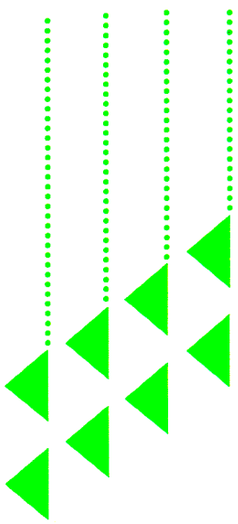
Fungal contamination in public buildings: A guide to recognition and management



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Federal-Provincial Committee on Environmental and Occupational Health

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FUNGAL CONTAMINATION IN PUBLIC BUILDINGS:
A GUIDE TO RECOGNITION & MANAGEMENT

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Foreword

In the field of environmental health, there are times when heightened awareness of a particular issue creates public concern. One example of this is the recent experience of Prince Edward Island with respect to indoor air quality in its schools. A major issue was fungal contamination — in particular, how to interpret laboratory data in terms of the effects of fungal contamination on human health.

The Federal-Provincial Committee on Environmental and Occupational Health was asked for assistance in resolving this issue. As part of its working mandate, this committee examines health hazards associated with chemicals, radiation, environmental microbials, tobacco, and hazardous products. Depending on the topic, fulfilling the committee's mandate may require the establishment of subcommittees, such as the one on drinking water, whose focus is to develop and continuously update the Canadian Drinking Water Guidelines. When an issue requires a report with a specific time-line, working groups are established.

At Prince Edward Island's request, and with the support of the Federal-Provincial Committee on Environmental and Occupational Health, a working group was authorized to review mycological air quality in public buildings. To provide a broad perspective of the problem, members were chosen with specialization in respiratory and occupational medicine, medical microbiology, occupational health and safety, medical mycology, environmental microbiology, and environmental health.

The Working Group on Mycological Air Quality in Public Buildings held a series of six meetings over a 10-month period.
Each member was assigned a topic and wrote a section of the report, which was then discussed by the group to achieve consensus. Much more remains to be understood about the significance of indoor air fungi and their impact on human health, and several recommendations for further research are contained in this report.

The Working Group has attempted to base its final report on existing scientific data, while recognizing that these data are limited and imperfect. The resulting guidelines are general, and considerable judgement is required to determine how they should be applied in specific situations. The guidelines emphasize the elimination of fungal amplification points and the protection of building occupants.

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Executive Summary

The health implications of the fungal contamination of indoor air have become an issue of increasing concern in recent years. At the request of the Government of Prince Edward Island, and with the support of the Federal-Provincial Committee on Environmental and Occupational Health, a working group was established to develop an interim guide to assist public health, occupational health, and building maintenance officials in the interpretation of fungal contamination data from public buildings with respect to health.

The guide has been designed to assist in the recognition and management of fungal contamination problems in public buildings. It also strives to further the understanding of the health significance of fungi detected in the course of building investigations. This guide applies to indoor air in all public buildings, excluding hospitals and industrial settings.

The Working Group has reviewed the health effects associated with fungal contamination of indoor air, reviewed existing indoor air quality guidelines, and provided guidance on procedures for the investigation and interpretation of indoor fungal contamination and for remediation and preventive maintenance of buildings.

Published literature describing the health impacts of exposure to indoor air fungi was critically reviewed. Epidemiological studies have consistently detected an association between respiratory symptoms and home dampness and mould growth, but causality in these studies has not been established. The burden of illness in the population attributable to fungi in private homes and public buildings is not yet known. While the magnitude of the population risk is unknown, it would seem prudent, based on current evidence, to remediate indoor sources conducive to fungal growth.

A guide to the interpretation of fungal sampling data was described in *Indoor Air Quality in Office Buildings: A Technical Guide*, published by Health Canada in 1993. The Working Group strongly supports the approach described in that document, which has been used on a regular basis to investigate and solve mycological indoor air quality problems. Accordingly, questions on the interpretation of data have arisen. In an attempt to answer these questions, the Working Group reviewed this approach and in this document proposes slightly revised courses of action. Their essence remains the same as stated in the original 1993 document.

The guiding principle during investigative or remedial actions is to ensure that no procedures contribute to further fungal contamination of the public building, thus minimizing the health and safety risks to occupants. The procedures for the investigation of possible mycological contamination of indoor air can be grouped broadly into the following phases:

Assessing the magnitude of health problems

A determination of the occurrence and severity of health problems may be obtained from discussions with health professionals, building engineers, managers, employees, union representatives, and building maintenance staff. Health questionnaires are sometimes used as a tool to assemble more comprehensive information.

Identifying problems in the building environment

Fungal proliferation is most often found in buildings in which there is excess moisture, often in the presence of water-damaged material. Investigators should look for areas in buildings where moisture and substrates may encourage fungal growth — for example, areas containing cellulosic materials, air filters, heat exchangers, humidifiers, water sumps, perimeter heating and cooling units, wetted carpet, porous duct lining materials, etc. An attempt should be made to correlate these conditions with high-symptom areas and to designate possible hot spots of contamination.

Identification of indoor fungal amplifiers

Thorough visual inspection of a problem building, combined with some surface samples for microscopic analysis of apparent mould colonies and of deposits in HVAC systems, may obviate the need for air sampling. Where such inspections yield negative results, air sampling should be considered.

Destructive testing is necessary when certain structures of the building have to be taken apart in an attempt to locate the source of suspected contamination. During this phase, the contamination status of the building is expected to be altered by the actions taken by the investigative team, possibly through exposure of previously cryptic contaminants and redistribution of such contaminants via the HVAC system or by other means. All individuals within the building should be protected from exposure.

A key element of the report is a detailed step-wise protocol to assist professionals who may be asked to investigate a building with a potential fungal amplification problem. This protocol covers investigation of building history, visual inspection, sampling and culturing of airborne propagules, examination and culturing of materials and assays for ergosterol and mycotoxin. A recent reference describing pathogenic and toxigenic fungi is also provided. In this protocol, special attention has been placed on the toxigenic fungus *Stachybotrys chartarum (=atra)*, which is frequently isolated from water-damaged buildings in Canada. The protocol includes a logic chart to aid in the investigation of buildings where *Stachybotrys chartarum* has been isolated or where health symptoms suggest its presence.

Risk communication

Lines of communication with building occupants, workplace health and safety officials, building managers and owners, employers, and union representatives should be established as soon as health complaints related to indoor air quality are received. Staged plans for investigation of the source of the problem should be presented and agreed to by all parties involved. If fungal contamination is detected, discussions should occur on the health risks and remedial measures to be carried out. Building occupants should be kept up to date during the investigative, remedial, and follow-up stages.

Remedial actions

Strategies for the remediation of indoor air quality problems caused by fungi are based on the elimination of conditions that promote the amplification of these potentially hazardous organisms. Remediation of fungal hazards may involve cleaning affected areas, decontaminating the HVAC systems, removing contaminated materials, repairing or replacing damaged materials or structures, and modifying the environmental conditions in the affected area. During this phase, the contamination status of the building is expected to be altered by the actions taken by the investigative team, possibly through disturbance of newly exposed heavy concentrations of contaminants and redistribution of such contaminants via the HVAC system.

The design, construction, and maintenance of public buildings should minimize conditions that allow the accumulation, amplification, and dissemination of micro-organisms in indoor air. Building maintenance personnel and building managers should be aware of the potential health problems associated with contaminated indoor air, including the importance of the proper design, installation, operation, and maintenance of HVAC systems to minimize accumulation, amplification, and dissemination of micro-organisms. Prevention of fungal contamination is one of the most desirable strategies for risk management.

Although the report is based on the data available in the literature, the Working Group recognizes that these data may be limited and imperfect. Nevertheless, the report should have general usefulness if it is applied with reasonable judgement to specific situations. The report emphasizes the elimination of fungal amplification sources and the protection of building occupants.

Much more remains to be understood about the significance of indoor air fungi and their impact on human health. Several recommendations for further research are contained in this report.

I. Introduction

The health implications of indoor fungal contaminants have become an issue of increasing concern in recent years. The purpose of these guidelines is to assist in the recognition and the management of fungal contamination in public buildings. This includes an understanding of the significance of fungal measurements performed in the course of building investigations. In the absence of national standards, guidelines published by other organizations have been used to interpret fungal studies in indoor air.

It is recognized that fungi can cause a spectrum of illnesses in humans, ranging from rhinitis to invasive diseases.[1] If associated with fungal contamination of buildings, relatively well defined diseases are considered "building-related illnesses" (BRI). The role of fungi is not well defined in "sick building syndrome" (SBS), but it is this syndrome that most frequently prompts building investigations. The result of these building investigations is commonly the isolation of environmental fungi for which the health risks are not well defined in these circumstances.

The present guidelines have been developed with reference to previously published recommendations.[2] The Working Group consulted many experts active in the performance and interpretation of mycological testing of indoor air environments. The literature concerning the health impact of exposure to indoor air fungi was critically reviewed. The present guidelines address potential health effects, recommended procedures for investigation and interpretation of indoor fungal contamination, remediation, and preventive maintenance.

In the course of the development of the guidelines, the methodology for collection of air samples was critically reviewed. The guidelines are linked to the method of data collection. Methods may be chosen on the basis of the limitations and usefulness of the data collected, the cost and availability of equipment, and current practice in Canada. The applicability of these guidelines to results derived from other sampling methodologies has not, however, been investigated.

