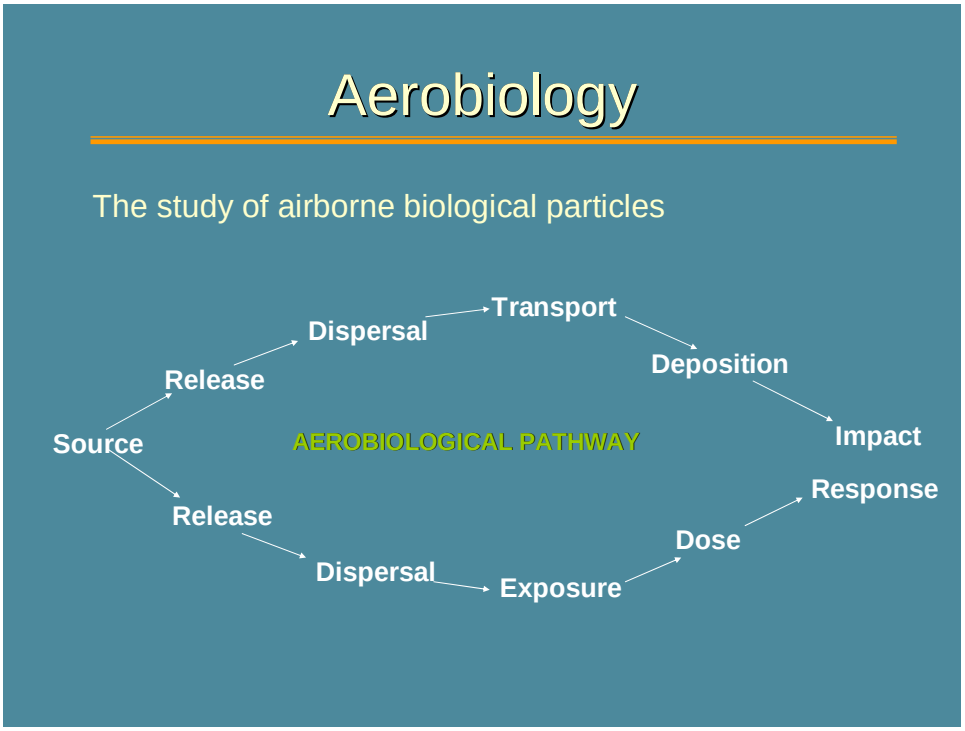

Figure 4. The pathway of airborne fungal spores from their source to their final impaction and potential elicitation of an immunological response. (Rogers, 2003).

The aeroallergen exposure becomes a risk factor in the prevalence of asthma and allergies. The relationship between A*lternaria* and poor respiratory health has been characterized by both allergen sequencing and the presence of IgE-antibody in serum and has been adopted in medically-related guidelines (Kurup, Shen, & Vijay, 2002a).

Currently, policy makers often rely on data collected, along with visual inspection, to describe fungal contamination within damp buildings rather than health effects. Testing for health effects is difficult due to variation in allergenic variability of different fungal isolates within a species, degree of immunological sensitivity of the

individual, subjectivity of questionnaires, and quality of fungal-based allergen extracts used in clinical hyperreactivity testing.

The quantification and speciation of fungal colonization usually is based upon environmental observations and assessments followed by laboratory culturing and counting with corresponding calculations to airborne concentration. Quantitative standards and guidelines are usually based on a variety of sampling techniques with corresponding good laboratory practices and calculations.

As immunological bioassays and protein sequencing advance in their technologies, more allergens are being described and identified in indoor environments. Other biomarkers of fungal colonization are also of scientific and medical interest.

An extensive review of published literature citing fungal agents and biomarkers of fungal colonization associated with wetted building environments along with documentation of poor respiratory health may aid in establishing a direct causal relationship between fungal agents and symptoms of nasal hypersensitivity (health outcome) in buildings. Meta-analysis was employed as the statistical tool to sew a common thread between fungal colonization in indoor environments and the development of symptoms of poor upper respiratory health.

Employing meta-analysis accumulates findings across studies and estimates and characterizes the amount of artifact variation between studies, thus arriving at the true relationship between variables (Wanous, 1989). The effect size is an index of the relationship between the treatment (detectable fungal agent) and the outcome (symptoms of nasal hypersensitivity). An extensive literature review has identified current scientific

and medical thought to better understand and describe the relationship between exposures to fungal agents and symptoms of nasal hypersensitivities exhibited by occupants of indoor environments.

Research Outcomes*:*

1). Built a computer-based reference database from published journal articles, books, scientific presentations at meetings, and other credible sources. Placed and organized relevant information into a literature review.

2). Identified search terminology for evidence acquisition.

3). Reviewed evidence-based research studies then excluded some from further review and included others into a meta-analysis.

3). Performed a quantitative analysis of the evidence-based literature employing Review Manager (version 5.0.24) software with two modifiers: children and adults.

4). Reviewed and discussed quantitative results of meta-analysis.

5). Discussed meta-analysis results and their potential significance towards community respiratory health policy development within the State of North Carolina.

6). Concluded that support funding is needed to investigate the causal relationship between fungal agents and poor respiratory health in local municipalities.

Chapter 2: Literature Review

In the United States during the 1970's, the collection of evidence of fungal sources within occupied buildings was reviewed and improved as researchers realized that traditional older approaches were not representing the true prevalence of fungal levels inside homes (Solomon, 1975). More recently, experts who authored Health implications of fungi in indoor environments (Samson et al., 1994) commented on their observations of fungi and poor occupant health, suggesting an association between the two. This book was compiled by a world-wide collective of scientists who offered limited evidence of an association between fungi observed in indoor environments and adverse health implications. In 2004, the Institute of Medicine of the National Academies stated in its publication Damp indoor spaces and health (Clark et al., 2004) that there is sufficient evidence of an association between health outcomes and the presence of mold or other agents in indoor environments.

In order to further investigate the relationship between fungal colonization of indoor environments and adverse health effects where the microbe interfaces with the human occupant, an environmental understanding of the fungal species complex is needed along with an operational understanding of an adverse upper respiratory health effect. By discussing the attributes of each side of the relationship, the foundation for statistical meta-analyses is laid.

Worldwide observation and reporting

Scientists world-wide suspect that the presence of certain fungi in indoor spaces causes upper respiratory health effects due to environmental exposures. Investigators from different regions of the world have published research findings attempting to connect the inhalation of fungal components with the onset of clinical symptoms after occupants have remained within structures compromised by wetted building materials for a period of time.

In Europe, Finnish authors (Koskinen, Husman, Meklin, & Nevalainen, 1999) (Pirhonen, Nevalainen, Husman, & Pekkanen, 1996) (Jaakkola, Jaakkola, & Ruotsalainen, 1993); British authors, (Jaakkola, Hwang, & Jaakkola, 2005); Italian authors (Simoni et al., 2005); Danish working group (Meyer et al., 2003); Swedish scientists (Bornehag et al., 2005) (Engvall, Norrby, & Norback, 2002) have led research investigations to better understand the conjunction of mold growth indoors with respiratory distress exhibited by occupants of buildings.

In Asia, investigations have observed relationships between mold and poor respiratory health (Saijo et al., 2009) (Saijo, Yoshida, & Kishi, 2009) (Li, Hsu, & Lu, 1997). Environmental factors and allergy in schoolchildren were studied in depth in Western Australia (Palmer, Valinsky, Pikora, Zubrick, & Landau, 1999) with similar conclusions.

Specific sites where exposure to fungal agents may occur are also of interest. School buildings compromised by fungal colonization are favorite research targets

pursuant to which the U.S. Environmental Protection Agency has tabulated five research studies dedicated to mold-related risk factors in water-damaged schools (Mudarri & Fisk, 2007). School buildings where children spend many hours indoors are environments where several studies have investigated environmental contaminants (Sheehan et al., 2008) (Santilli & Rockwell, 2003) (Moglia, Smith, MacIntosh, & Somers, 2006) (Levetin et al., 1995).

Another area of interest is the workplace, where NIOSH (National Institute for Occupational Safety and Health) lead by Kay Kreiss of the Division of Respiratory Disease Studies continues to study the highly suspected association between fungi colonization and respiratory adverse health effects (Sauni et al., 2009) (Cox-Ganser, Rao, Park, Schumpert, & Kreiss, 2009) (Park, Cox-Ganser, Kreiss, White, & Rao, 2008) (Park, Cox-Ganser, Rao, & Kreiss, 2006).

In the United States, a team of researchers measured fungal concentrations in water-damaged structures and suspected that those environmental conditions played a major role in the onset of allergic rhinitis in children with family history of allergic sensitivities (Stark et al., 2005). This investigation was included with two other published findings (Bornehag et al., 2005) (Simoni et al., 2005) during a quantitative meta-analytic investigation led by Lawrence Berkeley National Laboratory (Fisk, Lei-Gomez, & Mendell, 2007). The 2007 meta-analyses publication compiled the three 2005 publications with the nine independent single studies (Engvall, Norrby, & Norback, 2001) (Koskinen et al., 1999) (Jedrychowski & Flak, 1998) (Li & Hsu, 1997) (Yang, Chiu, Cheng, & Lin, 1997) (Pirhonen et al., 1996) (Jaakkola et al., 1993) (Brunekreef et

al., 1989) (Waegemaekers, vanWageningen, Brunekreef, & Boleij, 1989) described in the 2004 Institute of Medicine's book on dampness and mold (Clark et al., 2004) to associate dampness and molds with upper respiratory tract symptoms.

Evidenced-based bio-markers of environmental exposure and health effect

Fungal colonization on wetted building materials may occur after a nutrient-loading event such as flooding occurs. Then, after a time lapse, the fungal biomass becomes a contributor to the indoor air environment where human occupants interface with spores, hyphal fragments, and other small micron-sized fungal particles in their breathing zones. Whether fungal particles become detrimental contributors to the indoor air environment by lowering the quality of air depends on the susceptibility of the individual.

Whether environmental exposure results in ill health follows a time sequence. First, an event must occur that increases the moisture content of a cellulose-building material within the envelope of a building. Next, if fungal spores do not already exist in the building material, they are introduced to the building substrate along with nutrients during a flooding event or are carried inside by wind turbulence, air handling units, or insects. Once fungal colonization is established, the fungal biomass load in the indoor air generally increases as fungal biomarkers such as spores and hyphal fragments break apart from established fungal colonies. The fungal biomarker can be quantified through environmental assessment.

The susceptibility of the individual is extremely important when analyzing health effects associated with upper respiratory hypersensitivities. Many determinants, including genetic factors, nutrition, and age, may characterize the susceptibility of an individual person (Pier, Lyczak, & Wetzler, 2004).

Environmental exposure of a susceptible individual occurs when the fungal agent biomarker is inhaled and internalized by the human host. At this point in the exposure process, the fungal agent becomes a xenobiotic. This internal dose may remain as the initial xenobiotic biomarker directly eliciting immune or non-immune responses, or the xenobiotic biomarker may metabolize into a component which triggers immune hypersensitivity responses.

By eliciting an immunological response, the internal dose becomes a bio-effective dose. This internal dosing evolving into bio-effective dosing may be repeated if more fungal agents remain in the indoor environment and are continually inhaled by the occupant. The bio-effective dose activates a ferris wheel of receptors resulting in a variety of health effects marked by sneezing, itchy nose, nasal congestion, nasal discharge, coughing, and other symptoms of upper respiratory hypersensitivities. Disease may result from environmental exposure and bio-effective dosing of fungal agents (Campbell, Thrasher, Gray, & Vojdani, 2004).

This dissertation investigation attempted to connect the temporal relationship of environmental exposure to symptoms (health outcome endpoint) exhibited by the individual. (See red boxes of Figure 5). Information has been gathered by published authors to characterize both sides of this temporal relationship, with the environmental

exposure followed by the onset of health effects associated with poor upper respiratory health of exposed individuals.

Evidence-based biomarkers representing both sides of the environmental exposure and health effect relationship are generally defined by aerobiology and medical disciplines. Although information is gathered by different methodologies, each discipline has widely-accepted practices within each area of expertise. Information has been gathered to characterize the indoor air fungal component as one which may potentially elicit a health effect which can be clinically or self described.

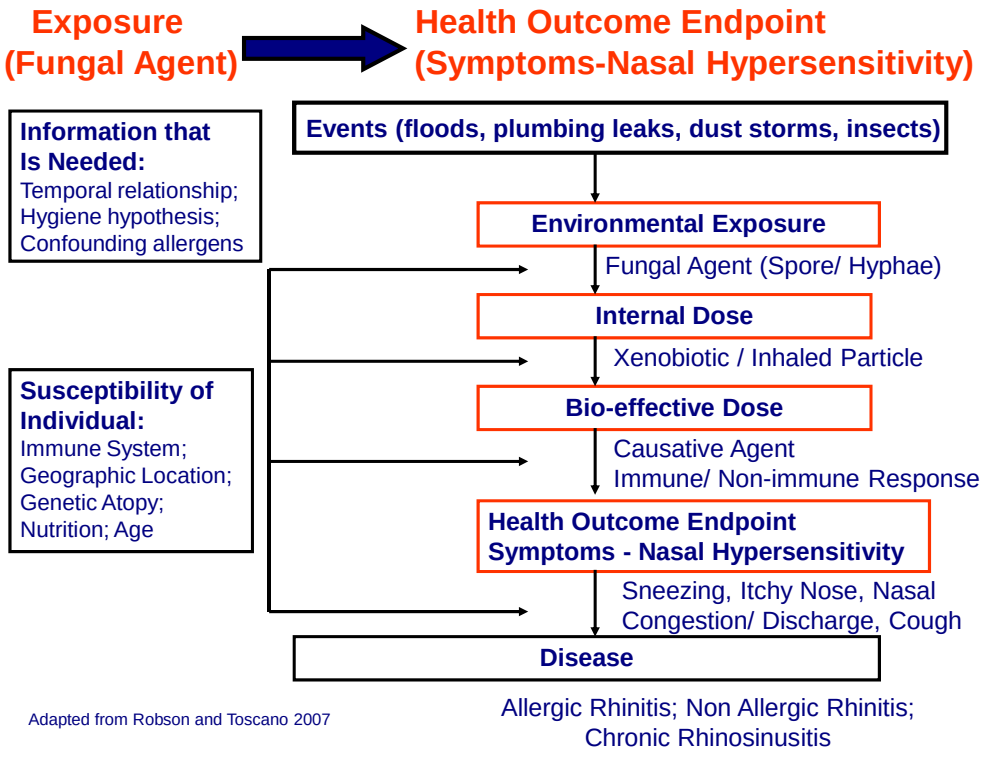

Figure 5 - Flowchart of evidenced-based bio-markers of environmental fungal exposure and subsequential upper respiratory health effects exhibited by occupants of contaminated buildings. (Adapted from Robson and Toscano 2007).

Events initiating environmental exposure to fungal biomarkers

Events such as natural disasters may cause flooding to structures, bringing nutrient loads inside buildings as soils are transported from outdoors to indoors. A single storm event may cause severe flooding when water channels are unable to accommodate large volumes of water during peak flow (Meyer, Sale, Mulholland, & Poff, 1999).

Hurricane events are often responsible for flooding buildings, such as homes which remain under water or partially under water for months, as seen in 2005 with

Hurricanes Katrina and Rita in New Orleans, Louisiana (Rao, Riggs, Chew, Muilenberg, Thorne, VanSickle, et al., 2007). Flooding following earthquake/tsunami events may initiate environmental exposure to fungal biomarkers.

Events associated with building maintenance such as plumbing leaks, roof leaks and condensation accumulation may result from poor building design and untimely building repairs allowing water to damage buildings which subsequently sustain fungal growth (Gravesen et al., 1999). In 1994, the American Society for Testing and Materials published an extensive manual to provide information enabling for engineers to design and maintain buildings free of moisture problems (Trechsel, 1994).

Another event which may introduce fungi to an indoor environment is wind turbulence transporting outdoor allergens to inside spaces. Sources of fungal spores and hyphae are released into the outside air by wind, rain, mechanical disturbance, or by actively discharge mechanisms within the fungal structure (Burge & Rogers, 2000). Dust events have the potential to increase the load of respiratory particles such as fungal spores into the atmosphere in a very short time (Polymenakou, Mandalakis, Stephanou, & Tselepides, 2008).

Fungal spores, as Primary Biological Aerosol Particles (PBAPs as PM_{10} mass), are transferred into the atmosphere without changing chemical composition and are major contributors to the overall composition of atmospheric PBAPs (Winiwarter, Bauer, Caseiro, & Puxbaum, 2009). Open doors and windows or poorly filtrated ventilation systems provide easy access for outdoor fungal agents to become indoor fungal agents. Once it settles onto a suitable surface, the fungal agent may propagate and colonize

indoors. This ability to transport from the outdoors to the indoors is an important factor when determining mold contamination within structures (Baxter et al., 2005).

Additionally, fungal spores, hyphae and other fungal-based propagules can be carried indoors by insects. Saprophytic fungi, such as *Aspergillus* and *Penicillium*, are carried on the surface of the insect's exoskeleton as it moves from place to place (Greif & Currah, 2007).

Fungal colonization within indoor environments

When buildings are wetted by natural or other events which can compromise their integrity, the damp, cellulosic building materials can provide the vital nutrients and substrate required for fungal colonization. Water-damaged, aged organic materials such as wooden floors, beams and plywood as well as wallpaper, jute, insulation, plaster and cardboard have been observed to support fungal growth, including *Aspergillus* and *Penicillium* species (Gravesen et al., 1999) (Ezeonu, Price, Simmons, Crow, & Ahearn, 1994).

Household dust may contain fungal agents and may become classified as respirable dust when it becomes airborne during normal occupant activities such as sitting in an upholstered chair or walking on a carpet (Elfman, Riihimaki, Pringle, & Walinder, 2009) (Pitkaranta et al., 2008) (Park et al., 2006). The presence of fungi indoors may be hidden in behind walls, inside ventilation systems, on air filters, behind wall décor, or under carpets, hampering easy visual detection (Ahearn et al., 2007) (Li & Yang, 2004)

(Spurgeon, 2003). Fungal concentrations within indoor spaces may also change within-day and within-season as temporal and spatial differences were noted by a team of investigators (Hyvarinen et al., 2001).

In order to colonize a cellulosic substance, fungi generally require free water or available water for growth. Many Acomycetes, such as cellulosic-seeking *Asperigillus* and *Penicillium* species, need wooden surfaces to contain some degree of moisture in order for those surfaces to be suitable for colonization. Mycologists have classified fungal species into categories based on each species' need for specific available water activity (a_w) requirements for sporulation and hyphal production. The a_w is expressed as a decimal fraction of the amount of water present in a substrate, such as wetted drywall, which is in equilibrium with relative humidity. Thus, a mold species index from indoor spaces is indicative of the extent of water intrusion and subsequent damage to a building (Thrasher & Crawley, 2009).

A low water activity (≤ 0.85) is a category for true xerophilic fungal species such as *Eurotium rubrum*. An intermediate range of water activity (0.85-0.90) groups typical fungal colonizers of wetted building materials such as *Aspergillus flavus, A. nidulans, A. sydowii,* and *A. versicolor*. Although fungal spores and hyphal fragments may accumulate in dry floor dust, they require a wetted surface with available nutrients in order to establish colonies inside buildings where occupants live, work, study, and play (Levetin, Horner, Carpenter, & McGinnis, 2002).

Environmental exposure

Fungal agent biomarkers within indoor spaces are identified and quantified during indoor air quality investigations assessing occupant respiratory health risk. The fungal agent biomarkers associated with fungal contamination of wetted buildings are as follows: microbial volatile organic compounds (mVOCs); mycotoxins; glucans; ergosterol; allergens; extra-cellular materials; fungal fragments; and, fungal spores. General indication of the presence of fungal growth is often indicated by visible mold growth which may be observed by an occupant, but it is the aerobiologist who quantifies and better describes fungal colonization activity within a moisture-damaged building.

Fungal agents:

Microbial Volatile Organic Compounds (mVOCs)

A person entering a building sustaining active mold growth may detect an odor. Mushroom-like or musty odors are common subjective descriptors of mVOCs such as 1-octen-3-ol and 2-octen-1-ol (Strom, West, Wessen, & Palmgren, 1994). These odors prominently are due to Carbon-8 compounds, Carbon-6 compounds and other organic compounds emitted by fungal colonies inhabiting damp, interior spaces (Nilsson et al., 2004) (Nilsson, Larsen, Montanarella, & Madsen, 1996) (Larsen & Frisvad, 1995) (Bjurman & Kristensson, 1992) (Borjesson, Stollman, & Schnurer, 1990). Typically,

mVOCs are low molecular weight alcohols and are dominant during early growth stages associated with the colonization of suitable substrates (Borjesson et al., 1990).

These minute volatile particles can permeate porous walls in buildings, allowing mVOCs to be suitable biomarkers for both visible and hidden fungal colonization (Dillon, Heinsohn, & Avise, 1996). One study concluded that the indoor concentration of mVOCs was significantly higher when compared to outdoor concentrations despite the lack of visible observation of mold growth in the indoor areas investigated. Although this finding was significant, the authors concluded that it was unclear to what extent mVOCs are suitable fungal biomarkers for fungal colonization of indoor spaces (Kim, Elfman, Mi, Wieslander, & Norbach, 2006).

Fungal mVOCs are generally referred to as side-products of primary metabolism of microbes but are not exclusive to secondary metabolism. Factors that control fungal growth (colonization of substrate) also influence mVOC production. Moisture conditions (relative humidity of the indoor air and water activity of the substrate) affect fungal growth and therefore mVOC production. Although indicative of microbial activity, a single mVOC cannot be related to a certain microbial species since the same mVOC may be produced by different microorganisms. Fifteen mVOCs are of most investigative interest when characterizing moisture and microbial damaged buildings (Korpi, Jarnberg, & Pasanen, 2009).

Volatile organic compounds generated by fungi responsible for moldy odors often described in occupant complaints may be present in the indoor air at concentrations too low for collection and identification by available standard analytical techniques (Korpi et

al., 2009). Moreover, compounds detected cannot be directly associated with a specific microbial species also identified within the same interior space (Polizzi et al., 2009). Sampling rates for measuring mVOCs in indoor air have not yet been defined or standardized (Araki et al., 2009). Even though two mVOCs (2-methyl-1-butanol and 1-octen-3-ol) showed significant association with mold status of a building, the authors concluded that diagnostic sensitivity and specificity for most mVOCs are too low for valuable and reliable results when characterizing the air quality of a building. At present, odor perception is more subjective than objective (Schleibinger, Laussmann, Bornehag, Elis, & Rueden, 2008).

Mycotoxins

Environmental exposures may occur when occupants come in contact with mycotoxins, *i.e.* toxins produced by fungi, during routine activities. These compounds are not volatile at ambient temperatures as are microbial volatile organic compounds. Inhalant exposure to mycotoxins can occur when dust, fungal components and other airborne particles are introduced into the upper respiratory tract during breathing. Mycotoxins are secondary metabolites and therefore are not indicators of early fungal growth and colonization. Mycotoxins may be present in air, settled dust and mycelium in indoor environments (Polizzi et al., 2009).

Even though certain fungal species are known to produce mycotoxins, some strains within those species are incapable of doing so. Sterigmatocystin and trichothecenes are two mycotoxins that occur frequently in water-damaged cellulosic construction materials (Tuomi et al., 2000) (Gravesen et al., 1999). Sterigmatocystin is a mycotoxin produced by numerous species of Aspergilli and shares a metabolic pathway with a well-known food-related mycotoxin, aflatoxin (Klich & Cleveland, 2000). Trichothecenes are potent irritants and may act as haptens; if bound to a carrier protein, an immune response may be elicited (Trout, Bernstein, Martinez, Biagini, & Wallingford, 2001).

Although different mycotoxins have been identified by both bulk and air sampling, standards of reference for analytical quantification are not available for most fungal metabolites (Polizzi et al., 2009). Investigators have had difficulty in determining mycotoxins as biomarkers of fungal exposure in building-related respiratory illnesses (Trout et al., 2001). Environmental assessment by profiling mycotoxins is of limited use without the combination of fungal identification and enumeration of fungal species (Tuomi et al., 2000). The use of mycotoxins as potential fungal agents is limited.

Glucans

Beta glucans are insoluble polysaccharides that provide structural support for fungal cell walls and generally comprise up to 60% of the cell wall of most fungal genera (Alexopoulos, Mims, & Blackwell, 1996). Specifically, research has focused on the

linkage (1,3)-β-D-glucan as a suitable fungal biomarker (Adhikari et al., 2009) (Elfman et al., 2009) (Rao, Riggs, Chew, Muilenberg, Thorne, VanSickle, et al., 2007) (Park et al., 2006) (Douwes, Thorne, Pearce, & Heederik, 2003) (Wouters et al., 2000) (Dillon, Miller, Sorenson, Douwes, & Jacobs, 1999) (Rylander, 1999). All acomycetes produce glucans but may vary in their glucan contents. For example, a culture of *Eurotium* species showed a higher level of glucan as compared to its anamorph *Aspergillus* (Milton, Alwis, Fisette, & Muilenberg, 2001).

The fungal burden of an environment may be characterized when quantifying the amount of glucans in environmental samples. The linkage (1,3)-β-D-glucan can be chemically separated from other polysaccharides, such as (1,6)-β- glucan, mannan, and bacterial-originating curdlan, during analytical analysis (Rylander, 1999). Glucans can be collected in the air and on moldy substrates (Seo, Reponen, Levin, Borchelt, & Grinshpun, 2008). Substrates may be vacuumed for the collection of glucans but the vacuuming of carpeting and flooring was found to significantly overestimate the inhalation exposure risk to glucan because this type of sampling collects both aerosolizable and deeply penetrated polysaccharide particles which would not be released into the breathing zone during normal occupant activities (Adhikari et al., 2009).

Sampling for glucan levels in airborne dust is preferable to sampling dust reservoirs such as carpeting since analytical results may be more representative of real inhalatory exposure (Noss et al., 2010). Air and floor dust sampling appear to be a better measure of current exposures as compared to upholstered chair sampling which may represent cumulative exposures (Rao, Cox-Ganser, Chew, Doekes, & White, 2005). One

study design had a low detection limit 0.1 ng/m^3 for glucan collected in air samples (Elfman et al., 2009).

The development of research tools to assess airborne fungal glucan exposure relating to upper respiratory response is currently being pursued by different teams of investigators with the common goal of developing a cost-effective analytical tool which can provide real-time assessments of indoor environments. (1,3)-β-D-glucan is a biologically active molecule and its detection and quantification in indoor environments remains important in the characterization of those environments.

Ergosterol

Ergosterol is a sterol compound found almost exclusively in the plasma membrane surrounding each fungal cell (Carlile & Watkinson, 1994). This sterol can be a specific measure of fungal mass (Borjesson et al., 1990) and it is not present in vascular plants (Dillon et al., 1999). Seasonality of fungal activity was noted in interior spaces where concentrations of ergosterol were higher in spring and summer (Pitkaranta et al., 2008).

Ergosterol is a biomarker for fungal biomass which indicates fungal growth, which in turn indicates active colonization of substrates. Correlations for airborne ergosterol and visible mold were higher than correlations for glucan and visible mold (Foto et al., 2005). Dust samples from floors and shelving may contain ergosterol in quantifiable amounts (Cox-Ganser et al., 2009) (Park et al., 2008) (Sebastian & Larsson,

2003) (Larsson, Burge, & Milton, 1997). Air samples taken from indoor environments and fungal spore suspensions contain measurable ergosterol levels providing assessments for fungal biomass in observed environments (Miller & Young, 1997).

Measurements of fungal metabolic activity are high during initial substrate colonization as empty hyphae fill with cytoplasm. During growth, a cell membrane comprised of ergosterol contains streaming cytoplasm and defines the branching shape of the hyphae. At these early stages of colonization, the correlation between fungal activity and biomass levels is strong (Borjesson et al., 1990).

Allergens

Allergens are proteins or glycoproteins that stimulate an IgE-mediated allergenic response. Fungi produce allergens that may express antigenic variability and cross-sensitivity. The amount of allergen release may increase with spore germination as noted with *Alternaria* (Mitakakis, Barnes, & Tovey, 2001). Fungal spores, spore fragments, hyphae, and hyphal fragments are thought to contain most of the 189 allergens currently identified. However, as fungal allergens become better characterized, detection of specific allergens may become important for environmental sampling and clinical diagnosis (Horner, Barnes, Codina, & Levetin, 2008). Fungal-produced allergens are identified through biochemical and immunochemical processes for characterization. Most known allergens are derived from anamorphs of ascomycetes (Horner, Helbling, Salvaggio, & Lehrer, 1995).

Extra Cellular Material

Fungi are found to release a matrix composed of extra cellular material (ECM) upon contact with a substrate or during germination. Usually, the ECM is comprised of a complex of high molecular weight glycoproteins, and its primary function is adhesion to the substrate (Nicholson, 2001). Saprotrophic enzymes (ectoenzymes) produced by fungi to recycle nutrients from decaying wood timbers in outdoor environments have the potential to degrade cellulosic materials in indoor environments during active colonization (Sinsabaugh et al., 1993). Proteases, pectinases, and amylases are enzymes with proven allergenic reactivity to Ig E antibodies in sera collected from mold-allergic individuals (Horner et al., 2008). Enzymes have been documented as an occupational health concern in soap and detergent industries (Nicholson, Taylor, Oliver, & Cathcart, 2001) (Pepys, 1992). Extra cellular materials may be useful as a quantitative biomarker for fungal colonization because heat-stable, water-soluble glycoproteins are essential cell-wall components and usually have antigenic specificity at the genus level (Dillon et al., 1999).

Fungal fragments (fine particles)

In general, fungal fragments include small pieces of hyphae which break away from colonized surfaces and then become suspended in the air or deposited onto other surfaces. These fragments of mycelium are non-sporulating but are detected in

environmental samples and require consideration when assessing environments for allergen contents. The dematiaceous hyphal fragments are small, with a particle size of less than 1.6 microns, and have been shown to have potential immunological reactivity containing both mycotoxins and antigens (Cho, Seo, Schmechel, Grinshpun, & Reponen, 2005) (Gorny et al., 2002). Fungal fragment characteristics include: small size, release rate, aerosolization suspension, and respiratory deposition, all of which are important factors influencing their xenobiotic nature in indoor environments (Cho et al., 2005).

Fungal spores

Ascomycetes may be divided into two groups: those which are known to reproduce only by mitospores (asexual reproductive propagules) or no spores at all; and, those which are capable of reproducing by meiospores (sexual reproductive propagules) (Taylor, Jacobson, & Fisher, 1999). Basidiomycetes, an entirely different category of fungi, also reproduce sexually by spore production (Horner et al., 1995).