The guidelines described in this document apply to all indoor air environments in all buildings, with two exceptions: hospitals, as the role of environmental fungi in causing opportunistic infection in immunocompromised patients was not investigated; and industrial settings, which are outside the scope of this document. The range of climates in Canada, together with the variability of buildings and the purposes to which they are put, makes it impossible to address every circumstance. Adherence to the guidelines, however, should minimize the impact of exposure to fungal materials and guide building maintenance personnel to address structural and potential environmental problems in buildings. The importance of avoidance of fungal growth by ongoing preventive maintenance of buildings is emphasized. Potential sources of fungal contamination should be eliminated wherever possible, even in the absence of air sampling results exceeding these guidelines, and conditions permitting growth should be investigated.

The complexity of indoor air mycology should be recognized. Although fungi are known to cause disease, their contribution to the presence of SBS is not well defined. Measurements of fungal materials can be difficult. Symptoms associated with indoor air quality concerns are not specific. The clinical significance of many mycotoxins has not been established in this setting. In addition to these issues, other questions remain, including the degree of bioavailability of toxins in fungal materials and the role of toxins, individually and in combination, in causing illness. The advances being made in our understanding of this field require that these guidelines be given regular review.

II. Human Health Effects of Indoor Fungi

Several excellent reviews have compiled the diversity of illnesses caused by fungi.[3–7] A detailed review of the literature on the human health effects of indoor fungi is provided in Appendix A. Mediators of disease include mycotoxins, allergens, biologically active cell wall components, and polyclonal cell activators. Antigenic properties of fungi have been implicated in asthma, allergic bronchopulmonary aspergillosis, extrinsic allergic alveolitis, and humidifier fever.[8–12] Relevant to the residential setting, Tarlo et al. reported that 14 of 26 allergic subjects (rhinitis, asthma) tested positive, in skin prick tests, to fungi in their homes.[12]

Mycotoxin-producing fungi are not uncommon in residential buildings. In a study of 52 Canadian homes, Miller et al. found evidence of mycotoxin production in three homes containing *Aspergillus fumigatus*.[13] Trichothecene mycotoxins have also been isolated from the ventilation systems of three "sick" buildings in Montreal.[14] *Stachybotrys atra*, a hydrophilic mould that can produce highly toxic macrocyclic trichothecenes,[11] was thought to be responsible for chronic health problems in a family dwelling.[8] Symptoms were consistent with a toxin etiology, toxins were isolated from the air, and workers apparently became ill while removing contaminated materials. Other adverse human health effects from mycotoxin inhalation, documented in uncontrolled case reports, include renal failure,[15] tremorgenic encephalopathy,[16] and organic dust toxic syndrome.[17] Living in a damp and mouldy home has been associated with non-specific complaints such as headache, sore throat, alopecia, flu symptoms, diarrhea, fatigue, dermatitis, malaise, cough, rhinitis, epistaxis, and fever.[8,18,19] ß-1,3-glucan, a constituent of fungal cell walls, may be related to dry cough and irritation of the skin, eyes, and throat.[20] Epidemiological studies from several countries have demonstrated associations between questionnaire-reported respiratory symptoms and questionnaire-reported mould growth in the home. This finding has been remarkably consistent across different climates, societies, housing characteristics, and scientific investigators. One Canadian study also showed a dose–response effect.[21,22] The odds ratio for cough was 1.61 (95% confidence interval [CI] 1.36–1.89) for the presence of one-versus-no mould sites and 2.26 (95% CI 1.80–2.83) for two-versus-no mould sites. Unlike home studies, results from the few studies of public buildings have been inconsistent. It would therefore be prudent, based on current evidence, to remediate indoor sources conducive to fungal growth.

It is well established that fungi cause several diseases, such as systemic infections and asthma. However, cases of these diseases associated with fungal exposure in public buildings are rarely, if ever, reported. On the other hand, fungi have been raised as one of the possible causes of SBS, which is frequently reported. Symptoms of SBS typically include:

- eye irritation (itching and watering eyes)
- nasal irritation, nasal congestion
- throat irritation
- cough, wheeze

- hoarseness, changed voice
- skin irritation (stinging sensation, itching, dry skin)
- headache
- nausea
- drowsiness, tiredness
- reduced mental capacity, mental fatigue
- changed sensation of odour or taste.

The symptoms of SBS are non-specific and have been associated with many factors, including temperature and humidity extremes. (These factors and their sources are listed in Table 1 on page 11 of the report *Indoor Air Quality in Office Buildings: A Technical Guide*, Health Canada, 1993.[2]) Broder et al.[23] identified a number of factors associated with decreased well-being among office workers. In order of descending magnitude, these were stress in the workplace, female gender, exposure to volatile organic compounds (VOCs), and other contributing factors. Fungal spore counts were not associated with decreased well-being. Skov et al.[24] investigated the influence of personal characteristics, job-related factors, and psychosocial factors on SBS. They found that being female, job category, work functions (handling of carbonless paper, photocopying, work at video display terminals), and psychosocial factors of work (dissatisfaction with supervisors or colleagues and quantity of work inhibiting job satisfaction) were associated with work-related mucosal irritation and work-related general symptoms, but these factors could not account for the differences between the buildings as to the prevalence of the symptoms. The building factor (i.e., the indoor climate) was strongly associated with the prevalence of the symptoms. Thus, in a given building, more than one factor may be at play, and practice has shown that it is usually difficult to attribute symptoms to specific agents. Even when the air quality in a building may be deemed unsatisfactory by investigators, the causes of symptoms may be difficult to establish, and determining them may become an exercise of exclusion.

III. Guidelines

3.1 Summary of Currently Available Guidelines for Mycological Indoor Air Quality

The following chart is a compilation of guidelines that the Working Group consulted.

SOURCE	ISSUE ADDRESSED	INSPECTION PROCEDURES	SAMPLING INFORMATION	BASIS	GUIDELINE	REMEDIATION
GENERAL						
ACGIH Guidelines for the Assessment of Bioaerosols in the Indoor Environment **1989**[25]	Bioaerosols of interest in indoor air, e.g., fungi, mycotoxins, bacteria, endotoxins	On-site investigation; could lead to remedial actions without further investigation	Description of air sampling; routine sampling of bioaerosols not recommended	***Medical Preassessment*** pp. 1–9	**ACGIH *Fungi*, p. 8** No numerical guidelines*	General remedial actions; biocides characterized and discussed separately
Indoor Air Quality in Office Buildings **Health Canada 1993**[2]	General technical guide	Individual checklists for various contaminants	Sampling considerations and strategies; RCS (4 min)	Guidelines based on occurrence of micro-organisms in non-residential buildings	Numerical guidelines presented; see Appendix B of this document	Individual remediation strategies for various contaminants
Biological Particles in Indoor Environments **Commission of the European Communities (CEC) 1993**[26]	Investigation of four main categories of bioaerosols (mites, dander from pets, fungi, bacteria)	Recommendations for different indoor environment studies: homes, non-industrial	Available samplers and analytical methods for air, dust, and surface sampling reviewed	No methods to adequately assess the exposure to biological particles; health effects and occurrence discussed	Values are given for representative categories (CEC, p. 35), but no health guideline can be set based on current knowledge	Not applicable
Airborne fungal populations in non-residential buildings in the United States. **1993**[27]	Fungal bioaerosols in non-residential buildings	Not applicable	Andersen N6 single-stage sampler (1 min) and malt extract agar (2%)	Occurrence based	Proposed limit of 200 CFU/m^3 for fungal aerosols**	Not applicable
SPECIES SPECIFIC						
Guidelines on Assessment and Remediation of *Stachybotrys atra* **New York 1993**[28]	*S. atra* contamination event	Evaluation of microbial contamination based on environmental assessment (visual inspection, bulk sampling, air monitoring)	For routine assessment, air sampling for *S. atra* not recommended	Chronic exposure to airborne *S. atra* poses a risk of debilitating health effects	Any concentration exceeding outdoor levels should be considered positive	Extensive remedial procedures for safe removal of *S. atra* with different levels of containment

Note: Additional standards: ASHRAE Standard 62-1989. Ventilation for Acceptable Indoor Air Quality[29]; OSHA Indoor Air Quality — Proposed Rule, 1994[1]

* ACGIH 1989[25] reported that fewer than 100 CFU/m^3 (colony-forming units per cubic metre) was considered of no concern, but ACGIH 1993[30] indicated that a general Threshold Limit Value (TLV) for a concentration of culturable or countable bioaerosols is not scientifically supportable at this time. The presence of unusual levels of a toxigenic fungus should trigger environmental sampling for specific toxins[25].

** Based on results from more than 2000 indoor and outdoor samples, a 200 CFU/m^3 guideline for fungal bioaerosols is recommended, because 75% of indoor samples yielded fungal concentrations less than 178 CFU/m^3. A critical analysis of results is required if pathogenic or toxigenic fungi are detected.

3.2 Interpretation of Results from the 1993 Health Canada Technical Guide[2] by the Working Group

Health complaints by building occupants may have many diverse etiologies, which may be identified by visual inspection of the building or by knowing the maintenance history. Identifiable promoters of fungal growth require correction, and any visible fungi require removal.