In general, spores are produced by fungi for reproductive purposes, enabling the fungal species to colonize new surfaces. Ascoymycetes asexual propagation occurs by forming blastic conidia from conidiophores and thallic conidia from somatic thalli (Ulloa & Hanlin, 2000). Their size, shape, chemical composition, color, germination requirements are used for species identification (Samson & Hoekstra, 2002) (Klich, 2002) (Samson & Pitt, 2000) (Pitt, 2000) (Harris, 1999).

Indoor air quality assessments may utilize techniques that focus on the collection of fungal spores. These assessments draw conclusions from the quantification of spores collected from volume of air sampled or weight of sample collected. Several air and bulk sampling techniques are utilized by research and commercial laboratories. Collection methodologies and subsequent interpretation of sampling results are important factors to review when determining validity of indoor environment assessments which are dependent on spore sampling and/or comparisons to spore sampling (Adhikari et al., 2009) (Green et al., 2009) (Horner, Barnes, Codina, & Levetin, 2008) (Peters, Muilenberg, Rogers, Burge, & Spengler, 2008) (Portnoy, Barnes, & Kennedy, 2004) (Muilenbereg, 2003) (Spurgeon, 2003) (Sime, Abbott, & Abbott, 2002) (Macher, 2001) (Zhou, Whong, Ong, & Chen, 2000) (Dales, Miller, & McMullen, 1997) (Muilenberg & Burge, 1994) (Aylor, 1993).

Target: *Alternaria, Cladosporium, Aspergillus, and Penicillium* fungal species representing active colonization

The scientific community may be able to provide additional information linking fungal biomarkers to the health outcome endpoint of nasal hypersensitivity by specifically focusing on indoor environments supporting active *Alternaria, Cladosporium*, *Aspergillus,* and *Penicillium* colonization. Allergens, mycotoxins, mVOCs, extra cellular materials, glucans, enzymes, ergosterol, fungal fragments and other fungal agents are already well documented as being produced by these four filamentous Ascomycetes.

Two ecological groupings can be represented in quantitative evaluations by focusing on *Alternaria* and *Cladosporium*, typically known as above-ground decay fungi; and *Aspergillus* and *Penicillium*, typically known as common soil fungi (Su, Rotnitzky, Burge, & Spengler, 1992). These four genera also represent the three water requirement categories for optimal growth rates: i.e., low water activity ($a_w \leq 0.85$) xerophilic mold *Eurotium rubrum* (teleomorph of *Aspergillus*); intermediate water activity (a_w (0.85 - 0.90) mesophilic molds several *Aspergillus* and *Cladosporium*; and high water activity ($a_w > 0.90$) hydrophilic mold *Alternaria* (Levetin et al., 2002).

The identification, classification and sampling protocols for detection and quantification of fungal components and by-products produced by *Alternaria, Cladosporium*, *Aspergillus* and *Penicillium* are well documented and are topics of current research efforts (Klich, 2002) (Samson & Hoekstra, 2002) (Samson & Pitt, 2000) (Klich & Cleveland, 2000) (Macher, 1999) (Kozakiewicz & Smith, 1994) (Klich, 1993).

Allergen detection from the hyphae of *Alternaria, Cladosporium, Aspergillus,* and *Penicillium* species in cultured spore sampling has shown that the quantity of allergen eluted increases as soon as hyphal growth is initiated after spore germination (Green, Mitakakis, & Tovey, 2003). Based on this study, a generalization may be extracted that wetted building substrates are colonized by over-lapping life-cycles of germinating conidial spores. Exposure to fungal allergens may be continuous because surfaces are colonized by germinating spores as long as conditions remain conducive for fungal colonization. Specific allergens produced by *Alternaria, Cladosporium, Aspergillus,* and

Penicillium ascomycetes have been recognized and identified by biochemists (Horner et al., 1995) (Arruda, Mann, & Chapman, 1992).

Mycotoxins produced by *Aspergillus* and *Penicillium* are commonly acknowledged by scientists and mycologists (Frisvad and Gravesen, 1994). Aflatoxin B_1 produced by *Aspergillus* species is regulated by the US Food and Drug Administration and lower levels may impair immune systems (Klich, 2002). This toxic form of aflatoxin was identified in air, settled dust and mycelium collected from water-damaged buildings (Polizzi et al., 2009). Whereas environmental monitoring focuses on mycotoxin concentrations in exposure media, biological monitoring centers on assaying tissues, fluids, and excreta collected from exposed individuals to identify fungal residues, adducts, and metabolites (Bennett and Klich, 2003).

Aspergillus and *Penicillium* spores in airborne dust can be directly linked to mycotoxin (ochratoxin) exposure of occupants in indoor spaces (Bennett & Klich, 2003) (Engelhart et al., 2002). Spores from different isolates of the same species may or may not exhibit toxicity as demonstrated by testing different strains of *A. fumigatus* (Anderson, et al., 1994).

Microbial volatile organic compounds isolated and identified from *Aspergillus* when grown on water agar differed from mVOCs emitted by *Aspergillus* cultures grown on rich malt extract media (Bjurman & Kristensson, 1992). Volatile metabolites of *Penicillium* varied as growth substrates changed (Borjesson et al., 1990).

Species-specific extracellular polysaccharides from *Aspergillus* and *Penicillium* spp. were identified and quantified along with (1, 3)-β-glucans from a mold mixture of

Penicillium, Cladosporium, Aspergillus, and *Alternaria* and subsequently utilized as biomarkers for fungal colonization in a European study (Gehring et al., 2007). Ergosterol from spores collected from eleven species of *Aspergillus, Penicillium,* and *Cladosporium* was analyzed and compared to pure ergosterol as an external standard in the bioassay. When applied to field investigations, ergosterol was quantified and represented the fungal biomass in indoor air environments (Miller & Young, 1997).

Fungal fragments (fine particles) of *Aspergillus, Penicillium ,* and *Cladosporium* genera were found in higher quantity than spores collected at the same time from agar media inoculated with each genera. These fine and ultra fine fragment particles may represent hyphal fragments, pieces of spores and fruiting bodies, or secondary metabolic by-products (Gorny et al., 2002). Approximately 25% of all hyphal fragments expressed detectable allergen using IgE immunostaining (Green et al., 2006).

Conidiation producing easily recognizable moniliaceous amerospores of *Aspergillus* and *Penicillium* enables a quick visual count during environmental monitoring (Green et al., 2009). Even during drying periods where hyphal growth is halted, microcycle conidiation may comprise a survival mechanism enabling fungal colonization on building materials to continue between wetting periods (Ahearn et al., 2007). The cylindrical, blunt end shape of *Cladosporium* spores is visually unique and recognizable. The larger-sized, septated spore of *Alternaria* is quickly counted in direct microscopy (Haines, Escamilla, Muilenberg, Gallup, & Levetin, 2003). Using acid fuchsin staining methodologies, the characteristic shape of *Alternaria alternata* spores is easily observed under 40X magnification. (Figure 6).

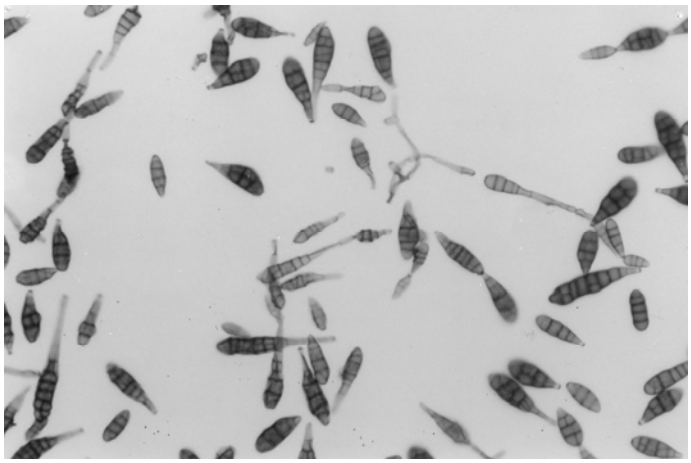

Figure 6: Characteristic *Alternaria alternata* spores under light microscopy at 40X magnification. (Bonny Dodson original photograph).

Sampling for each fungal spore concentration is ideal and common in environmental sampling of indoor air. Challenges common to investigations relying on spore sampling and subsequent cultural analysis involve reproducibility; sample storage; lack of allergen detection due to nonviable allergen components (ergosterol); lack of colony-forming units due to selective growth media, incubation temperature and humidity; community competition; and, cross-reactivity among several fungal species (Peters et al., 2008).

Although specific protocols for sampling methodologies are currently being debated among experts, the general consensus is that multiple sampling periods representing both temporal and spatial variation of fungal concentrations are needed for an accurate description of fungal colonization in indoor environments (Araki et al., 2009) (Zeng, Westermark, Rasmuson-Lestander, & Wang, 2006) (Dubey, 2005) (Horner

et al., 2004) (Lin & Li, 2003) (Gorny et al., 2002) (Sime et al., 2002)(Hyvarinen et al., 2001) (Burge & Rogers, 2000) (Macher, 2001) (Burge, 1995) (Dubey, 2005). Environmental sampling is vital step in the process leading to better scientific and medical understanding of fungal allergic sensitization, given the complexity of fungal life cycles, their rich biodiversity, and variability of fungal allergens (Green et al., 2009) .

Internal dose

The main natural route of entry for fungal xenobiotics that trigger upper respiratory responses is the nose with its two nostril openings. The nasal cavity and paranasal sinuses are lined with epithelial cells and are part of the respiratory tract of all mammals (Ooi, Wormald, Carney, James, & Tan, 2006). Fungal agents, such as spores, hyphae, and extra cellular materials suspended in air may easily be inhaled as these particles are light in weight and small in size (Vincent, 1995).

Once the fungal agent is inhaled it may become a xenobiotic. A xenobiotic is a substance foreign to a living organism whose metabolism is altered due to the presence of the xenobiotic (Smith, 2000). Xenobiotics are important factors in risk assessment for environmental health investigations.

Inhalation is the most rapid and complete natural portal of entry for absorption of xenobiotics because the only barrier between an airborne xenobiotic and the bloodstream is the thin, highly vascular respiratory membrane (Robson & Toscano, 2007). Once a fungal agent is inhaled it becomes a xenobiotic. Therefore, in indoor spaces, an

individual's breathing zone is the most critical zone for environmental assessment and respiratory health protection in moldy environments.

Individual exposure by inhalation is greatly influenced by the combination of several factors including particle size distribution, airborne concentration of particles, morphology, mineralogy, and chemical composition. Particle size influences how a particle enters the body during inhalation and how the particle penetrates and subsequently deposits in the respiratory tract (Cho et al., 2005). The concentration of particles in indoor spaces governs how many particles potentially may be deposited into the respiratory tract. Thirdly, the biological characteristics of the particles govern the subsequent fate and biological response of the host tissue (Vincent, 1995). For example, the concentration of ergosterol per one hundred fungal spores may vary between fungal species (Miller & Young, 1997).

A mold spore is a complex assortment of chemicals and proteins (Robbins, Swenson, Nealley, Gots, & Kelman, 2000). Respiratory allergy to fungal spores has been suspected by the medical community since the 1930's (Hyde, Richards, & Williams, 1956) (Bernton, 1930) (Hopkins et al., 1930). When fungal spores are inhaled, so are their fungal proteins composites. These proteinaceous xenobiotics may have allergenic potential which initiates immune responses in the host system. Generally, a proteinaceous xenobiotic that initiates an allergenic response is referred to as an allergen.

Bio-effective dose

Once inhaled fungal particles have entered the nasal passages, certain metabolic pathways may or may not be initiated depending on the susceptibility of the individual. The pathogenic effects of the fungal xenobiotic are greatly influenced by the integrity of nonspecific and specific host defenses. In one Korean study, *Alternaria, Cladosporium, Aspergillus,* and *Penicillium* were cultured from nasal lavage aspirated from 63.0% test individuals with chronic rhinosinusitis and likewise from 62.2% test individuals with non-hypersensitive immune systems (Shin, Ye, & Lee, 2007). If a metabolic pathway is initiated, then a bio-effective dose of the causative agent (for example, an allergen-containing fungal spore) has triggered an immunological response within the physiology of the host (Karp, 2010).

Allergens as xenobiotics

Allergen databases identify approximately 190 fungal species that produce allergenic protein substances capable of initiating immune responses in humans following exposure. This is a conservative number of known fungal allergens which can greatly be increased with the addition of genomic evidence identifying species of fungi which contain classes of proteins that cross react with already identified allergens. Clinically important allergens identified from *Aspergillus* and *Alternaria* species are often utilized

in medical diagnosis for fungal allergies present in a very limited number of species (Benndorf et al., 2008) (Horner et al., 2008a) (Bowyer, Fraczek, & Denning, 2006). Several fungal genera, including *Ulocladium, Curvularia, Epicoccum,* and *Stemphylium* produce allergens that show cross-reactivity to *Alternaria* –produced allergens in immunoassays (Peters et al., 2008).

Patterns of allergen expression vary among fungal species. Both spores and hyphae may elude allergens. The amount of allergen eluded may increase as spores germinate, depending on the fungal species. Localization and concentration of an allergen may vary along the length of the hyphae. For some species, certain sections of a hyphal strand may elute larger amounts of allergen, whereas in other species, allergen elution occurs in equal amounts among the entire length of the hyphae (Green et al., 2003).

The fungal species *Alternaria alternata* produces the major allergen Alt a 1 with several conformational and structural isoforms and variants. This fungal allergen has a molecular size of 29 kD and may easily bind with human Ig E in atopic individuals (Kurup et al., 2002) (Curran, Young, Burton, & Vijay, 1993). The structure and function of the host response to an allergen may vary if the protein undergoes any post-glycosylation. Binding initially may be with Ig E anti-bodies with an identified protein-allergen, but after glycosylation, binding with Ig G antibodies may occur more often, as demonstrated using molecular techniques (Kurup, Shen, & Vijay, 2002). Therefore, any change in the structure and function of an allergen may influence the Ig E or Ig G titers in

collected serum or nasal lavage while clinically assessing environmental exposure to fungal colonization in interior spaces.

Currently, the International Union of Immunological Societies Allergen Nomenclature Sub-Committee, in conjunction with the World Health Organization, maintains an official list of allergens along with nomenclature for allergen molecules (www.allergen.org). This list is continuously updated and reviewed by respected scientists (International Union of Immunological Societies Allergen Nomenclature Sub-Committee in conjunction with the World Health Organization, 2010).

As fungal agents, aeroallergens (allergens suspended in the air) have a great potential for absorption by their host system given their proteinaceous nature, small size, and quantifiable concentrations. Inhaled aeroallergens may be quickly be absorbed by the highly vascular respiratory membrane lining the host respiratory system (Robson & Toscano, 2007). Once absorbed, the aeroallergen may trigger an immune and inflammatory response in the host system.

Immune response

For decades, clinical diagnosis has relied on diagnostic allergen-containing reagents to better understand the immune and inflammatory responses in a host system (Green et al., 2003) (Matsson et al., 1996) (Vincent, 1995) (Horner et al., 1995) (Fogelmark, Sjostrand, & Rylander, 1994) (Lebowitz, 1992) (Pepys, 1992). Unfortunately, standardization of commercially available extracts has been hindered due

to the lack of understanding of the metabolic pathways involving fungal xenobiotics and the sensitized individual (Esch, 2004). Current scientific investigations are focusing on allergic symptomatic responses and metabolic pathways involved in the symptomatic responses as well as the chemical makeup of the fungal allergen diagnostic reagents (Green et al., 2009).

Traditional immunodiagnosis may fail to associate the fungi present in a contaminated indoor environment with the occupant's fungal allergic sensitization due to the lack of availability of effective diagnostic reagents. A low or normal total serum Ig E value does not eliminate the possibility of Ig E mediated mechanisms in allergic respiratory diseases. In contrast to total serum Ig E, the detection of an allergen-specific Ig E antibody in the serum of an individual may lead to the prediction of an allergic sensitization when that individual is re-exposed to the same allergen (Matsson et al., 1996) .

The sensitivity and specificity of both *in vivo* (clinical bioassays- generally a skin, bronchial, or nasal provocation bioassay test) and *in vitro* (laboratory immunoassays for detecting a substance by using an antibody reactive with that substance) diagnostic tests rely heavily on the characterization and definition of the actual allergen initiating the biological response. If the diagnostic reagent is not an exact match to the allergen responsible for the environmental exposure, then both the *in vivo* and *in vitro* may yield false negatives (Matsson et al., 1996).

Cross-reactivity of fungal allergens, compounded by their variability, may further complicate the refining and manufacturing effective diagnostic tests that mimic real-time

fungal causative agents responsible for environmental exposures and the subsequent allergic fungal sensitizations (Green et al., 2009) (Simon-Nobbe, Denk, Poll, Rid, & Breitenbach, 2008) (Horner et al., 1995). Ig E cross-reactivity which involves Ig E antibody recognition of a protein with an epitope similar to that of an initially sensitizing allergen may be a common clustering artifact in the molecular phylogenetic relationships of fungal species (Soeria-Atmadja, Onell, & Borga, 2010) (Bowyer, Fraczek, & Denning, 2006). Current methodologies focus on improving sensitivity and specificity of diagnostic and therapeutic reagents to avoid false negatives. By increasing the sensitivity of the epidemiological testing, fewer inaccurate clinical allergograms will result (B. Green et al., 2009) (Chapman et al., 2008a) (Horner et al., 2008) (Karvala, 2008). (Figure 7).

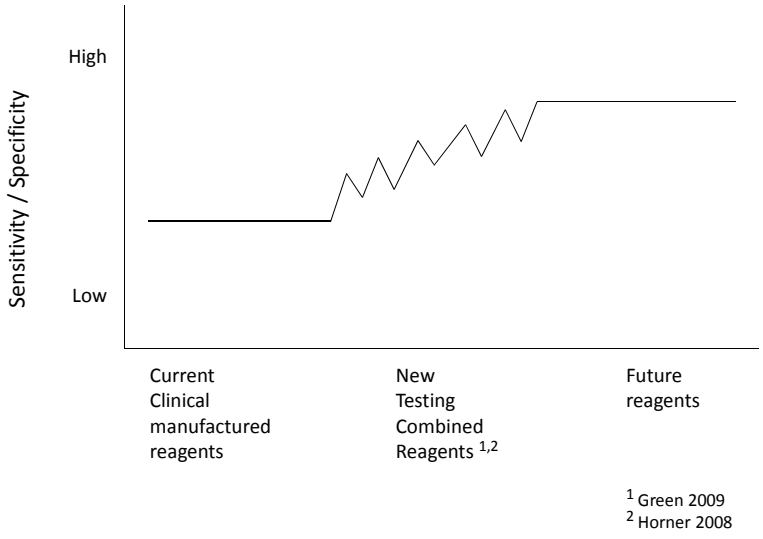

Figure 7. Mycological-based reagents for epidemiological sensitivity testing.

Immunopathogenesis

The respiratory immune response to fungal allergens and other fungal particles encompasses both the upper respiratory system and the lower respiratory system. "Innate immune systems," recently coined by Dr. Karp, includes all cells and all biological pathways that make up innate immunity (formerly referred to as cellular immunity). In contrast to the multi-system makeup of innate immunity, adaptive immunity (formerly referred to as humoral immunity) is a single system comprised of its antigen receptors (T-cell receptors and immunoglobulins), and cells (T and B cells). Both have undergone changes due to evolutionary pressure and have immune effector mechanisms. Contrary to earlier teachings, efficient activation of adaptive immunity is dependent on the innate immune systems. Moreover the activation of adaptive immunity is not associated with the inactivation of the innate immune systems (Karp, 2010).

By studying mucosal cells (dendritic cells that line airway epithelium and lamina propria) and their use of pattern-recognition receptors for surveillance of microbial particles, scientists have provided evidence for the linkage of the innate immune systems to the adaptive immune system in respiratory immune responses. These two branches of respiratory immune responses are not mutually exclusive but rather are closely linked to each other (Holt & Strickland, 2010).

As the primary interface between indoor environments supporting active fungal colonization, airway mucosa vulnerable to potentially inflammatory components of biological dust (Holt & Strickland, 2010). On the surface of airway epithelial cells are

adhesion molecules which bind foreign molecules, such as fungal allergens and glucans. Nasal swelling and increased titers of interleukin-8 (IL-8) were observed in a study where household dust was spiked with glucans (Beijer & Rylander, 2005). Surfactant Protein D, a member of the collectin family, is associated with allergic hypersensitivity inflammation (Ooi et al., 2006) (Haczku, Vass, & Kierstein, 2004) (Schaub et al., 2004) (Madan et al., 2001). In a nasal explants model using human tissue biopsies from chronic rhinosinusitis patients, both *Aspergillus* and *Alternaria* showed dose-dependent increases in nasal tissue surfactant protein D mRNA expression (Ooi et al., 2006).

Chronic inflammation of the nasal and paranasal sinus mucosa may result from humoral and cellular immune responses that initiated the production of immunoglobulin E and immunoglobulin G, and IL-5, respectively. In serum collected from test subjects diagnosed with chronic rhinosinusitis (CRS), less than thirty percent had specific Ig E antibodies to *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* detected in their serum, whereas specific Ig G antibodies to these four fungi were detectable in all CRS test subjects. Increased humoral Ig G levels strongly correlated with increased cellular IL-5 levels in response to *Alternaria* (Shin et al., 2004).

Fungal xenobiotics act as etiologic agents in immune responses without tissue invasion in rhinitis. The spectrum of fungal allergy is very broad, including hypersensitivity reactions of types I, II, III, and IV (Simon-Nobbe et al., 2008). Hypersensitivity denoted by detection of Ig E mediated hypersensitivity (atopy) and Ig G mediated hypersensitivity occurs as an early immune response, whereas eosinophilic mediator release occurs as a later phase reaction to fungal etiologic agents (Taj-Aldeen,

Hilal, & Schell, 2004). Early-phase responses concur with histamine release and late-phase responses concur with IL-6, IL-8, and eosinophil release (Marple, 2008). Neutrophils recognize microbe-associated molecular patterns with their Toll-Like Receptors and recruit additional immune cells to the sites of pathogen detection (Hayashi, Means, & Luster, 2003).

Sensitized individuals, regardless of atopy and elevated Ig E levels, may exhibit tissue inflammation in their nasal passages. This inflammatory response to certain fungi inhaled and deposited onto nasal and sinus tissue is largely due to the presence of eosinophils. This immune reaction targets the fungi in the mucous by initiating eosinophil migration from the blood vessels to nasal and sinus lining of the upper respiratory tract. Next, the eosinophils enter the nasal airway mucus and release granular effector mediators that initiate inflammation of the airways, causing obstruction in the sinonasal passages. This eosinophilic degranulation appears to cause local epithelial tissue damage through the release of toxic major basic protein (Kern et al., 2007).

Eosinophil degranulation induced by *Alternaria* is greatly influenced by calcium-dependent pathways. Reduction of extra-cellular calcium de-accelerates eosinophil degranulation whereas increased intra-cellular calcium enhances the eosinophils response to *Alternaria*. The binding of *Alternaria* protein(s) to the eosinophils surface depends on the presence of free calcium ions (Inoue, Shin, Ponicau, & Kita, 2003). The binding of the eosinophil to a fungal protein, for example proteases, signals that a bio-effective dose has initiated an immune response, for example, Th2 response within the susceptible individual (Bush, 2008). Eosinophils, but not neutrophils, possess G protein-dependent

cellular components that directly respond to *Alternaria* and *Penicillium* protein product(s) and activate cellular and effector functions (Inoue, Matsuwaki, Shin, Ponikau, & Kita, 2005). In support of the hypothesis that eosinophils have defense functions against fungi, nasal secretion collection and subsequent use of a mucolytic agent that breaks the mucus disulfide bond, allowing direct contact of fungi to the cultural growth medium, was an important step in accurately identifying fungal species present in the collected lavage (Shin et al., 2007).

Interleukins may also be inflammatory effector molecules in the upper respiratory systems of individuals who work in moisture-damaged buildings. In the early stages of immunological antigen response, mast cells and basophils synthesize cysteinyl leukotrienes. In the later phases of the immune response, eosinphils and macrophages synthesize these lipid mediators. Levels of cysteinyl leukotrienes in nasal secretions may become elevated in patients with allergic rhinitis. These leukotrienes act locally and systemically to promote allergic inflammation (Peters-Golden & Henderson, 2005).

Nasal lavage samples indicated elevated IL-4 cytokine levels in individuals working inside a fungal-contaminated office building. Both air and bulk sampling confirmed *Aspergillus* fungal contamination within the office building whereas skin hypersensitivity testing using *Aspergillus* reagents yielded negative results (Roponen et al., 2001). More responsive fungal testing reagents, combined with culturing for fungi present in nasal lavage samples, may yield results linking the suspected fungal causative agent with the elevated IL-4 immune response observed in those workers.

The extraction of fungi directly from nasal mucus followed by the purification of fungal proteases may aid in the determination of the effects of individual protease activity. Fungal components in the nasal cavity maintain or enhance the inflammatory process through the activation of nasal epithelial cells and production of chemical mediators, such as IL-8 for neutrophils. A dose-dependent increase in chemical mediators IL-8 and GM-CSF production was detected from 0 to 50 µg/ml fungal extracts of *Alternaria* and *Cladosporium*, whereas the dose-dependent increase tapered at a lower threshold (25µg/ml) for *Aspergillus* extracts (Shin, Lee, & Alexopoulos, 2006). Future investigations linking fungal proteases with the expression of protease activated receptors, adhesion molecules, cytokines, co-stimulating mediators, and other molecules associated with the up-regulation or down- regulation of various metabolic pathways in the respiratory system in exposed individuals may further promote scientific understanding of fungal sensitivity within indoor environments.

Non- immune response

Clinical observations by medical personnel may reflect non-immune responses in patients previously exposed to fungal colonization in indoor environments. Some fungal xenobiotics have the potential to possess virulence (non allergic factors) which facilitates fungal colonization of the respiratory system by reducing respiratory cilia beat frequency. This example of virulence may facilitate a subsequent allergic reaction by providing a

suitable surface for fungal spore germination and subsequent fungal colonization within the respiratory tract and compounding exposure to newly produced allergens (Arruda et al., 1992).

Health outcome endpoint: Symptoms of nasal hypersensitivity

Generally, the "allergic" status of an individual is determined by skin hypersensitivity testing. If the fungal-based testing reagent is biased or non-reactive due to ineffective extract technology and yields a false negative, the patient's true exposure sensitivity may be misrepresented. An individual being diagnosed as "non-allergic" to fungi based on a misrepresentation may derail an indoor environment- health outcome investigation. Moreover, diagnosing hypersensitivity solely based on Ig E serum titers may clinically misrepresent the true sensitivity being expressed by other immunological mechanisms. Recent ideology includes an intermediate subgroup of rhinitis referred to as "mixed" which is positioned between allergic and non-allergic subgroups (Carr, Nelson, & Hadley, 2008) (Nassef, Shapiro, & Casale, 2006).

Subjective symptoms may indicate a response to an agent from a fungal xenobiotic and may be considered as a health outcome endpoint for meta-analysis purposes. Symptoms of rhinitis may be clinically observed and recorded or self-reported (Haverinen, Husman, Pekkanen, et al., 2001). The utilization of checklists during office visits or questionnaires during on-site investigations may provide insight of what xenobiotics may have elicited symptomatic responses. The Rhinitis Outcomes

Questionnaire (ROQ) is currently used routinely in clinical practices for diagnosing rhinitis (American College of Allergy and Immunology, 2008).

Rhinosinusitis, a current terminology that combines the terms "sinusitis" and "rhinitis" since they usually coexist and are concurrent in most cases, is a symptomatic disorder of the nose and paranasal sinuses. Major symptoms of rhinosinusitis include nasal obstruction or discharge, facial congestion and pain, loss of smell, and cough (Dykewicz & Hamilos, 2010) (Bousquet et al., 2008) (Wallace & Dykewicz, 2008) (Meltzer et al., 2004).

Enhanced hyperreactivity of nasal mucosa to allergen stimulation may be observed during early or late phase responses. Sensitized individuals may present symptoms of sneezing, runny nose, itchy nose, and/or reduction of air-flow due to mucosal swelling during early-phase response which usually occurs within minutes following initial exposure. Late-phase responses may include resurgence of early-phase responses along with nasal inflammation, blockage, cough, and congestion. Late-phase symptoms may last for days after exposure (Marple, 2008). Risk factors for classic allergic rhinitis include inhalant allergens from fungal sources from indoor and outdoor environments (Bousquet et al., 2008).

Disease

Chronic rhinosinusitis (CRS) can be divided into three clinical subtypes with distinctive but overlapping clinical features. The three subtypes are: CRS without nasal polyposis; CRS with nasal polyposis; and allergic fungal rhinosinusitis (Dykewicz & Hamilos, 2010).

The International Classification of Disease (ICD) is the international standard diagnostic classification for all general epidemiological and clinical uses. ICD-10 (slated for complete adoption in 2012) classifies disease and other health problems. ICD-10-Chapter X, classifies diseases of the respiratory system into separate sections. Upper respiratory diseases of interest are classified in the following sections: Acute upper respiratory infections (J01- Acute sinusitis); (J06- Acute upper respiratory infections of multiple and unspecified sites); (J30- Vasomotor and allergic rhinitis); (J31- Chronic rhinitis); (J32- Chronic sinusitis); (J33- Nasal polyp); and (J39- Other diseases of upper respiratory tract). The classification J39.3, "upper respiratory tract hypersensitivity reaction, site unspecified," is a category which uses the general term" hypersensitivity" and not the term "allergy" (World Health Organization: ICD-10, 2007).

The natural history of disease in an individual is a timeline progression starting with the biological onset of disease, followed by the development of signs and symptoms. Once the signs and symptoms are observed, the clinical phase begins with diagnosis and therapy, lengthening the timeline before the disease is fully defined and assigned an ICD-10 classification, or the individual approaches death. For most diseases, a theoretical

critical point exists on the individual's timeline where treatment will be more beneficial if administered before the point is reached, rather than after (Gordis, 1996).

Better understanding of the relationship between the onset of upper respiratory hypersensitivity diseases and fungal xenobiotics within indoor environments would enable the critical points for sensitized individuals to better be determined so that action to prevent disease progression due to environmental exposure could be undertaken more swiftly.

Information that is needed

Temporal relationship

Unless a temporal timeline is established, the determination of a causal relationship between fungal xenobiotics residing within indoor environments and symptoms of nasal hypersensitivity presented by occupants would be difficult. The environmental exposure of fungal allergens must be characterized and precede the onset of nasal hypersensitivity exhibited by the occupants.