Ideally, sampling should be done to identify a situation that is unhealthy for the building occupants. Although it is clear that pathogenic and toxigenic fungi can cause disease, the health risks associated with a given measured level are, for the most part, unknown. Rather than trying to quantify health risks, sampling can be used to indicate the presence of an indoor fungal amplifier. The presence of high fungal concentrations, certain species mixes, and particular individual species provides the evidence necessary for experienced indoor air investigators to diagnose an indoor fungal amplifier.

The nature of the technique used in the search determines which species are found. *Stachybotrys atra* may be growing in wall cavities but may not be found in air samples taken in adjacent rooms. Contamination of a small localized area in a large space may be missed if air is sampled only from the middle of the space. The choice of media is crucial. The fungi observed are heavily dependent on the media used.

Fungal counts should not be interpreted in isolation. The detection of pathogenic and toxigenic fungi requires further investigation or remediation. Observed counts are heavily influenced by many factors and have a great deal of variability, making it difficult to set limits for acceptable levels. Some of these factors include the type of medium the sampler used, sampling time, activities of the occupants, season, natural ventilation versus ventilation provided by a heating, ventilation, and air conditioning (HVAC) system, and geographic location.

Canadian guidelines were published in *Indoor Air Quality in Office Buildings: A Technical Guide* in 1993.[2] As described in that document, the guidelines are based on a large data set gathered over a period of several years using a Reuter centrifugal sampler with a four-minute sampling time. These guidelines have been found useful by workers in the field and are used on a regular basis. Accordingly, questions of interpretation and clarification have arisen. The guideline statements are listed below with the intention of clarifying these issues,

but the essence of the guidelines remains as stated in the original document (see Appendix B), which the Working Group strongly supports.

1. Bird or bat droppings accumulating in air intakes, ducts, and/or rooms frequently contain virulent pathogenic fungi, such as *Cryptococcus neoformans* and, in some geographic areas, *Histoplasma*, as well as fungi of lower virulence such as the toxigenic *Aspergillus fumigatus*. These organisms are not all reliably detected by sampling air or droppings; accumulated droppings should automatically be regarded as hazardous sources of pathogenic fungi. Appropriate action should be taken for the safe removal of any accumulations of bird or bat droppings.
2. The persistent presence, demonstrated on repeated sampling, of toxigenic fungi (e.g., *Stachybotrys atra*, toxigenic *Aspergillus*, *Penicillium*, and *Fusarium* spp.) indicates that further investigation and appropriate action should be taken.
3. The confirmed presence of one or more fungal species occurring as a significant percentage of a sample in indoor samples and not similarly present in concurrent outdoor samples is evidence of a fungal amplifier. Appropriate action should be taken.
4. The "normal" air mycoflora is qualitatively similar to and quantitatively lower than that of outside air. The number of fungal isolates in outdoor air is affected by the sampling technique, the season, weather conditions, activities, etc. Published data on the range of "normal" values in different parts of Canada are not available, and those that are available may be based on sampling techniques unlikely to be applied in modern indoor studies.
5. If more than 50 CFU/m^3 (in either indoor or outdoor air) of a single species (other than *Cladosporium* or *Alternaria* spp.) are detected, there may be reason for concern. Further investigation should be considered if a repeat sample confirms the finding and establishes that it is based on an indoor source. (As the sampling error is high for low colony counts, repeated sampling can reduce variability of the results and assist in distinguishing situations that warrant action.)
6. Up to 150 CFU/m^3 is acceptable if there is a mixture of species reflective of the outdoor air spores. Higher counts suggest dirty or inefficient air filters or other problems.
7. Up to 500 CFU/m^3 is acceptable in summer if the species present are primarily *Cladosporium* or other tree or leaf fungi. Values higher than this may indicate failure of the filters or contamination in the building.

8. The visible presence of fungi on mouldy ceiling tiles, humidifiers, diffusers, air supply ducts, or other surfaces (including microscopically visible fungi in humidifiers) requires investigation and remedial action regardless of the airborne spore load.

9. The sensitivity of air sampling for the detection of fungal amplifiers is imperfect, and false negative results can occur; some species are detected particularly poorly. If a fungal amplifier is suspected, other means of investigation should be used, as described elsewhere in this document.

IV. Recommended Procedures for the Investigation and Interpretation of Indoor Fungal Contamination

4.1 Background

A safe workplace is mandated by law in Canada under various legislative frameworks. These include various provincial occupational health and safety acts, the Worker's Right-to-Know, the Workplace Hazardous Materials Information System (WHMIS), the Canada Labour Code, and Transport Canada's Transportation of Dangerous Goods (TDG) Act and Regulations. It is of paramount importance to have an operating procedure that will protect the health and safety of visitors, occupants, and the general public, as well as the workers performing their duties in the investigation of possible fungal contamination in public buildings (excluding hospitals and industrial workplaces).

4.2 General Principles

The guiding principle is to ensure that work or procedures during the investigative or remedial phase do not contribute to further contamination of the public building and do not endager the health and safety of building occupants. This is a multi-stage process, and, as the investigation proceeds, employees and occupants must be kept informed on a continuing basis. (For further details, see Appendix C.)

Procedures for the investigation of possible mycological contamination in indoor air can be grouped broadly into the following six phases:

1. PHASE I — *Assessing the magnitude of health problems and taking the building history:* An estimate of the prevalence and severity of health problems may be obtained from discussions with managers, employees, union representatives, joint occupational health and safety committees, and building maintenance staff. Advice should be sought from knowledgeable health professionals. Health questionnaires are sometimes used as a tool to assemble more comprehensive information. The value of such data is reduced by the fact that in so-called healthy buildings, a significant minority of occupants will describe symptoms that they attribute to the building environment. During this phase, the contamination status of the building is not expected to be altered by the actions taken by the investigative team.
2. PHASE II — *Identifying problems in the building environment:* Fungi require water and nutrients for growth and proliferation. They are most often found in buildings in which there is excess moisture, often in the presence of water-damaged material. Humidity may be high. There may be visible condensation on windows. Colonization of walls and other exposed surfaces may be visible. There may be a distinctive fungal odour. Investigators should look for areas in buildings where moisture and substrates may encourage fungal growth — for example, areas containing

cellulose material (paper, cardboard, wood, etc.), air filters, heat exchangers (condensation on cooling coils), humidifiers, water sumps, perimeter heating and cooling units, wetted carpet, porous lining materials, etc. An attempt should be made to correlate these conditions with high-symptom areas and to designate possible hot spots of contamination. During this phase, the contamination status of the building is not expected to be altered by the actions taken by the investigative team.

3. PHASE III — *Sampling (see also Appendix D):*
   a. Transparent tape surface sampling.
   b. Scrapings of contaminated materials.
   c. Routine air sampling.
   d. Bulk sampling.

   During this phase, the contamination status of the building is not expected to be altered by the actions taken by the investigative team.
4. PHASE IV — *Risk communication:* Risk communication has been defined as "the act of conveying or transmitting information between interested parties about the levels of health or environmental risks; the significance or meaning of such risks; or decisions, actions or policies aimed at managing or controlling such risks."[31] Lines of communication between building occupants, workplace health and safety officials, building managers and owners, employers, and union representatives should be established as soon as health complaints related to indoor air quality are received. Steps for investigation of the source of the problem should be presented and agreed to by all parties involved. If fungal contamination is detected, discussions should occur on the health hazards and remedial measures to be carried out. Individuals involved in the investigation and remediation should have received appropriate training as required by the Occupational Health and Safety Act appropriate to the particular province and WHMIS. Building occupants should be kept up to date during the investigative, remedial, and follow-up stages. Detailed information on effective communication strategies for dealing with indoor air quality problems is available.[2,32,33] During this phase, the contamination status of the building is not expected to be altered by the actions taken by the investigative team.
5. PHASE V — *Destructive testing:* Destructive testing occurs when certain structures of the building have to be taken apart in an attempt to locate the source of suspected contamination. During this phase, the contamination status of the building is expected to be altered by the actions taken by the investigative team, possibly through exposure of previously cryptic contaminants and redistribution of such contaminants via the HVAC system or by other means. All individuals within the building should be protected from exposure.
6. PHASE VI — *Remedial actions:*
   a. Removal of contaminated material.
   b. Decontamination of the HVAC and other systems as required.
   c. Repair or replacement of damaged materials and/or structures.

   During this phase, the contamination status of the building is expected to be altered by the actions taken by the investigative team, possibly through disturbance of newly exposed heavy concentrations of contaminants

and redistribution of such contaminants via the HVAC system.

4.3 Sampling Methods for Indoor Fungal Amplifiers

Air sampling is very useful in the assessment of indoor fungal amplification problems, but it must be used strategically. When there is no reason to suspect fungal problems, such sampling is unnecessary. For example, when a non-fungal cause of air quality problems is evident in routine analysis, air sampling for fungi will generally be superfluous. When indoor fungal proliferation is considered possible — for example, where there is a history of high humidity or water damage or where basic air quality tests fail to suggest a likely cause of perceived air problems — air sampling may be considered. It is no longer used in isolation, however, and it is not regarded as the sole means of assessing the presence of fungi. Often, thorough visual inspection of a problem building, combined with some surface samples for microscopic analysis of apparent mould colonies and of deposits in ducts, may supplement information obtained in air sampling or, in some cases, even render air sampling unnecessary. This is particularly the case when flourishing amplifiers of aerially dispersing moulds are found in visual inspection, leading to the working assumption that they are the predominant sources of indoor-derived inoculum in the building. In such cases, prompt remediation may eliminate the problem and, if symptoms experienced by affected persons are alleviated, void the need for further sampling. Where visual inspections give negative results (including cases where some previously visible mould has been cleaned up) but an air quality problem still persists, the prospect of doing air samples must be given further consideration. The possibility of non-fungal factors being responsible for the problem must also be thoroughly revisited. Because fungi may proliferate out of sight in ducts and other hidden sites, particularly in larger buildings with complex HVAC systems, a negative visual inspection alone does not allow one to conclude that problem fungal amplifiers are absent. Also, a building may contain both grossly evident and hidden amplifiers. Air sampling may be the most reliable and accessible way of detecting fungal amplifiers that are not seen in initial visual inspection. Detailed suggestions regarding when and when not to perform air sampling can be found in Section 4.4. Also, for further details on sampling methodology, see Appendix D.

4.4 Protocols for Investigation of Indoor Fungal Amplifiers

4.4.1 The Purpose of These Protocols

The following protocols are not intended to be read as background information, but rather are designed to serve as "recipes" to assist the investigator in detecting potentially problematic indoor fungal amplifiers. Each protocol is numbered and is referred to by number in the text. This device, although necessary for brevity and procedural clarity, may make the text difficult to read discursively, and it is recommended that a copy of the relevant section of the Table of Contents be made and kept to one side to be used as a guide to the numbers. Each protocol has a preamble partially outlining its rationale.

The intended user of these protocols is a knowledgeable member of any of the various professions (occupational health and safety inspector, building inspector, mycologist, health professional, building

engineer, occupational hygienist, etc.) who may be called in to investigate a building with a possible fungal amplification problem. Such a building either may be associated with health complaints or may be a place where mould growth has been noted visually or detected through some sort of routine sampling procedure. No information on fungal identification is provided here. An excellent review of the pathogenic and toxigenic fungi known to be of concern has been published by Gravesen et al.[34]

Although it is mentioned elsewhere in this document, it must be stressed again here that this material is not intended to assist in the search for opportunistic fungal pathogens in hospitals. In that situation, the search may be directed towards numerically rare types of fungal propagules; in general indoor fungal amplification problems, on the other hand, numerically abundant propagule types are most often the object of concern. Detection strategies in the two cases are necessarily quite different.

Several of the protocols are directed towards the diagnosis of infestations caused by *Stachybotrys chartarum* (=*atra*). This particular mould is used as a model for developing the algorithm for assessing indoor mould proliferation. It is singled out not because of its relatively high toxicity, but rather for purely technical reasons: unusual techniques are often needed to detect it accurately. Unlike other toxigenic moulds associated with indoor proliferation, it frequently is represented in the environment predominantly by conidia and other materials that, although actively toxic, do not grow on fungal culture media. The majority of conidia in many amplifiers appear either to be non-viable or to be otherwise inhibited in germination. Only uncommonly are numbers of colonies obtained that give a fair approximation of the amount of *S. chartarum* present. In some cases, air or dust sampling may yield no *S. chartarum* at all, yet considerable quantities of still-toxic conidia may be evidenced on direct examination of dust or may be found within a wall cavity of a room whose occupants have been distressed. The episodic culturability of this species necessitates several atypical search strategies, outlined in protocols #11–#14. Other common toxigenic indoor moulds tend to be culturable, although in a few cases growth may be contingent on the water activity of the media used. Protocols for these moulds are generally less elaborate.

4.4.2 How to Use These Protocols

Begin at #1 and follow the suggestions in that section that direct you to other protocols that may apply. For each protocol you are directed to, read the short introductory paragraph(s) and then each of the numbered subsections below it (e.g., #8a, #8b, #8c, #8d). Choose only the ones that apply to your situation and follow them, ignoring the ones that do not apply. The subsections will tell you which other numbered protocols to go to.

A suggested protocol marked with an asterisk (*) is usually a valuable technique that is very labour intensive, knowledge intensive, or costly and that would ordinarily be done only after simpler techniques had proven unable to resolve the situation. Techniques marked with an asterisk can therefore ordinarily be ignored in early investigations and in investigations that turn out to be straightforward, lacking unusual complicating factors.

4.4.3 Symbols and Expressions

Symbols and expressions used in the various protocols are explained below.

4.4.4 Protocols

#1: Why Investigate?

Investigation of a potential fungal contribution to indoor air problems may follow either 1) the appearance of symptoms compatible with exposure to fungal bioaerosols (see Chapter 2), or 2) the detection of potentially problematical fungal material in a building environment. Apart from the necessarily rapid diagnosis and clean-up of gross contamination, fungal investigation of public buildings should not normally proceed in isolation from investigation of other parameters influencing air quality (see protocol #2).

a) If symptoms are compatible with those outlined in Chapter 2, then do a visual inspection (#3) and check the building history (#2). If these do not reveal an obvious source of the problem, then do air sampling (#4) or thorough dust sampling (#6)*. Also, do examination of settled materials (#5).

Symbol/expression	**Explanation**
*	- optional, thorough protocol, labour intensive, and not always done, but an option available for the most thorough examinations.
rely on	- do protocol if not done before; if done before, refer to those results.
remediation protocol	- general protocol for mould clean-up (see Section 5.1).
benchmark or action level	- established threshold at which contamination exceeds tolerable levels and action must be taken.

b) If there are no symptoms, take no action unless a moderate to large quantity of mould is seen (do #2, #3, #7, #8*, #4*, #5*) or high counts are revealed in preliminary or routine air samples (do #2, #3, #5) or *Stachybotrys* is detected in preliminary or routine air samples, dust samples, sampled visible mould or cultured swabs, surface materials, or contact plates (proceed according to instructions for *Stachybotrys* in protocol #4 for air samples, #6 for dust samples, #7 for macroscopically or microscopically visible *Stachybotrys*, and #8 for swab/surface material cultures and contact plates).

#2: Building History Investigation

Building history (general): Indoor air problems may have various etiologies and should not be assumed automatically to be of microbial origin. Important factors such as non-fungal chemical contamination (e.g., volatile organic compounds of industrial or commercial origin, tobacco smoke, ozone, nitrogen dioxide, carbon monoxide) are outside the scope of these protocols, as are the potential effects of sociological issues, such as labour/management disputes on perceived environmental tolerance. Also beyond the scope of these protocols, but possibly germane in investigated cases, is the evaluation of bacterial diseases, allergies, and intoxications (including endotoxin exposure), viral diseases, arthropod (e.g., insect, mite) allergies, protozoal allergies, and allergies to any aerosolized animal and plant parts or products. A thorough investigation should consider as many of these factors as may be applicable.

Building history (relevant to fungal growth): Inquire about sites associated with symptoms; any previous flooding or other water damage; seasonal humidity; winter condensation; summer condensation on cold plumbing fixtures and pipes; frequency of carpet cleaning, duct cleaning, HVAC maintenance; humidifier, aerosolizer, or mister type and maintenance; and long-time colonization of attic or other areas by birds or bats. Interpret any water damage seen in visual inspection (#3) as a historical indicator.

a) The building history suggests there is an area of readily discernible mould growth (e.g., water-damaged surfaces or stored materials, poorly maintained ducts or HVAC, etc.); perform a visual inspection (#3) wherever this has not been done already.

b) The building history suggests there is a hidden locus of mould growth (e.g., previously flooded wall cavity or false ceiling; previously flooded carpet backing; unshielded cold water pipes or leaky pipes in false ceilings; previous fire extinguished by water, possibly affecting wall cavities; insulation materials present inside ducts). In these and similar cases, where symptoms warrant only, do an extraordinary physical search (#9). Where a hidden locus of mould growth is suggested but the area is not associated with symptoms and is unlikely to be a source of mould inoculum to areas associated with symptoms (e.g., is not connected to vents that may distribute mould inoculum to rooms where symptoms have been noted), either take no action or perform #9b*.

c) The building history suggests long-time bird or bat colonization of the attic or elsewhere: inspect visually (#3) while wearing a high-efficiency particulate air (HEPA) filter respirator, or call an expert.

d) The building history is not suggestive, or information is lacking or incomplete; rely on #3, #4 (or #6), #5. Procedures #4 and #6 (air and dust sampling) are relatively labour intensive and should be undertaken only where complaints, history, or physical conditions are suggestive of a potential mould problem, but where the source of this problem is not immediately evident upon walk-through (#3) and microscopic (#5, #7) visual inspection, or where the bioaerosol significance of visually evident mould growth is queried.