Epidemiological studies that include buildings cannot assume that the entire building has homologous fungal concentrations over time and space. Researchers determining that a building has been wetted and can sustain fungal colonization may avoid this "snapshot in time" by making multiple environmental assessments at different times and days. In one pilot study, the total concentration of viable fungi (*Aspergillus*

and *Penicillium*) was higher in a residence with moisture damage than in a building without moisture problems. Both within-day (higher values in the morning) and within-season (beginning of winter) differences of total concentrations of fungi were noted (Hyvarinen et al., 2001).

Efforts to correct building moisture problems may decrease exposure to fungal xenobiotics and subsequent symptoms of nasal hypersensitivity. Upper respiratory symptoms may be clinically noted before renovation, but their prevalence should decrease after the renovation removes both the fungal xenobiotics and the conditions conducive for fungal colonization. Two studies concur that occupants who had symptoms of increased airway hyperresponsiveness reported a decrease therein after renovation (Savilahti, Uitti, Laippala, Husman, & Roto, 2000) (Rylander, 1997).

"Hygiene Hypothesis"

The term "hygiene hypothesis" was first formulated from work initiated by David Strachan in 1989 with the objective of providing a plausible explanation for the prevalence of hay fever over a thirty year period in Britain (Strachan, 1989). His insights into possible connections between symptoms of hay fever, early childhood microbial exposures and subsequent airway infections, and hygienic practices within the home sparked a new idea within the immunologic community. This new concept suggested that infection frequency was an influencing factor discriminating high-risk and low-risk populations for allergic sensitization (Holt & van den Biggelaar, 2010).

The idea was that exposure in early childhood could influence future sensitivity due to a protective mechanism. This concept was demonstrated in active farming families and their risks of sensitization investigated by researchers from various geographic locations (Ernst & Cormier, 2000) (Kilpelainen, Terho, Helenius, & Koskenvuo, 2000) (Riedler, Eder, Oberfeld, & Schreuer, 2000) (Braun-Fahrlander et al., 1999). One study summarized that an objective doctor's diagnosis of hay fever, along with the subjective reporting of hay fever symptoms, and the reporting of the variable of indoor dampness, showed a lower disease prevalence among the farm children than the suburban children observed (Von Ehrenstein et al., 2000).

The hygiene hypothesis concept has been discussed since its inception (Karp, 2010) (Linneberg, 2008) (Kusel et al., 2007) (Yazdanbakhsh, Kremsner, & van Ree, 2002) (Wills-Karp, Santeliz, & Alexopoulos, 2001) (Martinez & Holt, 1999) (Holt, Sly, & Bjorksten, 1997) (Martinez, 1994). Although the hygiene hypothesis is itself a complex theory, broader understanding of the relevance of timing of exposure, type and intensity of airway sensitizations, and allergy pathogenesis has been gained by the worldwide community as a result of debate surrounding this theory (Holt & van den Biggelaar, 2010).

Confounding allergens and stimuli

Many different sources of allergens or stimuli may impact the health of occupants exposed to indoor environments. These factors may influence the onset of symptoms

associated with hypersensitivities but may not be the xenobiotic targeted for the environmental research study. Allergens produced by non-fungal organisms inhabiting the same environment which sustains fungal colonization, and are also known to elicit hypersensitivity responses, are referred to as confounding allergens.

House dust mites, cockroaches, mice, dogs, cats, plant pollens, grasses, bacterial endotoxins, and household dust may also be sources of aeroallergens in occupied areas in addition to fungal sources (Chapman, 2010) (Chruszcz et al., 2009) (Pate, Hamilton, Ashley, Zeldin, & Halsey, 2005) (Shin et al., 2004) (Macher, 1999) (Platts-Mills, Woodfolk, Chapman, & Heymann, 1996). The selection of assay methodologies utilized to identify bacterial endotoxins as potential confounders is an important factor in the validity of an investigation, since it is known that fungal β-glucan can yield false positive results when employing the Limulus Amebocyte Lysate (LAL) endotoxin assay for the detection of bacteria in the environment (Vassallo & Limper, 1999).

The importance of confounding factors during environmental assessments linking fungal colonization and the onset of symptoms associated with nasal hypersensitivities has yet to be determined. One study investigating symptom prevalence associated with reported mold growth concluded that the association between residential fungal colonization and respiratory/nonspecific symptoms was not confounded by dust mite antigens or bacterial endotoxins (Dales & Miller, 1999).

Potential confounding stimuli may be responsible for symptoms associated with vasomotor rhinitis (idiopathic non allergic rhinitis). Hyperreactivity of the nasal mucosa may result upon exposure to nonspecific stimuli from volatile organic compounds, strong

odorants, smoke, temperature fluctuations, and other sources (Claeson, Nordin, & Sunesson, 2008) (Nassef et al., 2006). As more studies are conducted and published, a better understanding of confounding allergens and stimuli will aid in risk assessment of indoor environments contaminated with mold.

Susceptibility of individual

Risk factors for rhinitis may occur at all ages in the susceptible individual. Genetics and family history influence the risk of atopy. Early-life risk factors, mostly allergens, may be related to rhinitis with reference to the hygiene hypothesis. The individual's occupation, along with his/her socioeconomic status, ethnic group, and emotional status, may influence the risk for rhinitis (Bousquet et al., 2008).

Research studies focusing on different ages and genders may offer insights into the early stages of the disease of rhinitis (Korhonen et al., 2006) (Lai, Hopp, & Lusk, 2006). For example, susceptibility to proinflammatory mechanisms in disease pathogenesis for asthma has been demonstrated in atopic teenagers (Hollams et al., 2009). In another study, populations from the ages of 19 to 68 were identified as susceptible to risk factors for nasal hyperreactivity in non-specific building-related illness (Shusterman & Murphy, 2007). The expansion of the number of older adults and the prevalence of geriatric rhinitis was of concern to the geriatric population in the United States (Pinto & Jeswani, 2010).

The susceptibility of individuals may relate to time spent indoors where exposure to fungal colonization may result. Exposure and potential sensitization may depend on how many hours per week are spent at home, at the workplace, at school, or any other place frequented by the individual. These parameters are important when describing conditions that may influence respiratory symptoms and diseases (Sauni et al., 2009).

Research investigations have also targeted different geographic locations in studying rhinitis in different populations (Randriamanantany et al., 2010). If the individual lived in a region that was designated as an Asthma Capital, such as Richmond, Virginia, in 2010, the risk for sensitization might be greater than in a region not considered a hub for respiratory diseases (Asthma and Allergy Foundation of America, 2010).

Individual variation in response can range from lack of response to multi-system hyperresponsiveness. Genetic polymorphism may be one explanation for the wide range of variation in response to certain antigens (Marinkovich, 2004). The role of diet and nutrition for each individual can also be an important factor in immune tolerance, shifting focus away from medical interest in preventing hypersensitivity to developing immune tolerance by avoiding (or supplying) specific nutrients in individual diets (Jennings & Prescott, 2010) (Adhikari et al., 2009).

Chapter 3: Methodology

Meta-analysis was the methodology selected to test the null hypothesis: there is no association between fungal agents and nasal hypersensitivity in indoor environments. If the null hypothesis was proven false, the degree to which the relationship is present in the population will be represented by an effect size greater than zero (Wolf, 1986). The effect size is an index of the tested relationship between treatment (intervention) and outcome (symptoms of disease) which can be compared across different studies (Dunlap, Cortina, Vaslow, & Burke, 1996). Under the null hypothesis, there are no differences in the intervention effect (changing exposure status from exposed to non-exposed) among selected studies in the meta-analytic review (Deeks & Higgins, 2007).

The second-level meta-analytic research seeks to generate new qualitative knowledge by integrating results from primary-level research studies. This systemized process of reviewing and selecting primary studies for an additional level of review has employed epidemiologic primary data to formulate healthcare public policy based on risk assessment.

Avoiding risk is paramount in community respiratory health policy formulation. However, before direction can be offered by policy-makers, scientific associations from environmental exposure to symptom expression must be examined, re-examined, and supported by the environmental scientific community, the medical clinical community,

and the policy-making community in order to achieve the common goal of protecting public health.

The goal of risk assessment is to provide an evidentiary base for making policy decisions to protect the public from identified health hazards (Robson & Toscano, 2007). Several tools are available for environmental risk assessment, such as odds ratio and relative risk methodologies. Both methodologies employ the same factors for calculation but differ in their mathematical formulaic approaches. Some research designs prevent the computation of relative risk, such as case-control designs that seek subjects based on their health outcome and not the exposure. Adjustments for confounding variables are difficult to make when using relative risk whereas covariate adjustments are easier when using odds ratio. If an event (symptom or health outcome) is uncommon (infrequent), then the odds ratio may be applicable for relative risk assessment for environmental health (Children's Mercy Hospitals and Clinics, 2001) (Gordis, 1996). The odds ratio is the independent repeated measure across studies and is converted to the effect size during the meta-analytic procedure.

A terminology list was constructed to guide evidence acquisition from electronic databases to insure consistency. Selecting keywords and databases to be included in a meta-analysis search for primary studies is critical and the investigator must have specialized knowledge and be current in his/her understanding of research parameters, methodologies and statistical applications surrounding the relationship being qualified (Lipsey & Wilson, 2001). (Table 3).

Table 3. Search terminology for evidence acquisition.

Databases	Environmental Key Words	Medical Key Words
ScienceDirect	fungi; fungus; environment	rhinosinusitis; rhinitis; sinus;
Ovid; PubMed	mycotoxin(s); spore(s); glucan;	nasal; congestion; discharge;
Agricola;	ergosterol; mold; mould; flood	allergy; allergen; hypersensitive
FirstSearch;	*Aspergillus; Penicillium*;	cough; respiratory; asthma
Embase	*Cladosporium; Alternaria*;	risk; risk assessment; ratio
	SBS; sick building syndrome;	hay fever; hygiene hypothesis;
author name	aerobiology; bioaerosol; fungal	exposure; mucus; symptom
journal archives	building; dust; indoor; PM_{10};	epidemiology; policy; health

Exclusion/Inclusion of primary research studies

Several databases were employed for initial searches using keywords encompassing environmental and medical terminologies. Multiple searches referred to numerous published books, papers and presentations along with their listed references and related studies. Additional papers were extracted from journal and book bibliographies to broaden the field of potential papers to be reviewed for inclusion in the meta-analysis.

Evidence-based investigations were identified and reviewed to insure that the science was current for both the mycological and medical aspects for the dissertation research. Articles were written in English or offered abstracts written in English.

Research studies published between 1985 and 2010, a 25- year time interval, were considered for further review. Each paper was reviewed for its scientific integrity, methodology, primary data, and objectivity. If a study had statistical analyses unfamiliar to the reviewer, references were consulted for further explanation (Bowers, House, & Owens, 2006) (Eudey, Su, & Burge, 1995) (Zolman, 1993). Attention was given to distinguish between primary investigations and secondary review investigation. A study was excluded from further review if it summarized primary studies and did not offer any new evidence, for example, a meta-analytical article (Fisk et al., 2007).

Some authors publish identical research results in different peer-reviewed journals to broaden their readership. Careful review identified several of these duplicated studies and only one was selected for possible inclusion (Haverinen, Husman, Pekkanen, et al., 2001) (Haverinen, Husman, Vahteristo, et al., 2001). Several studies provided multiple original data sets on the same population but at different time intervals, such as cohort studies. These were identified and reviewed as independent studies for inclusion consideration (Jaakkola et al., 2005) (Jaakkola et al., 1993) (Brunekreef, 1992) (Brunekreef et al., 1989).

After a re-review, some papers were excluded based on perceived procedural flaws such as mismatched objectives and results, methodological limitations, or incomplete data required for meta-analysis. The absence of needed data within a study resulted in the exclusion of that particular research investigation from further meta-analytic review. For example, standard error is calculated from original data and is a critical input for a meta-analytic review (Cortina, 2003). Therefore, if a study does not

clearly state the standard error nor provide data sets required to calculate the standard error, the study was excluded from the meta-analysis.

Studies that provided only building dampness information and not mold or fungal agent information were excluded, thus avoiding the assumption that building dampness was an indicator for fungal colonization. The theory that conditions conducive for fungal colonization, such as wetted building materials containing cellulose, could equate to the presence of fungal agents was rejected to avoid potential Type I errors (false positives) although some studies have based their primary investigation (Haverinen, Husman, Pekkanen, et al., 2001) or their secondary investigation (Fisk et al., 2007) on this flawed assumption.

Research variables were designated and ranked as topics during the search for included studies. The decision to include or exclude each independent or dependent variable was based on its ranking. Ones with A and B designated ranks were included whereas ones with C and D designated ranks were excluded from the study. (Table 4).

Table 4. Independent and dependent research variables.

Independent Variables	Rank †	Dependent Variables	Rank †
fungal spores identified	A	nasal congestion	A
fungal agents quantified	A	nasal discharge	A
green/ black spots	A	nasal symptoms	A
visible mold	A	rhinitis; allergic rhinitis	A
fungal species identified	A	rhinosinusitis	A
wetted ceiling	C	Sinusitis	A
wetted flooring	C	Cough	B
wetted wall	C	Headache	C
water leaks	C	watery eyes	C
musty odor	D	Fatigue	D

† *Ranks C – D not included in test statistics*

Some studies were excluded based on the selection of software chosen to provide the effect size of the tested relationship. Review Manager 5 (*Review Manager (RevMan) [Computer program]. Version 5.0. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration*, 2008) was the chosen software system for meta-analytic quantitative testing. If data points required by Review Manager (such as log odds ratios and standard error) could not be extracted or calculated from a primary study, then that study was excluded from the secondary analysis. (Table 5).

Table 5. Characteristics of excluded studies.

Author	Year	Reason for Exclusion
Issues related to nature of symptoms presented in review		
Ceylan	2006	Detailed data missing related to rhinitis symptoms
Strachan	1990	Discussed only wheezing as symptom of exposure
Vojdani	2003	Presented very vague upper respiratory symptoms resulting from exposure to mold
Issues related to distinct identification of mold/fungal exposure as risk factor		
Bornehag	2005	Discussed only dampness factors and no reference specifically to mold exposure
Engval	2001	Discussed dampness and moldy odor but no specific reference to mold exposure
Haverinen	2001	Assumed presence of wetness or dampness equivalent to fungal colonization
Jaakkola	2005	Assumed presence of wetness or dampness equivalent to fungal colonization
Jedrychowski	1998	No distinct delineation between mold and dampness as risk factors
Mommers	2005	No distinct delineation between mold and dampness as risk factors
Pirhonen	1996	No distinct delineation between mold and dampness as risk factors
Shusterman	2007	No distinct identification or discussion of mold exposure as a risk factor
Simoni	2005	No distinct delineation between mold and dampness as risk factors
Waegemaekers	1989	Discussed only dampness factors and no reference specifically to mold exposure
Walinder	2001	No distinct delineation between mold and dampness as risk factors
Yang and Lin	1997	Discussed only dampness with no details concerning exposure to mold
Issues with nature of study or nature of groups reviewed		
Cooley	1998	Provided no data on non-exposed group for study
Cox-Ganser	2009	Provided no data on non-exposed group for study
Fisk	2007	Meta-analysis and not original study

A group of studies was selected for further review after evaluation of each paper for its methodological merits. The odds ratio was examined in reviewed primary research

focusing on occupant exposures to fungal agents in indoor environments and the onset of nasal hypersensitivity symptoms or increased measurement of biomarkers associated with hypersensitization. Studies chosen needed to contain original data in order to calculate an unadjusted odds ratio along with standard error. In addition, studies with a 95% or more confidence interval were included to construct a viable meta-analysis (Cortina, 2003).