#3: Visual Inspection

Inspect for mould growth or water damage on walls, especially in basements, on window frames, and on carpets (check backing in water-stained areas if possible), on ceiling tiles, as well as on any currently or formerly damp material made of fibrous cellulose (wallpaper, books, papers, shredded newspaper insulation) and all accessible HVAC components; also check for any substantial indoor space with exposed soil such as unfinished basements or crawl spaces, extensive amount of indoor plants, contiguous greenhouses, attics with resident or seasonal birds, bats, or other animals (wear HEPA filter respirator when checking if building history (#2) indicates long-term bird or bat breeding or roosting in attic); and any other likely mould sources.

a) Visual inspection reveals mould or suspected mould: take scrapings or transparent tape mount for direct microscopic examination (#7) and scrapings, swabs, or contact plates for culture (#8*). If the attic contains substantial amounts of bird or bat guano, consult an expert.

b) Visual inspection reveals no mould but reveals damp materials or areas of exposed soil as indicated above: rely on #2, #4, and #5. If a substantial area of exposed soil is found, ensure that any air sampling includes samples taken near this area to probe its significance as a source of mould propagules. Sample of soil may also be analysed using the same techniques as for dust analysis (#6*).

c) Visual inspection reveals neither mould nor apparent dampness or substantial indoor soils: if symptoms are present in the building occupants, rely on #2, #4, #5, and #6*. If no symptoms are present and no other evidence of indoor fungal amplification exists, take no further action. If no symptoms are present but other evidence of fungal amplification exists, consider doing an extended visual inspection (#3); if a hidden building maintenance problem is suggested, do an extraordinary physical search (#9).

#4: Air Sampling

Vacuum/culture devices such as RCS, Andersen, and slit-to-agar samplers are recommended for air sampling in public buildings; note that current Health Canada guidelines are based on four-minute samples with the RCS. If no such device is available,

settle plates may be used for preliminary study, but they should be interpreted by an expert familiar with their limitations and with common local indicator species of indoor mould proliferation. Even as interpreted by such an expert, settle plates may reliably indicate only extreme (very mouldy, very clean) environments (heavy or minimal mould growth after one-hour exposure of a sufficient number of plates), whereas low to moderate indoor mould growth may give settle plate results that are difficult to interpret. See Appendix D for further background information on vacuum/culture and settle plate sampling.

In any case where the indoor environment is suspected to be the source of mould-related problems, analysis of air samples should concentrate on counting and identifying moulds of indoor origin or indoor accumulation, not transitory moulds of outdoor origin. This is accomplished by a species-by-species comparison with control samples of outdoor air. Disregard any species found indoors at a level similar to or lower than its contemporaneous outdoor level (exceptions: *Stachybotrys*, dimorphic systemic fungal pathogens, *Cryptococcus neoformans*) unless other evidence indicates the species is nonetheless proliferating indoors. Species need not be named in these comparisons, but isolates must be recognized as conspecific based on mycological analysis of macroscopic or microscopic characters.

Identification of *Aspergillus* species, *Penicillium* subgenera, and genera of other numerically significant fungi is recommended. Minor species present in proportions below 1% can be ignored provided any *Stachybotrys*, *Aspergillus*, or other pathogenic or toxigenic fungi known to be of concern are detected.

It is critical that outdoor controls be taken well away from the building being tested or any similar buildings and upwind of any possible air outflows. Heavily contaminated buildings will significantly adulterate "outdoor controls" and render them useless unless proper procedures are followed. Outdoor sampling should take place at least 6 m, and preferably 10 m, windward of the building of concern (and any similar buildings) if possible; investigators using electrical devices should carry enough all-weather extension cord to facilitate this. Where buildings are closely crowded, air sampling on the windward side of the roof may be tried; however, if high levels of moulds ordinarily associated with indoor sources are obtained, further investigation should be undertaken to determine the prevalence of these species outdoors in the general area (1 km^2) of the sample.

In general, well-established, medically meaningful benchmarks for acceptable indoor mould levels are lacking; in their absence, the best benchmark to use for the detection of indoor mould amplifiers is that no species should be at significantly greater levels indoors than outdoors (test significance with chi-squared test where applicable). A species more prevalent indoors than outdoors is usually proliferating indoors. Only rarely will outdoor spora sediment out and accumulate indoors to the extent that species of outdoor origin appear to be more common indoors than outdoors. This may, however, occur near large outdoor composting facilities. Indoor proliferation usually indicates a maintenance problem, which may also be associated with some degree of health risk.

Similarly, a species found indoors in a proportion of total spora much greater than its outdoor proportion is usually proliferating indoors and indicates a maintenance problem (e.g., if *Cladosporium cladosporioides* makes up 20% of the outdoor spora and 40% of the indoor spora, indoor amplification is likely and is probably occurring in an excessively moist area). As exposure-related health risk is currently difficult or impossible to quantify for indoor moulds, a better approach is to use mould sampling to detect building maintenance problems and to correct them with the assurance that any genuinely associated, mould-related health problems will be corrected at the same time.

Benchmarks or action levels based on absolute rather than relative numbers are a frequently discussed scientific ideal, but most such benchmarks heretofore proposed are based on unwarranted assumptions or have arbitrary elements. In recent years, most authorities have declined to give uniform and absolute standards for acceptable fungal propagule levels in indoor air. Indoor/outdoor comparisons are generally superior in the detection of potentially problematic proliferations. In some cases, counts of moulds of indoor origin in buildings with air quality problems have been compared with average mould levels in complaint-free buildings. Such comparisons with "normal" airspora levels are best interpreted in combination with indoor/outdoor comparison and amplifier detection as outlined above. At best, studies comparing buildings under investigation with results from unknown problem and non-problem buildings must be interpreted with caution. Not only must technology be highly standardized to compose the baseline data, but also the environments studied (e.g., type of building, geographic area) should be highly comparable. Some tentative benchmarks based on airborne fungal levels in essentially proliferation-free, normal Canadian office buildings were originally proposed by Health Canada[2] and appear in a slightly revised version in the current document (Section 3.2).

Growth media for air sampling fall into two physiological categories: high water activity (general fungal growth media such as malt extract agar or colony diameter restricting media such as Littman oxgall or rose bengal agar) (see Appendix D), and low water activity (medium with high solute concentration, e.g., dichloran 18% glycerol agar, used for detecting fungi growing on very dry material such as dust components and not growing well on general fungal media). Since many common indoor moulds grow on these low water activity media, their use in indoor mould sampling has increased in recent years. The usage of various media in indoor fungal sampling is further discussed in Appendix D. Antibacterial antibiotics are usually incorporated into the media in mycological studies.

a) Air sampling with vacuum/culture device shows moulds of indoor origin present and exceeding the benchmark level (e.g., significantly higher than outdoor levels for same species; in the case of *Stachybotrys*, any isolation is above the benchmark and should trigger further investigation). Go to #10, and, if any *Stachybotrys* is present, go to #4b–#4f and follow the most fitting choice.

b) Minimal *Stachybotrys* found indoors but not found in outdoor controls. (Minimal = a vanishingly small number detected: for example, a single isolated colony found under any circumstances, or two widely dispersed colonies in a large series of samples from different rooms. Note that "colony" means an actual fungal colony observed on the sampling medium after incubation, not a CFU/m^3 in calculation.) Go to #11.

c) Low to high numbers of *Stachybotrys* found ("low to high": at least three colonies in a multi-room sample or two colonies from a single room; or more colonies found under any circumstances) but either not found in outdoor controls or found in outdoor controls at statistically significantly lower levels (chi-squared test). Go to #12.

d) Minimal *Stachybotrys* found indoors (see #4b for definition of minimal) and also in outdoor controls at minimal or higher level. Go to #13.

e) Low to high (see #4c for definition) numbers of *Stachybotrys* found indoors and also in outdoor controls at insignificantly different (chi-squared test) or higher levels. Go to #14.

f) Air sampling shows moulds of indoor origin are absent or below benchmark; no *Stachybotrys* is present. Rely on #2, #3, and #5 (#6*) to detect any temporary and poorly disseminating amplifiers.

#5: Examination for Evidence of Fungi in Settled Materials: Dust, Biofilms, and Standing Water

Air sampling using culturing techniques has traditionally been favoured over direct sampling of potential fungal substrates and sediments indoors. This type of air sampling as the strength that it reflects (to a reasonable degree of approximation) the number of live fungal propagules a person's respiratory system is exposed to. Its disadvantages include its insensitivity to non-viable propagules, its reliance on potentially fluctuant airborne propagule populations, its short sampling times, making phenomena like diurnal cycles of propagule release difficult to discern, and its inability to discern the source of indoor propagules. On the other hand, the direct sampling of sediments and solid or liquid fungal substrata may facilitate the detection of non-viable fungal propagules, the confirmation of sites of fungal growth, the in situ quantification of deleterious fungal chemicals or of chemicals indicative of fungal biomass, and the discernment of patterns of fungal propagule deposition over moderately long periods of time. Note that significant biofilms and potentially contaminated standing water ordinarily should not be present in a building and should ordinarily be remediated immediately upon discovery. Sampling in such cases is only to establish possible links between growth at these sites and airborne or sedimented inoculum sampled elsewhere.

a) Initial procedures where symptoms are absent, analytical resources are limited, or a preliminary survey is being conducted: within air ducts and HVAC coils, etc., do #7 and #8*; visually inspect any functioning humidifier, aerosolizer, vaporizer, or mister; if water is turbid, take a sample for microscopic examination (#17) and culture (#18).

b) Examination for evidence of fungi in settled materials: dust, biofilms, and standing water. Rigorous procedure where symptoms warrant investigation and resources permit: do all procedures outlined in #5, as well as #6. Certain procedures currently under applied research investigation may become increasingly used in these investigations, including fungal ergosterol or glucan sampling (#15) or, in environments with mycotoxigenic species present or expected, direct mycotoxin sampling (#16) or cytotoxicity testing (#19).

#6: Dust Vacuum Sampling

Collect floor or carpet dust using a suitable vacuum device; also compare with outdoor dust control*. On a subsample of bulk dust, perform direct microscopic examination (#7); also do dilution series in sterile water or other appropriate diluent, beginning with a measured quantity, e.g., 1 g dust suspended in 10 mL fluid (1:10 suspension), subsampling 1 mL of suspension to 9 mL fluid to make a 1:100 suspension, and likewise 1:1000, 1:10 000, and 1:100 000*. Plate 0.1 mL from each suspension onto high water activity and low water activity growth media as per procedure #4, with replication. Analyse CFU/g dust based on counts on the dilution that has more than 25 and fewer than 100 colonies per plate, or a number close to one of these figures. Estimate the number of moulds of indoor origin, as gauged by species-by-species comparison with outdoor controls. Species need not be named in these comparisons, but isolates must be recognized as conspecific, based on mycological analysis of macroscopic or microscopic characters. Identification of *Aspergillus* species, *Penicillium* subgenera, and genera of other numerically significant fungi is recommended. Minor species present in proportions below 1% can be ignored provided any *Stachybotrys*, *Aspergillus*, or other pathogenic or toxigenic fungi known to be of concern are detected.

Note that some workers sample dust by plating it directly on growth medium (often dichloran 18% glycerol medium, which favours the growth of dust-associated xerophiles), since making aqueous suspensions may be difficult. Others prefer to gain an approximate measure of human exposure to settled dust by disturbing dusty materials and air sampling with a volumetric technique. In water dilutions and in air sampling of disturbed dust, clumps of hydrophobic spores or conidia may remain largely intact, yielding colonies that actually reflect agglomerations of potential colony-forming units. In dilutions where a wetting agent (e.g., dimethyl sulphoxide) is used, they may break up and give a stronger appearance of heavy infestation.

a) If moulds of indoor origin are present at negligible levels or below the benchmark level (see below), take no further action unless indicated by other procedures. The presence of any quantity of *Stachybotrys* always warrants further investigation — go to #6b. Re benchmark: there are currently no proposed benchmarks for

such studies, but experimental studies possibly yielding such benchmarks for different dust sampling techniques are in progress. If available, please use. In the meantime, experienced evaluators may judge whether moulds are present at unusual levels suggestive of indoor amplification, or whether individual species or species associations suggestive of indoor proliferation are present. If so, go to #6b.

b) If moulds of indoor origin are present at levels indicative of substantial indoor amplifiers or at levels above benchmark (see comment on benchmark under #6a, above) for total fungal count, or if individual species of concern or ecological categories (such as toxigenic fungi) of concern are present, or if species composition of dust spora suggests indoor proliferation, then go to the appropriate subprocedure of #6b:

bi) Moulds other than *Stachybotrys* are present indoors at levels considered to be of potential concern: go to #10.

bii) Minimal *Stachybotrys* found indoors but not found in outdoor controls. (Minimal = a vanishingly small number detected: for example, a single isolated colony found under any circumstances, or two widely dispersed colonies found in a large series of samples from different rooms. Note that "colony" means an actual fungal colony observed on the sampling medium after incubation, not a CFU/g in calculation.) Go to #11.

biii) Low to high numbers of *Stachybotrys* colonies found ("low to high": at least three colonies found in a multi-room sample or two colonies from a single room; or more colonies found under any circumstances) but either not found in outdoor controls or found in outdoor controls at statistically significantly lower levels (chi-squared test). Go to #12.

biv) Minimal *Stachybotrys* found indoors (see #6bii for definition of minimal) and also in outdoor controls at minimal or higher level. Go to #13.

bv) Low to high (see #6biii for definition) numbers of *Stachybotrys* found indoors and also in outdoor controls at insignificantly different (chi-squared test) or higher levels. Go to #14.

#7: Microscopic Examination of Mould or Dust

Mount dust sample, mould scrapings, transparent tape sample, or surface scrapings on a microscope slide with a hydrophobicity-reducing mounting medium (e.g., water + Roccal or other laboratory detergent; 25% sodium hydroxide; or ethanol followed immediately by water) and examine under 10× and 40× for characteristic structures of fungi, particularly fungi of known concern. In surface scrapings, a site of active fungal growth, i.e., an amplifier, is recognized by the presence of hyphae and (in most cases) conidiophores as well as conidia of the same species. Other fungal sporulating structures such as ascomata may also be present. Heavy deposits of conidia or spores alone may signify either heavy deposition from another source or an old, inactive amplifier in which structures of active growth have broken down; alternatively, it may simply indicate that the surface sampled has not been abraded aggressively enough to detach hyphal structures from the substratum.

Taking these and any other salient factors into account, the experienced investigator should make a judgement about whether the material examined is from an amplifier. For general remediation of mould amplifiers, see Section 5.1 of this document. A specialized remediation protocol for *Stachybotrys* has been formulated.[28]

a) Microscopic mount shows *Stachybotrys* from amplifier site: if site is known, go to *Stachybotrys* remediation protocol[28]; if unknown, or if more sites may exist, go to 7c.

b) Microscopic mount shows other mould from amplifier site: clean as warranted, based on type and extent of mould (see Section 5.1).

c) Microscopic mount shows no evidence of an amplifier but *Stachybotrys* conidia present, or mount shows evidence of a *Stachybotrys* amplifier for which the location cannot readily be traced (e.g., conidiophores from an unknown source present in bulk dust sample): rely on #2, #3, #5, repeating or extending if necessary, and, failing these, #9 or expert consultation, to locate source. When the source is found, go to *Stachybotrys* remediation protocol.[28]

d) Microscopic mount shows no evidence of an amplifier, but significant levels of fungal material present, or mount shows evidence of an amplifier for a fungus other than *Stachybotrys*, but the exact location of this amplifier is not apparent: rely on #2, #3, #4*, #5 (extended if necessary), #6* to assist in locating amplifier site(s). If an amplifier is located, go to remediation protocol (Section 5.1).

e) Microscopic mount negative for fungal structures or nearly so. Take no further action unless another procedure (#3, #4, #5, #6*) indicates significant indoor mould proliferation in other locations.

#8: Culture of Swabs, Scrapings, or Surface Dust; and Use of Contact Culture Plates

Surface dust culture, swabs, scrapings, or contact plates will all suffice to detect fungi on surfaces but will not distinguish between species growing on the surface and species that have merely been deposited there as inactive propagules. These techniques are substantially interchangeable, and their use should be tailored to the situation and the sampling materials available.

Sufficiently large quantities of dust or other material that can be suspended more or less evenly in water can be cultured by dilution series as outlined in #6. Swabs should be streaked on high water activity and low water activity media (see #4) as per normal microbiological inoculum attenuation procedure (e.g., limiting the original swab streak to one-third of the plate and cross-streaking with a sterile loop through three or four partial rotations of the plate). Small pieces from scrapings, particularly from mouldy-looking areas, should be suspended in sterile water; the suspension should be vigorously agitated or sonicated and plated out directly on high water activity and low water activity media. Contact plates should be applied to surfaces where mould growth or deposition is suspected and incubated at room temperature (or, where invasive opportunistic pathogens are specifically being sought, at 37°C) for at least seven days.

a) Culture shows *Stachybotrys* in scrapings, swabs, or contact plates; to find source, rely on #2, #3, #5, and #7, repeating or extending if necessary; failing these, rely on #9a, #9b, or expert consultation. For *Stachybotrys* in dust, proceed as per #6bii–#6bv as appropriate, or, where outdoor controls are lacking, consider proceeding as per #6*. If the original *Stachybotrys* in swabs, scrapings, or contact plates was minimal, seen only as one or two colonies in a large sample, and if #9a and #9b fail to show evidence of *Stachybotrys*, then do a new #3, #4 (or #6*), and #5. If these are again positive but minimal, consult the outdoor controls from #4 or #6 and proceed as per #6bii or #6biv, whichever is more appropriate. If the repeats are again positive but levels rise beyond minimal, then consult the outdoor controls and proceed according to #6biii or 6bv, whichever is more appropriate. If the repeats are negative, then disinfect the site where *Stachybotrys* was isolated. If no symptoms are reported, take no further action; if symptoms are reported and #9a has been done, consider doing #9. If #9 and all other procedures following the initial minimal isolation of *Stachybotrys* are negative but symptoms continue to be reported, consider monitoring again in 2–3 months in addition to testing non-fungal potential causes of symptoms.

b) Scraping, swab, contact plate, or surface dust culture grows profuse toxigenic or allergenic mould but not *Stachybotrys*: rely on #7 to confirm growth at sampling site. If #7 is positive, see instructions for #7. If #7 is negative (e.g., if growth is derived from small, sedimented conidia obscured by debris), rely on #2, #3, and #5 to locate the site of growth and also disinfect the broad area around the positive sample site as per Section 5.1. Select additional sample sites in similar or nearby areas and proceed according to #5, #7, and #8. Reiterate until no more sites of heavy mould inoculum are detected.

c) Scraping, swab, contact plate, or surface dust culture is heavily positive for bacteria or yeast, with few or no moulds present. There is a very moist environment, likely occurring indoors in conditions of high humidity, flooding, or condensation, which will also produce mould in less moist habitats. Rely on #3, #4 (or #6*), and #5 to detect the degree of mould growth and sites where it occurs.

d) Scraping, swab, contact plate, or surface dust culture is negative for mould or nearly so. Take no further action unless a previous protocol (#3, #4, #5, #6) has indicated significant indoor mould proliferation in other locations.