Two papers that were included at this point of the dissertation review had apparent typographical errors in the results section which I corrected (Gent et al., 2002) (Koskinen et al., 1999). One paper stated that the relative risk estimate was of similar value to the adjusted odds ratio offered in the paper so that paper was included for meta-analytic review (Brunekreef, 1992). Another study also supported its use of the adjusted odds ratio by stating that the findings remained significant after adjusting for possible confounders, and hence was included for the Review Manager review (Gunnbjornsdottir et al., 2006). For all included studies, published results were checked for validity and reasonableness against calculated results for the secondary analysis (Skorge, Eagan, Eide, Gulsvik, & Bakke, 2005).

Investigations that utilized reporting mechanisms such as questionnaires or surveys were included if these subjective efforts were supported by research organizations, such as the Rhinitis Outcomes Survey (American College of Allergy and Immunology, 2008) (Demuth, Nelson, Boucha, Demuth, & Dalan, 2008); the International Study of Asthma and Allergies in Childhood (ISAAC) standardized medical questionnaire (Randriamanantany et al., 2010) (Von Ehrenstein et al., 2000); the European Community Respiratory Health Survey I and II (Gunnbjornsdottir et al., 2006)

(Janson et al., 2001) (Braun-Fahrlander et al., 1999), and, the health survey developed by the American College of Occupational and Environmental Medicine (Vojdani, Campbell, Kashanian, & Vojdani, 2003). Both subjective and objective reporting of symptoms associated with nasal hypersensitivities was accepted for analysis.

Self-reporting questionnaires can provide accurate observations of mold growth within indoor environments when they are administered properly and the information obtained is verified by repeated question measures within the questionnaire (Haverinen, Husman, Pekkanen, et al., 2001).

Another criterion for inclusion was the age of the study population. Since age is a chief personal characteristic in epidemiological studies, most studies clearly indicated the age or age groups within their investigative reporting. For purposes of this meta-analytic effort, age was divided into two non-overlapping subgroups: children (birth to 17 years of age) and adults (18 to 90 years of age). Another meta-analytic report also considered age group as the categorical moderator (Fisk et al., 2007).

Measures of exposure and non-exposure targeted by primary studies had to contain biomarkers discussed within the literature review of this dissertation. The observation or measurement of fungal biomarkers represented exposure to fungal colonization and the absence of fungal biomarkers represented non-exposure or lack of allergic response to fungal colonization. Measures of health outcomes included symptoms of upper respiratory hypersensitizations, measurement of inflammatory mediators in nasal lavage, measurement of immunoglobulins in blood serum or, positive results from skin-prick reaction tests. Symptoms, and not clinical diagnosis of the

disease, were the health outcomes in several recent studies focusing on respiratory health (Jaakkola et al., 2005) (Belanger et al., 2002) (Nafstad et al., 1998).

When a single study offered more than one independent variable (risk factors indicative of fungal colonization) or more than one dependent variable (symptoms associated with nasal hypersensitivity as health outcomes), the author restricted to three the number of results from a single study. Although the risk factors were multiple independent variables, they all were examples of fungal xenobiotics. As such, although the symptoms were multiple dependent variables, they all may be symptoms of nasal hypersensitivity. Restrictions limiting the use of multiple statistics from one study have been placed in previous studies (DeCoster, 2004) (Wolf, 1986) (Gilbert, McPeek, & Mosteller, 1977). (Tables 6 and 7).

Table 6. Characteristics of included studies: Subgroup children.

Subjects	Author	Year	Group size – exposure	Group size - control	Risk Factor	Health Symptom Outcome
Children	Brunekreef	1989	1404	3221	Exposure to mold	Cough, Hay fever
	Brunekreef *	1992	♦	♦	Exposure to mold	Cough
	Cuijpers	1995	110	358	Exposure to mold	Cough
	Gent	2002	545	335	Exposure to *Cladosporium*	Cough
	Gent	2002	357	523	Exposure to *Penicillium*	Cough
	Jaakkola *	1993	75	2076	Exposure to mold	Cough, Nasal congestion, Nasal excretion
	Li and Hsu*	1997	♦	♦	Exposure to mold	Allergic rhinitis
	Randriamanantany	2010	189	6537	Sensitization to *Alternaria*	Hay fever
	Savilahti	2000	♦	♦	Exposure to mold	Sinusitis
	Stark	2005	152	246	Exposure to mold or mildew	Allergic rhinitis
	Strachan	1989	89	911	Exposure to mold	Hay fever, Nasal blocked – running, Nocturnal cough
	Yang and Kao	1997	2496	1668	Exposure to mold	Cough

** Use of adjusted odds ratio (OR) considered appropriate and acceptable due to insignificant impact on OR when adjustments made for various confounders*

♦ *Not specifically enumerated within the study – ln(OR) and 95% CI used*

Table 7. Characteristics of included studies: Subgroup adults.

Subjects	Author	Year	Group size – exposure	Group size - control	Risk Factor	Health Symptom Outcome
Adults	Campbell	2004	209	28	Exposure to mold	Cough, Sinus discomfort, Nasal symptoms
	Gunnbjornsdottir	2003	318	1465	Exposure to mold and water damage	Nocturnal cough
	Gunnbjornsdottir *	2006	♦	♦	Exposure to mold	Nocturnal cough
	Hirvonen	1999	32	25	Exposure to mold	Cough, Rhinitis
	Koskinen	1999	189	510	Exposure to mold	Nocturnal cough, Rhinitis, Sinusitis
	Niemela	1985	234	294	Exposure to mold	Cough, Allergic rhinitis
	Skorge	2005	107	2294	Exposure to mold	Chronic cough

* *Use of adjusted odds ratio (OR) considered appropriate and acceptable due to insignificant impact on OR when adjustments made for various confounders*

♦ *Not specifically enumerated within the study - ln(OR) and 95% CI used*

Review Manager 5.0.24 software

Review Manager 5.0.24 was chosen to assist in performing a meta-analysis to test the null hypothesis of the dissertation investigation. This software program was developed by The Cochrane Group, has creditability and wide acceptance among scientific and medical personnel, and is supported by a reader-friendly handbook and a web-based bulletin board (Higgins & Green, 2009). Categorical data extracted from each study was initially placed in a 2 x 2 epidemiological table for the calculation of odds ratio. If prevalence was given in a study, the prevalence percentage was applied to the

study population to determine the number of events. The log of the odds ratio was calculated because the log number yields a more symmetric distribution of results. Standard error was independently calculated either from the dichotomous data or from the differences between the natural logarithms of the upper and lower limits of the 95% confidence interval (Higgins & Green, 2009).

A generic inverse variance model was used for computing outcome. The use of subgroups, children and adults, is supported by generic variance outcome modeling which uses the inverse variance statistical method, a non-Mantel-Haenszel method. The individual effect sizes are weighted according to the reciprocal of their variance.

Heterogeneity was assessed using an estimate of the between-study variance in a random-effects meta-analysis, referred to as tau-squared (Tau^2); chi-squared distribution (Chi^2) with *k*-1 degrees of freedom (where *k* is the number of studies included in the review (or subgroup), and the I-squared statistic. Heterogeneity (I^2) is a statistic derived from Tau^2 which is related to random effects; Chi^2, which is related to subgroups; and, degrees of freedom (df) in Review Manager (Higgins & Green, 2009). Test for presence of an overall intervention effect is presented by the test statistic Z (Higgins & Green, 2009) (Deeks, Higgins, & Statistical Methods Group, 2007).

Each study statistic was weighted according to the population sample size to enhance the overall efficacy of the meta-analytic study. In the 1997 study (Yang, Chiu, Chiu, & Kao, 1997), the total population sample size contained 2496 children, whereas in the 1989 study (Strachan & Sanders, 1989), the total population sample size consisted of 89 children. Consequently, the meta-analysis results assigned a higher weighted

percentage of 8.3 to the larger-size study population, compared to a lower weighted percentage of 2.5 to the smaller-sized study population. Moreover, each study's statistic odds ratio effect measure was proportional to the total sample size. By weighing the variation of effects across studies, the overall treatment effect was strengthened (DerSimonian & Laird, 1986).

Diversity within a meta-analysis may be measured by estimating a between-study variance that is independent of the treatment effect metric. This variance, known as methodological or clinical heterogeneity, exists when results from different studies utilizing different study designs, study statistics, sample sizes, and study populations are pooled together to test a secondary hypothesis (Rucker, Schwarzer, Carpenter, & Schumacher, 2008) (Higgins, Thompson, Deeks, & Altman, 2003) (Higgins & Thompson, 2002).

Odds ratio in meta-analysis

When the outcome (health outcome endpoint) is a true dichotomy, the test statistic odds ratio can be relied upon to estimate the effect size (Hunter & Schmidt, 2004). In this meta-analysis, the dichotomous dependent variable was the existence of upper respiratory symptoms. Generally, symptoms listed on the Rhinitis Outcomes Questionnaire presented in each included study were reviewed for acceptances for input into the odds ratio for analysis (American College of Allergy & Immunology, 2008).

One of the key benefits of the utilization of the odds ratio is its invariability across sampling methods, reflecting the fact that it is not affected by unequal sampling sizes within the study statistic (Haddock, Rindskopf, & Shadish, 1998). All analyses were performed on the natural log of the odds ratio so that a positive value reflects a positive relationship (Lipsey & Wilson, 2001).

The 2 x 2 cross tabulation table is presented in the form of relative frequencies and proportions where a, b, c, and d indicate the cell frequencies, whereas p_a, p_b, p_c, and p_d, indicate the proportion of each group in each status (Lipsey & Wilson, 2001). (Figure 8).

Constructed 2 x 2 tables were useful in obtaining the odds ratio for each test statistic for all studies included. Within the child subgroup, the cross tabulation table for Randriamanantany (2010) was a = 569 [children not sensitized to *Alternaria* presenting hay fever symptoms]; b = 6537 [total children not sensitized to *Alternaria*]; c =37 [children sensitized to *Alternaria* presenting hay fever symptoms]; and, d = 189 [total children sensitized to *Alternaria*].

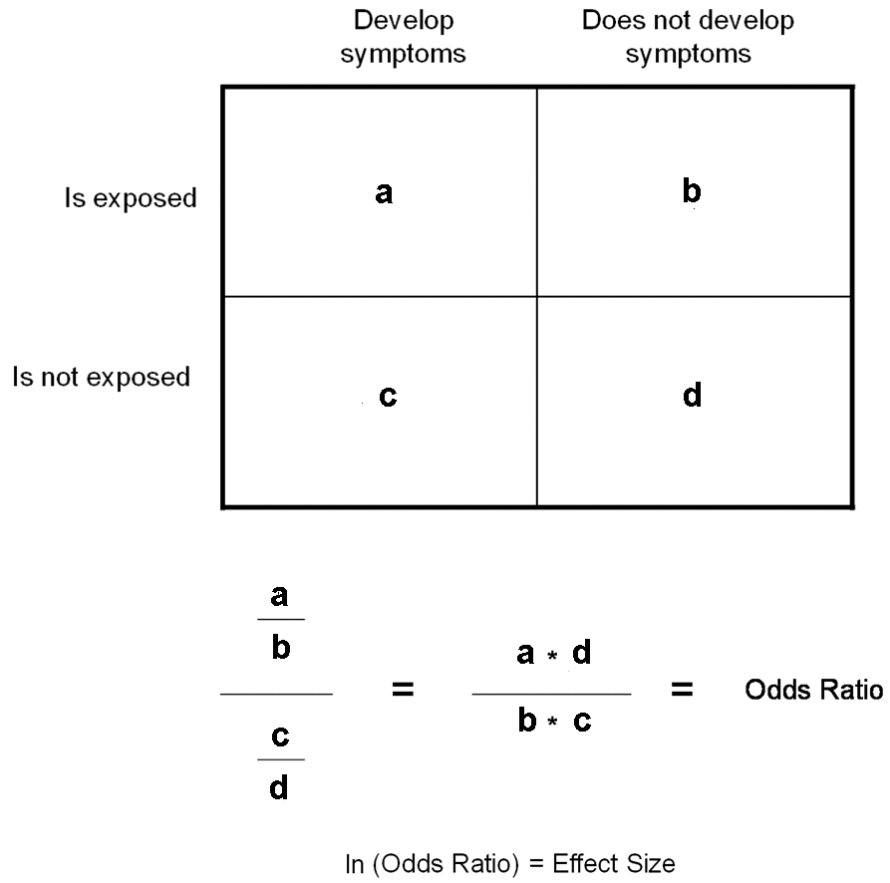

Figure 8. Calculation of odds ratio and effect size.

Another study, Savilahti *et al.* (2000), did not require a construct 2 x 2 table since the log of odds ratio was stated within the published results. The natural log of the odds ratio with standard error was calculated using the data given in the study. A visual check to boost confidence was taken as the published 95% CI was 0.34 - 3.91 and the independent calculated 95% CI was 0.34 - 3.92. (Figure 9).

$$SE\ (\ln OR) = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}}$$

$$SE\ (\ln OR) = \frac{\ln (CI\ upper\ limit) - \ln (CI\ lower\ limit)}{2 * 1.96}$$

Figure 9. Standard error calculations from dichotomous data and from 95% CI values.

Results from a third study, Campbell (2004), contributed three different statistics to be incorporated into three separate 2 x 2 epidemiological tables. Since the symptoms presented vary from individual to individual, the measures for the three symptoms of cough, nasal discomfort, and sinus discomfort were not averaged into one measure but were included as three separate statistics. Health outcomes endpoints in the thirty individual statistics were indicated by symptoms: cough (15); rhinitis (5); nasal congestion, discharge, discomfort (4); sinusitis and sinus discomfort (3); and, hay fever (3).

Random effects model within meta-analysis

Random effects modeling was chosen to draw unbiased and precise effect size estimates from the included studies identified from a universe of studies. For most effect sizes, differences in standard error between studies are mainly the result of differences in sample sizes within each study statistic. Generally speaking, the random effects model is

preferred over the fixed effects model where the intervention effect is fixed across studies (Schulze, 2007). In order to present a more conservative approach, the confidence interval around the random effects pooled estimate is established as wider than the confidence interval around a fixed-effect pooled estimate. A sensitivity analysis was not performed since case-control studies with a lower number of participants were not considered for inclusion (Green et al., 2009).

An estimate of between-study variances in a random effects modeling within Review Manager is represented by tau-squared (T^2). If publication bias influenced the overall effect size estimate (summary odds ratio) of a meta-analysis, then the random-effects model would exacerbate the effects of the bias. Funnel plots generated from data points showing symmetrical distribution without pronounced gaps indicate the absence of publication bias on an effect size estimate (Green et al., 2009). (Figure 10).