#9: Extraordinary Physical Search: Examination of Difficult Sites within Building

Previous flooding may have resulted in accumulations of mould conidia within wall or false ceiling cavities, in wall or duct insulation, on the backings of carpets, or in other inconvenient locations. Walls and false ceilings are sufficiently enclosed to serve as humid chambers promoting mould growth, yet they are often sufficiently porous to allow the dissemination of conidia or toxic or antigenic hyphal or conidial fragments. Actively growing mould may produce offensive, volatile, odour-causing substances. Flooding history or the results of air, dust, or surface samples may suggest

that easily accessible sites are not growing enough mould to explain the observed mould levels or mould-related symptoms, and that a possible cryptic mould reservoir exists. In such cases, difficult samples, requiring entry of ordinarily inaccessible spaces, must be undertaken.

a) History (#2) or physical inspection (#3) of the site suggests previous or current flooding in a poorly accessible site (e.g., wall cavity, false ceiling interior, wall or duct insulation, carpet backing): expose a representative portion of the environment (e.g., wall interior or carpet backing) in the area of concern and perform #7 and #8* in the newly exposed environment. If available, a rigid endoscope (borescope) may be used to examine cavity interiors with minimal damage; perform #7 and #8* adjacent to borehole. Where material suggestive of fungal amplifiers or spore deposition is seen using endoscope, drill additional holes as necessary and perform #7 and #8*.

b) In sites where no historic information is available and no water damage is recalled or seen but where investigation is still warranted by symptoms or other concerns, perform one or more of the following tests with stringency appropriate for the degree of health concern:

bi) Wall cavities: A low stringency test is to check a few representative wall cavities behind switch face plates. Use an alcohol-disinfected, flamed, bent wire to scrape small amounts of material from back or front of wall cavity, being careful to avoid electric wires, and perform #7 and #8*. This test is subject to false negative results if amplifiers are discontinuously distributed but can detect gross,confluent contamination or heavy general spore accumulation. More stringently, use a rigid endoscope (borescope) as outlined in #9a or cut an approximately 15 cm × 15 cm hole in representative wall sections and inspect visually. Perform 7, 8* on exposed interiors, and in particular on suspected amplifiers.

bii) Ducts: Use existing access openings or a rigid endoscope (borescope) to inspect duct interiors. Perform #7 and #8* adjacent to access hole or borehole. Where material suggestive of fungal amplifiers or spore deposition is seen at a distance from original entry point, use elongated scraping device or drill additional holes as necessary and perform #7 and #8*. Holes can be sealed with a plug.

#10: Mould (Non-*Stachybotrys*) Source Location and Clean-up

Procedures such as air sampling and dust sampling do not directly disclose the location of amplifiers (i.e., sites of indoor mould proliferation). If potentially problematic levels of moulds of indoor origin are revealed by these techniques, the sources of these mould propagules must be discovered. *Stachybotrys* is treated separately because a stringent clean-up protocol has been described.[28]

a) Find the source of (non-*Stachybotrys*) mould: rely on #2, #3, and #5 to suggest the location (follow any procedures branching from #5); if necessary, perform extended #3 and #9a* or #9b*. When the source is found, remediate (see Section 5.1).

#11: Minimal *Stachybotrys*, Source Not Located, Likele Indoors

Stachybotrys occurs on cellulose that has become moist. Although it may occur indoors in large quantities on structural materials and bulk stored materials, it may also occur sporadically on small cellulosic substrata or in low abundance in marginal habitats. For example, a library may contain a very low number of previously water-damaged books that may still shed a few *Stachybotrys* conidia. A few *Stachybotrys* isolates in an air or dust survey may derive from such origins. Likewise, dust of outdoor origin may serve as a reservoir for a small number of *Stachybotrys* conidia indoors, and one or two colonies may turn up on indoor surveys even when contemporaneous outdoor control samples are negative.

The isolation of a small number of *Stachybotrys* colonies is difficult to interpret. One of the attributes rendering *Stachybotrys* distinctive is the low viability of its conidia encountered in indoor situations. Hundreds of conidia seen in a direct examination may show only 2–3% viability in culture. Results from using only culture techniques alone may profoundly underestimate the presence of this organism and therefore its toxic effect. Dead conidia apparently remain toxic for some time, as do conidial fragments. Undoubtedly the decline in conidium viability occurs over time, and most or all conidia are likely viable when they are fresh. Yet *Stachybotrys* material investigated by indoor mould researchers tends to show low culture viability. It may be that this highly toxic material is less likely than other environmental contaminants to be degraded into an inoffensive form after it has lost viability.

One or two colonies of *Stachybotrys* in an indoor survey may be the viable representatives of large numbers of non-viable propagules, thus indicating a serious level of contamination. Alternatively, they may reflect fortuitous isolation from a minor source. In either case, repeated air or dust samples analysed by culture may yield a false negative result. The best procedure to use as a follow-up to positive cultures is a search for possible *Stachybotrys* sources. Only when this search gives a negative result is it reasonable to attempt repeat culture samples. This is done to exploit the possibility that, if significant amounts of *Stachybotrys* are present, they may recur at least some of the time in culture samples. In other words, when better techniques have failed, this imperfect technique may be used because of the chance that it may yield valuable information.

a) Rely on #2, #3, and #5 (and, failing these, #6*) to suggest the site of *Stachybotrys* growth. If #3 is negative and #5 or #6 is positive for visible conidia or culturable propagules, perform an extended #3 and #9*; if all tests are negative, repeat #4 or do #6 (repeat #6 if it was the original source of *Stachybotrys* isolation) in the area where *Stachybotrys* was detected. If the repeat test is negative, disregard *Stachybotrys*; if the repeated results of either #4 or #6 are positive but minimal (a single colony or two widely dispersed colonies in a large series of samples), rely on direct microscopy of dust as per #7 to determine if dead *Stachybotrys* conidia are present; also perform #9* or #9a if not performed already; if no *Stachybotrys* conidia or sources are evident, disregard *Stachybotrys*. If conidia are evident in #7, do an extended #3 and #9; if no source is found in these procedures, call

an expert. If #6 is positive for low (three or more colonies overall or two clustered colonies; or a single colony in a small sample) to high, follow procedure #12 if outdoor controls are negative or #14 if outdoor controls are positive.

#12: Low to High *Stachybotrys*, Source Not Located, Likely Indoors

The finding of low to high *Stachybotrys* colonies strongly implies that an indoor amplifier is likely to exist. In such cases, it is necessary to find the amplifier.

a) Rely on #2, #3, and #5 (and, if these fail, repeat #6 if it was done only once before) to suggest the site of *Stachybotrys* growth; if the source is found, remediate as per *Stachybotrys* remediation protocol[28]; if the source is not found, perform an extended #3 and #9 or #9b. If all tests are negative, repeat #4 or do #6 (repeat #6 only if it was done once before; if you already have a result of a repeat trial, rely on that); if the repeat #4 or #6 is negative, consider monitoring on one or more further occasions or, where symptoms are of concern, consult an expert; if the repeat #4 or #6 is positive, further extend #2, #3, #5, and #9 exhaustively or consult an expert.

#13: Minimal *Stachybotrys*, Source Not Located, Outdoors or Indoors

See comments under #11 on the significance of minimal numbers of *Stachybotrys* colonies. Note that *Stachybotrys* is common in certain agricultural situations, such as decaying straw, especially in horse barns. *Stachybotrys* may also occur on other natural moist cellulose substrata and hence may appear in outdoor control samples.

a) If a low-level or distant outdoor *Stachybotrys* source is possible, rely on #2, #3, and #5 (and, if these fail, #6, repeated if done before) to clarify indoor levels and sources. If new outdoor controls and #2, #3, and #5 are negative and a new indoor dust sample (#6) is positive but minimal (a single colony or two widely dispersed colonies in a large series of samples), consider monitoring on one or more further occasions; if the outdoor controls are negative but indoor levels are low (three or more colonies overall or two clustered colonies; or a single colony in a small sample) to high, the likelihood of an indoor source is heightened; follow #12. If the outdoor control is positive but the indoor control is negative, disregard indoor *Stachybotrys* procedures (if the outdoor source yields high levels, consider searching for and eliminating it). If both outdoor and indoor samples are negative, discontinue *Stachybotrys* procedures.

#14: Low to High *Stachybotrys*, Source Not Located, Outdoors or Indoors

See comments under #13 on possible occurrence of *Stachybotrys* in the outdoor environment.

a) Controls of previous studies have indicated that a nearby or relatively high level outdoor source of *Stachybotrys* is possible. Nonetheless, an indoor source also remains possible where conducive habitat conditions exist or have existed; an outdoor amplifier may have served as an inoculum source for indoor colonization. Rely on #2, #3, #5, and #9b* to detect any indoor amplifier; if the original *Stachybotrys*-positive controls in #4 or #6 were within 6 m of the test

building or downwind of possible air outflow from the building, repeat #4 or do #6 (repeat #6 only if done only once before; if you already have a result of a repeat trial, rely on that) with outdoor controls in more distant, windward (#4) or normally windward (#6) sites. If new outdoor controls are negative and indoor controls are positive for *Stachybotrys* at any level, go to #12. If outdoor levels in the repeat still differ insignificantly (chi-square) from or exceed indoor levels, discontinue indoor analysis or, if symptoms are of concern, consult with an expert. If new outdoor controls are positive for *Stachybotrys* but indoor levels are significantly greater (chi-square), then go to #12. If new indoor and outdoor tests are both negative for *Stachybotrys*, discontinue analysis.