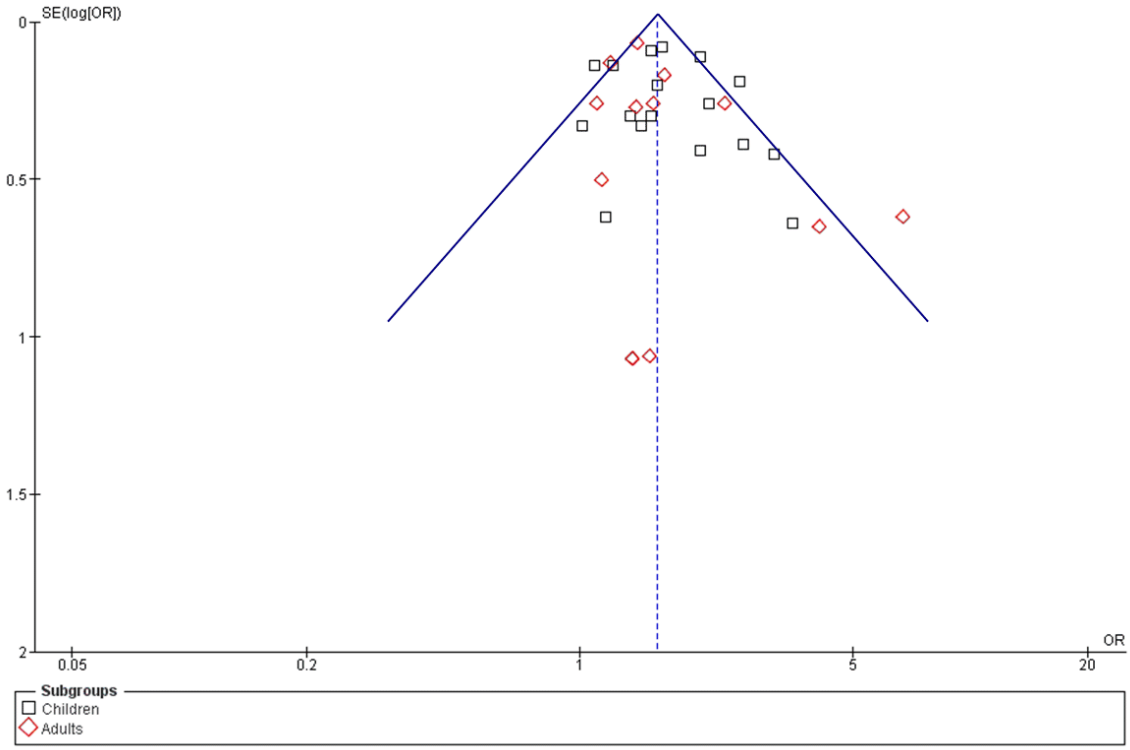

Figure 10. Funnel plot with demonstrated symmetric distribution. (Adapted from a plot generated by Review Manager).

Chapter 4: Results

The quantitative results of the evidence-based meta-analysis connect the occurrence of nasal hypersensitivities with fungal colonization in indoor environments. This consistency of association supports current scientific acknowledgement of poor occupant respiratory health upon exposures to fungal agents while studying, working, and living indoors.

The summary odds ratio derived with random effect inverse-variance was 1.58 with a 95% confidence interval of 1.42 upper limit and 1.75 lower limit. The odds ratio calculated for the child subgroup was 1.63 [1.41, 1.87] and the odds ratio calculated for the adult subgroup was 1.49 [1.27, 1.74]. Both subgroups had odds ratios that fell within the other subgroup's 95% confidence levels, lending support to evidence that occupants exposed to fungal agents are more likely to exhibit symptoms of nasal hypersensitivities.

The result for the Z-test for overall effect was 8.48 ($p<0.00001$). Since the test statistic of 8.48 is high (greater than 1.96 for a 95% confidence interval), the probability that H_o is true is small, so H_o was rejected. If there was no effect difference between exposure and non-exposure, Z would equal zero.

Variance is $(\text{Standard Error})^2$ and the smaller the variance the more significant the individual statistic. By taking the inverse of the variance (utilization of the generic inverse method within Review Manager), each statistic is weighted. The more weight

percentage assigned to a statistic, the more influence that statistic had on the quantification of the subgroup summary effect estimate or the overall summary effect estimate. All the percentages added up to 100%. (Table 8).

Review Manager calculated the heterogeneity of this meta-analytic investigation as $I^2 = 44\%$ [Subgroup (Children) = 51% and Subgroup (Adult) = 26%]. A clinical interpretation of 44% may represent moderate heterogeneity-attributed variance between studies rather than to sampling error (Higgins, Thompson, Deeks, & Altman, 2003) (Higgins & Thompson, 2002).

The odds ratios along with their 95% CIs from the thirty individual statistics extracted from the eighteen primary studies mentioned above are ordered alphabetically, separated into the two subgroups. The resulting forest plot (Figure 8) generated by Review Manager, has each contributing statistic along with two subgroup summary odds ratios and an overall summary odds ratio represented by three diamond-shaped visuals. The top diamond depicts the child subgroup; the middle diamond depicts the adult subgroup; and, the lower diamond shows the overall summary group of all ages. The visual qualification of the effect estimates supports the quantification of the effect estimates. The effect size estimates (odds ratios) line up beyond the 1.0 on the x-axis, an indication that each statistic lends support to evidence that occupants exposed to fungal agents are more likely to exhibit symptoms of nasal hypersensitivities. The three diamonds occupy almost identical footprints, representing agreement favoring reduced exposure, as each summary odds ratio is greater than one. (Figure 11).

Table 8. Review Manager statistical output measuring respiratory health outcomes resulting from the presence of fungal agents in indoor environments. (Table generated by Review Manager).

Study or Subgroup	log[Odds Ratio]	SE	Weight	Odds Ratio IV, Random, 95% CI
1.1.1 Children				
Brunekreef (1992) Cough	0.45	0.2	4.3%	1.57 [1.06, 2.32]
Brunekreef (1989) Cough	0.71	0.11	7.4%	2.03 [1.64, 2.52]
Brunekreef(1989) Hayfever	0.48	0.08	8.7%	1.62 [1.38, 1.89]
Cuijpers (1995) Cough	0.01	0.33	2.1%	1.01 [0.53, 1.93]
Gent (2002)- Clad/Cough	0.08	0.14	6.2%	1.08 [0.82, 1.43]
Gent (2002)- Pen/Cough	0.19	0.14	6.2%	1.21 [0.92, 1.59]
Jaakkola (1993) Cough	1.14	0.42	1.4%	3.13 [1.37, 7.12]
Jaakkola (1993) Nas excre	0.71	0.41	1.5%	2.03 [0.91, 4.54]
Jaakkola(1993)Nas congest	0.96	0.39	1.6%	2.61 [1.22, 5.61]
Li and Hsu(1997) Rhinitis	1.25	0.64	0.7%	3.49 [1.00, 12.24]
Randriamanantany(2010)HFv	0.94	0.19	4.6%	2.56 [1.76, 3.72]
Savilahti (2000) Sinus	0.15	0.62	0.7%	1.16 [0.34, 3.92]
Stark (2005) Rhinitis	0.29	0.3	2.5%	1.34 [0.74, 2.41]
Strachan (1989) Hay Fever	0.36	0.33	2.1%	1.43 [0.75, 2.74]
Strachan (1989) Nasal	0.76	0.26	3.1%	2.14 [1.28, 3.56]
Strachan (1989) Noc Cough	0.42	0.3	2.5%	1.52 [0.85, 2.74]
Yang (1997) Cough	0.42	0.09	8.3%	1.52 [1.28, 1.82]
Subtotal (95% CI)			**63.9%**	**1.63 [1.41, 1.87]**
Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 32.88$, df = 16 (P = 0.008); $I^2 = 51\%$				
Test for overall effect: Z = 6.82 (P < 0.00001)				
1.1.2 Adults				
Campbell (2004) Cough	0.31	1.07	0.2%	1.36 [0.17, 11.10]
Campbell (2004) Nasal	0.41	1.06	0.3%	1.51 [0.19, 12.03]
Campbell (2004) Sinus	0.31	1.07	0.2%	1.36 [0.17, 11.10]
Gunnbjornsdottir(03)Cough	0.18	0.13	6.6%	1.20 [0.93, 1.54]
Gunnbjornsdottir(06)Cough	0.34	0.07	9.1%	1.40 [1.22, 1.61]
Hirvonen (1999) Cough	1.41	0.65	0.6%	4.10 [1.15, 14.64]
Hirvonen (1999) Rhinitis	1.9	0.62	0.7%	6.69 [1.98, 22.54]
Koskinen (1999) Noc Cough	0.85	0.26	3.1%	2.34 [1.41, 3.89]
Koskinen (1999) Rhinitis	0.5	0.17	5.2%	1.65 [1.18, 2.30]
Koskinen (1999) Sinusitis	0.33	0.27	2.9%	1.39 [0.82, 2.36]
Niemela (1985) Cough	0.1	0.26	3.1%	1.11 [0.66, 1.84]
Niemela (1985) Rhinitis	0.13	0.5	1.0%	1.14 [0.43, 3.03]
Skorge (2005) Cough	0.43	0.26	3.1%	1.54 [0.92, 2.56]
Subtotal (95% CI)			**36.1%**	**1.49 [1.27, 1.74]**
Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 16.31$, df = 12 (P = 0.18); $I^2 = 26\%$				
Test for overall effect: Z = 4.86 (P < 0.00001)				
Total (95% CI)			**100.0%**	**1.58 [1.42, 1.75]**
Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 51.95$, df = 29 (P = 0.006); $I^2 = 44\%$				
Test for overall effect: Z = 8.48 (P < 0.00001)				

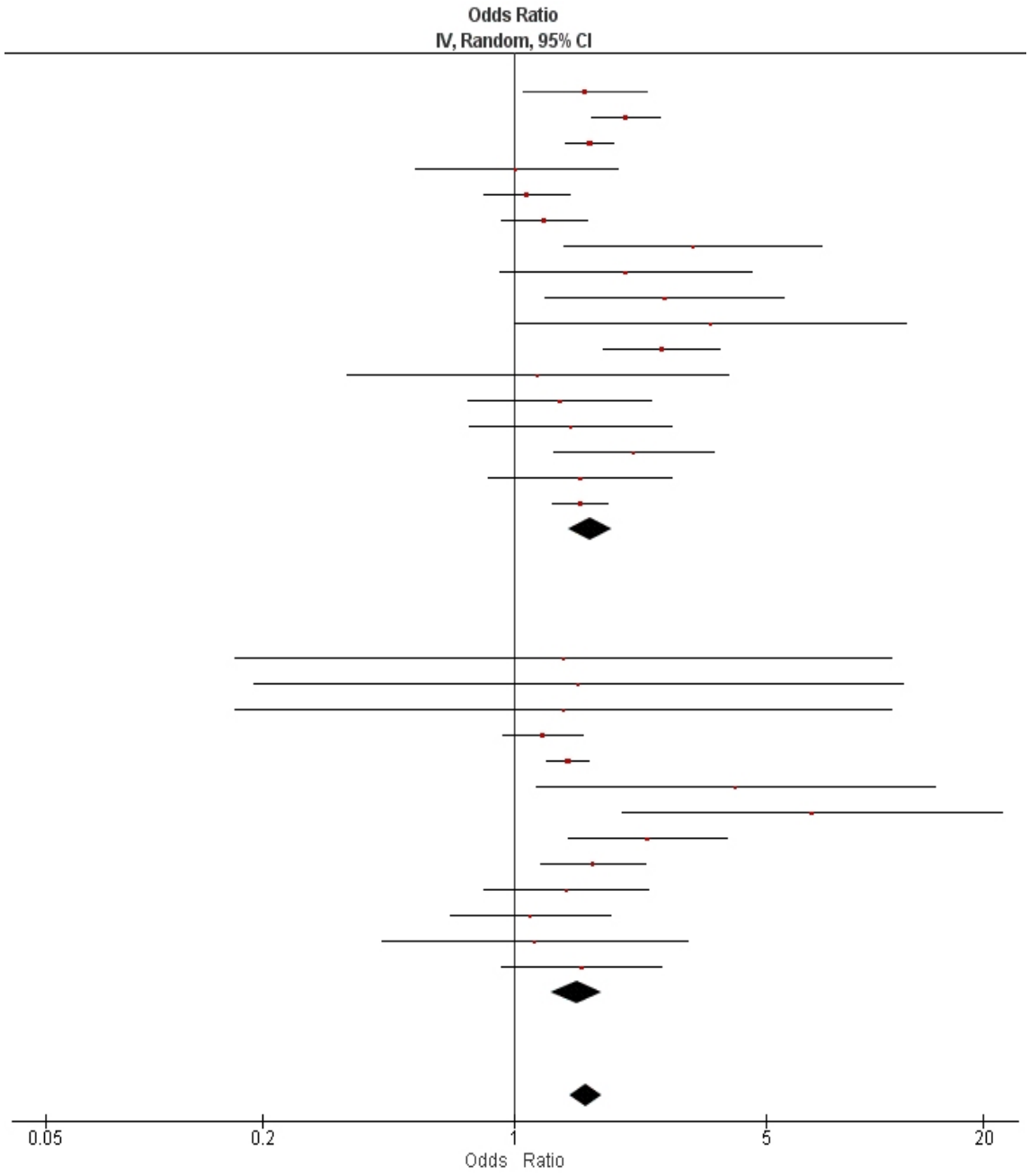

Figure 11. Forest plot results for the measurement of health outcomes resulting from the presence of fungal agents in indoor environments. (Plot generated by Review Manager).

To assess the possibility of publication bias, an inverse funnel plot was constructed. The relative symmetry of the generated funnel plot supported the absence of publication bias. (Figure 12).

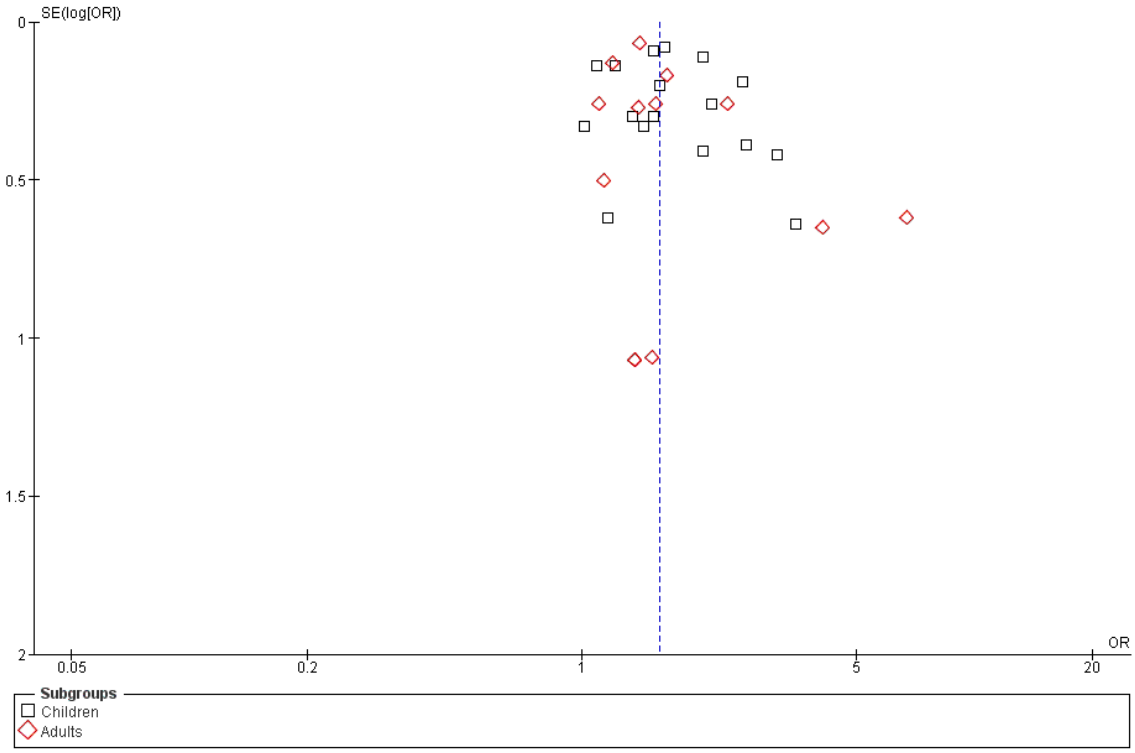

Figure 12. Funnel plot with relative symmetry of data points indicating absence of publication bias. (Plot generated by Review Manager)

Other artificial biases were considered in addition to publication bias. Recall bias, such as over-reporting by questionnaire respondents, was considered unlikely since most studies stated that the sample population was not aware of possible association between respiratory health effects and damp, moldy buildings (Fisk et al., 2007) (Jaakkola et al., 1993) (Brunekreef, 1992). Centre bias resulting from pooled data in large-scale studies

was ruled out by two individual studies (Gunnbjornsdottir et al., 2006) and (Bornehag et al., 2005). Another bias considered but not expected to manifest was selection bias, since none of the included studies analyzed sought specific study subjects, such as atopic individuals, alone.

The strength of evidence of the included studies is supported by primary studies which contain both specific data quantifying and/or qualifying the presence of fungi within buildings, and documentation of the incidence of respiratory health effects. The studies also contain controls where occupants were not exposed to observed fungi. The included studies were conducted by investigators from accredited organizations throughout the world and were not limited to one methodology or one way of thinking. Contrary to Terr (2004), who summarized several research investigations connecting sensitivities with indoor molds as misleading, investigators could not exclusively base symptom observations on clinical wheal-flare skin tests due to their specificity and sensitivity limitations which might generate a high percentage of false negatives. Questionnaires are subjective instruments that collect reliable and useful data (Lim, Kobzik & Dahl, 2010) (Sahakian, Park & Cox-Ganser, 2009).

The meta-analysis of the parameters was similar to established meta-analytical literature with regards to the subgrouping of children and adults (Fisk et al., 2007). Most included studies were retrospective studies except for Stark, et al. (2005), which was a prospective cohort study. Methodologies for environmental assessments were current when each primary study was conducted.

The study factor of dampness with regards to respiratory health was treated as an independent variable and not considered in this investigation. Primary studies which included dampness as a co-variable to fungal contamination were excluded from the meta-analysis. Contrary to theories supported by Fisk (2007) and IOM (2004), dampness was treated as a condition conducive to fungal colonization on cellulosic building materials. Definitions of *dampness* vary among research studies as they are conducted in diversely constructed buildings and climatic regions (Hagerhed-Engman, et al., 2009). In Sweden, dampness was found to be an important independent risk factor regarding symptoms associated with sick building syndrome (Sahlberg, Wieslander, & Norback, 2010).

The event of flooding, due to natural or man-made causes, is necessary to provide water requirements to sustain fungal growth. This dampness or wetting may be an independent variable in relation to the dependent variable of the outcome of fungal colonization. Dampness may also be an independent variable in relation to the dependent variable of the health outcome of respiratory symptoms, and if so, a separate meta-analysis should be conducted to test the relationship between dampness and respiratory illness. By conducting multiple meta-analyses to test for multiple relationships, investigators can avoid inflating the Type I error rate by not lumping different classes of outcomes together into one meta-analysis quantitative study (Wolf, 1986).

Limitations to this dissertation's investigation may have resulted from lack of adjustment for any possible confounders. The decision not to adjust for confounders was based on the findings of a 1999 study which concluded that the association between

residential fungal contamination and respiratory symptoms was not confounded by dust mites, bacterial endotoxins or other known disease-causing agents (Dales & Miller, 1999).

Confounders were not considered in the meta-analysis because known and unknown confounders would have been distributed randomly between sample groups. Potential confounders that have been tested include housing factors such as the presence of air conditioning units and dehumidifiers, ventilation designs, and types of floor covering. Inconsistencies have been noted in relationships between housing factors and fungal measures (Burge, Chew, Muilenberg & Gold, 1995). Other possible effect modifiers in primary studies, such as ventilation rates, were not considered in the secondary dissertation study (Hagerhed-Engman, et al., 2009). Therefore, housing factors were not stratified in the meta-analysis.

Several studies which adjusted for confounders presented stratified results. One study, Gunnbjornsdottir (2006), stated that findings for both adjusted and unadjusted odds ratios were significant. The test statistic generated from the reported adjusted odds ratio was assigned a weight of 9.1% within the adult subgroup which had a weight of 36.1% . (Table 7).

Chapter 5: Discussion and Concluding Remarks

Relationship strength between fungal agents and upper respiratory illness

Evidence presented in this meta-analysis strengthens the relationship between the presence of fungal agents and symptoms of nasal hypersensitivity. The summary effect size estimate of odds ratio 1.58 [1.42 – 1.75] supports earlier meta-analytical findings published by Fisk (2007) of odds ratio 1.70 [1.44 – 2.00].

Although both meta-analytical studies had similar results and some overlapping, the two studies utilized different statistics, different risk factors, and different categories of health outcomes. This meta-analysis focused on the relationship of fungal agents with symptoms of nasal hypersensitivity. Test statistics from primary investigations generated from categories of "dampness or mold" and "dampness only" were not included in the meta-analysis. The Fisk meta-analysis regarded dampness as a risk factor that assumed the presence of mold whereas this dissertation's meta-analysis did not acknowledge dampness as a risk factor but as an independent condition conducive for fungal colonization.

The Fisk meta-analysis separated the health outcome, cough, into a separate health outcome category whereas this dissertation included cough in the symptom pool for analysis. Also, the two meta-analytical investigations shared statistics from twelve

included studies, whereas the dissertation's meta-analysis included six more studies published between 1985 and 2010.

The dissertation's meta-analysis had two non-overlapping subgroups, children and adults, and all study statistics had odds ratios greater than 1.0. The two distinct subsamples yielded two independent pooled estimates: child odds ratio 1.63 [1.41- 1.87] and adult odds ratio 1.49 [1.27 – 1.74]. Collectively, the two-folded meta-analytical evidence from the dissertation's analysis represented by effect size estimates of odds ratios 1.49, and 1.63 are greater than 1.0 thus supporting the position that there was a trend in both age groups between exposure and non-exposure to fungal agents among all occupants.

The trend for the relationship between the presence of fungal contamination and poor respiratory health has been acknowledged by IOM (2004), Fisk (2007), and this dissertation. According to Swaen and van Amelsvoort (2009), the probability that an association is causal is primarily based on three elements: criteria strength, consistency of the association, and experimental evidence. This meta-analytical study contributes to two of the three criteria, i.e. the consistency of the association and the experimental evidence.

The evidence-based research presented in this dissertation confirms the relationship between fungal agents and upper respiratory symptoms. The relationship begins when cellulosic substrates become conducive for fungal colonization and an individual occupying the contaminated area inhales a fungal agent or agents. The occupant might have repeated exposure during subsequential visits. The specificity of the association was confirmed by respondents who observed mold in the building or who

by environmental sampling identified the presence of fungal agents. The temporal sequence of the association was inferred by lack of observation of mold growth before wetting of building occurred coinciding with the onset of respiratory symptoms (Jaakkola et al., 1993).

The biological plausibility of this association has been demonstrated by the incorporation of sensitization testing using fungal extracts collected from contaminated environments (Osborne et al., 2006). The coherence of the association was apparent as confounders were considered in adjusted odds ratios, but findings remained significant. The reversibility of the association seems possible because studies have demonstrated that when buildings are remediated and the sources and sinks of fungal colonization are removed, respiratory health improves (Cox-Ganser et al., 2009) (Fung, Tappen, & Wood, 2000).

Public health policy perspective at the local level

From a public health perspective, understanding the relationship between fungi in domestic interiors and respiratory health of occupants is key in protecting the health of the population (Su et al., 1992). In order to prevent upper and lower airway hypersensitivities, Simons (1999) stated that medical advice should focus on the avoidance of airborne environmental triggers in indoor environments. Public policies often match medical advice. Risk management encompasses evidence-based and risk-informed public policy formulation (Pollard, Davies, Coley, Lemon, 2008). By accepting

the evidence-based trend that the relationship between occupants and fungal agents is likely to result in poor respiratory health of the individual, policy-makers in a community may formulate a specific course of risk management to protect public health.

With policies in place, especially at the local level, situations recently documented from flooding events such as hurricanes (Rao, Riggs, Chew, Muilenberg, Thorne, Van Sickle, et al., 2007) can be minimized with shorter time intervals between the event and remediation efforts. Successful programs preventing and/or mitigating moldy conditions would also be effective in reducing the public health risks associated with such building conditions (Mudarri & Fisk, 2007). In order to reduce risk to respiratory health, decontamination procedures to remove fungal colonization, combined with continual environmental monitoring to insure effective remediation, are examples of risk management reduction policies directed towards the reoccupation of flooded buildings and the protection of public health (Dixit, Lewis & Wedner, 1995).

Because of hurricanes, coastal states may be more at risk for flooding events than land-locked states. The frequency and intensity of hurricanes are expected to have a substantial impact on coastal areas in the future, if the global climate changes as anticipated (Michener, Blood, Bildstein, Brinson, & Gardner, 1997).

Coincident with this ecological awareness, the state health director of North Carolina convened a task force in 1998 to address the increase of asthma prevalence in the state. In 1999, Hurricane Floyd caused flooding in the eastern part of the state. Two years later, the task force broadened its objectives and became organized as the Asthma Alliance of North Carolina. The North Carolina Department of Health and Human

Services (NCDHHS), Division of Public Health, has partnered with the Alliance and adopted a joint mission: to reduce asthma morbidity and mortality for all people in North Carolina through a comprehensive public health approach. (Figure 13).

The Alliance, a partnership of local and state government agencies, academic institutions, local asthma coalitions, non-profits and private industry working collaboratively to address the burden of asthma, has acknowledged the importance of fungal contaminates and dampness as environmental triggers through its educational brochures, seminars, and policy-making. The Department of Epidemiology of the University of North Carolina, an academic partner of the Alliance, conducted state-wide adolescent asthma surveillance and illustrated that a statewide surveillance using the International Survey for Asthma and Allergies in Childhood (ISAAC) was feasible to described the burden of asthma in North Carolina (Yeatts, Shy, Wiley, & Music, 2000).

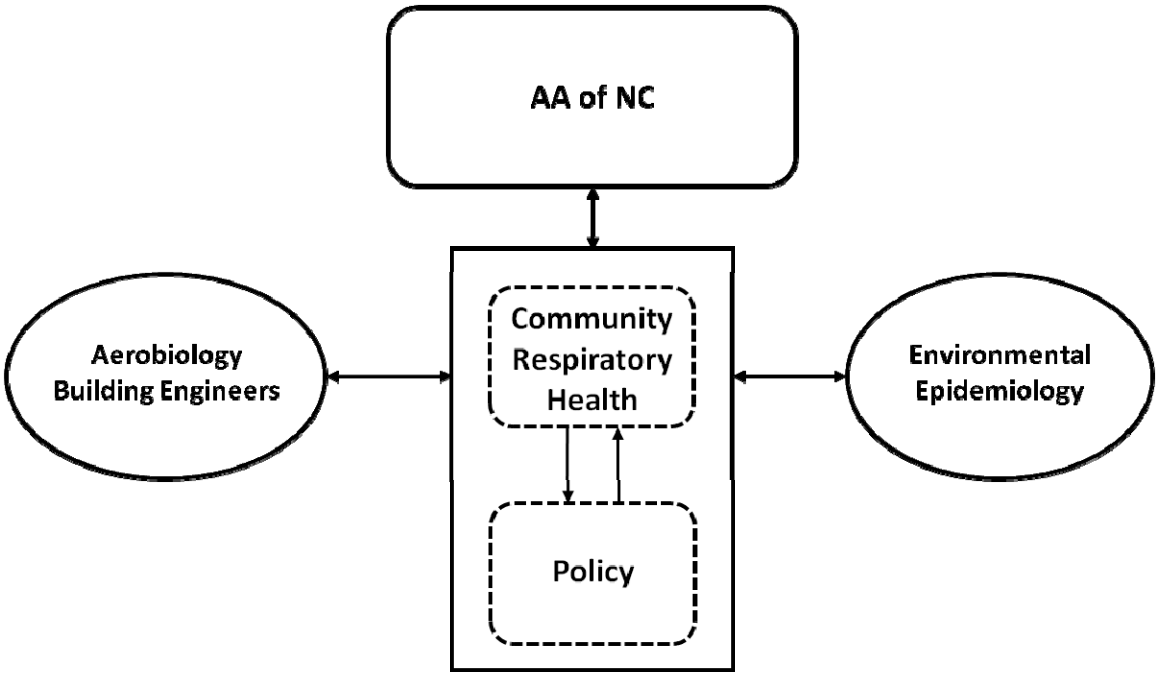

Figure 13: Community respiratory health policy formulation.

The Alliance co-authored two publications with NCDHHS. The first publication entitled "The burden of asthma in North Carolina 2006" sought to build epidemiological evidence by describing the prevalence of asthma among the citizens of North Carolina, both children and adults (Jensen, 2006).

The second co-authored publication, entitled " The North Carolina asthma plan 2007-2012" is a comprehensive document addressing the goals, objectives and priorities of a strategic policy plan aimed at reducing the burden of asthma within all counties of North Carolina (Yeatts & Hathcock, 2007).

The development of community respiratory health policies may be able to assist the Asthma Alliance of North Carolina (AA of NC) in achieving its goals as set forth in the forementioned publications. Intervention favoring reduced exposure to fungal agents in indoor environments may be one part of an Allergen Intervention Management (AIM) program that mitigates existing fungal colonization in indoor environments and prevents fungal contamination from occurring in flooded buildings.

Concluding Remarks

The results from this investigation acknowledged the consistency of association between fungal agents and poor upper respiratory health of the occupants of indoor environments through meta-analytic quantitative analysis.

Funding is needed to strengthen the criteria of the relationship, to standardize environment assessment procedures and reporting, and to develop better clinical diagnostic tools. The utilization of questionnaires, especially those which are interactive through touch screen technology, will continue to provide additional information needed for evidence-based decision-making regarding remediation efforts in homes, schools, and workplaces to reduce exposures to fungal colonization in wetted buildings.

With continuing environmental and medical interests in the relationship between the presence of fungal agents and poor respiratory health in indoor environments, it is hoped that funding for additional primary investigations may become available to provide additional test statistics for secondary meta-analytical studies.

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Curriculum Vitae

As a second-generation pest management professional, the interests of Bonny Lynn Dodson have ranged from inspecting buildings from an environmentalist point of view to facilitating integrated pest management strategies to improve the quality of indoor environments. Skills such as watching colonizing ants and termites, inspecting for mammalian, microbial, and arthropod habitats, and, highlighting the importance of preventing and remediating fungal colonization in occupied buildings to protect public health complete her professional interests. Her professional responsibilities have included being a technical specialist for pest management firms providing technical and policy support for trade associations and government agencies, educator, entrepreneur, safety trainer, consultant, facilitator, writer, building inspector, and other related responsibilities. Both her academic and professional efforts will continue to protect public health and the environment.

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Master of Business Administration in Science Technology and Innovation 1995

Virginia Polytechnic Institute and State University
Master of Science in Entomology 1987

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