#15: Ergosterol or Glucan Sampling

Ergosterol is a cell membrane component (analogous to human cholesterol) that is specific to fungi. Quantitative analysis of ergosterol in an environment can be used to estimate the level of fungal contamination present. It cannot identify whether this contamination derives from indoor amplifiers, sedimentation of outdoor airspora, or fungi in imported mud and debris (e.g., from shoes, dog or cat feet). Nonetheless, markedly high indoor levels generally indicate problematic fungal amplifiers, and this inference is easily confirmed with species-by-species analysis of a small number of air or dust samples. For further information, see Miller et al.[13]

β-1,3-glucan is another fungal biochemical, a cell wall component, that may be measured in an attempt to assess the total quantity of viable and non-viable fungal biomass. A well-established protocol for interpreting β-1,3-glucan results from allegedly mould-contaminated buildings does not yet exist but is an area of active research.

#16: Mycotoxin Sampling from Air or Dust

In certain cases, significant levels of a particular mycotoxin may be expected to occur in an environment, either because of high occurrence of the associated mould or because aerosols deriving from a substrate for toxigenic mould growth (e.g., peanut or corn dust in the case of *Aspergillus flavus*) are suspected to be present. Direct mycotoxin detection mechanisms ranging from simple medium tests to complex chromatography and spectrometry may be used to determine mycotoxin levels. A large literature exists on analytical techniques for various mycotoxin classes, but review of this literature is beyond the scope of this document.

In many contaminated indoor environments, the substances responsible for causing symptoms are not well known and cannot be detected directly. Most moulds produce complex mixtures of mycotoxins and may produce toxic proteins and irritating antigens in addition to the classic small mycotoxin molecules. Hence, direct mycotoxin monitoring is applicable only where there is a clear environmental dominance of an individual toxin-producing species or a special concern regarding a particular toxin or class of toxins. An example of such a concern is the possibility of exposure to aflatoxins, predisposing workers to liver cancer, in a peanut processing factory where symptoms and adverse health effects suggest exposure to mycotoxins.

#17: Humidifier Water Direct Microscopy

Water from humidifiers and misters may

contain profuse growth of micro-organisms, including moulds and yeasts, as well as bacteria and protozoans. Clean humidifier water should appear clear. If it does not, the cause of turbidity may be biological or chemical. Chemical turbidity usually consists of a sediment of calcium carbonate crystals; in areas where water is high in calcium, this sediment may heavily encrust evaporation-based humidifiers. Chemical turbidity arising from the suspension of fine carbonate particles may be difficult to distinguish from biological turbidity. Microscopy and culture may be used to make this distinction.

Water should be taken in a sterile container. Mounts should be made and examined microscopically, looking for distinctive fungal filaments, yeast cells, and conidia. Centrifugation may be used to concentrate the biological matter for examination.

#18: Humidifier Water Culture

Water should be plated directly onto any general fungal growth medium (ideally, media restricting colony diameter such as Littman oxgall agar, rose bengal agar, or inhibitory mould agar) containing antibacterial antibiotics. Prior centrifugation should not be necessary, as the contamination is dense in significantly contaminated humidifiers. Indeed, a dilution series (as in #6, simply dilute aqueous suspensions 1:10, 1:100, and so on) may be desirable to obtain well-separated, countable colonies that are relatively free of overgrowth by antibiotic-resistant bacteria. Sampling of bacteria and endotoxin should also be considered.

#19: Cytotoxicity Testing

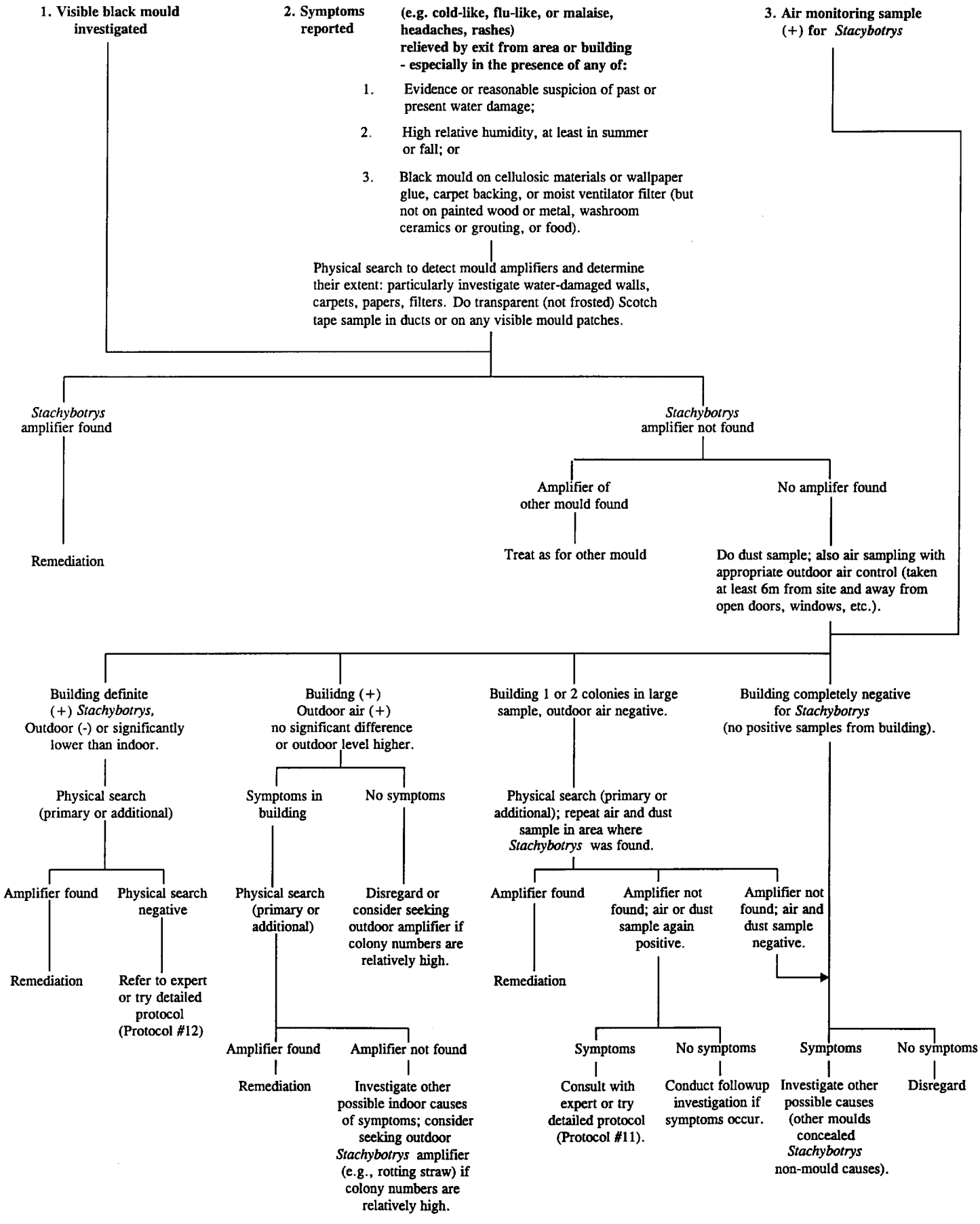
Dust collected by means of a vacuum device may be tested directly for toxic components without targeting particular toxins. A technique for accomplishing this utilizes a sensitive cultured human cell line and exposes it to chemical extracts of the dust.

Miller et al.[13] adapted a technique employing human HeLa cells for dust cytotoxicity studies; the reader is referred to that publication for further information.

Cytotoxicity analysis detects overall levels of toxins in dust, not just mycotoxins, and the relative importance of mycotoxins in known cytotoxic samples must be inferred from fungal analysis (culture and/or direct microscopy) of the same samples.

Abbreviated *Stachybotrys* algorithm

(for detailed methods see protocols #11-14)



V. Remediation and Preventive Maintenance of Buildings

5.1 Remediation

Strategies for the remediation of indoor air quality problems caused by fungi are based on the elimination of conditions that promote the amplification of these potentially hazardous organisms. Typically, amplification occurs in environments where excess water is available.[2,33] This situation is commonly found in areas that have been flooded or where condensation has taken place. Air ducts in which moisture has collected, dampened ceiling tiles, wall panels, carpets, and insulation also provide ideal conditions for the growth of fungi. Remediation of fungal hazards involves cleaning, removal of contaminated materials, and modification of affected environments.

Clean-up of fungal contaminants associated with hard or non-porous surfaces such as walls, air ducts, cooling coils, and drain pans that collect water should be carried out when the affected area is unoccupied.[28] Trained clean-up personnel should use appropriate protective equipment such as respirators and gloves during cleaning activities (see Appendix C). The use of biocides for clean-up of fungal contaminants is usually discouraged because of the potential toxic effects for cleaning personnel and other individuals who may be exposed. However, if well-characterized biocides are used according to manufacturers' specifications, and if they are properly applied, they may provide a valuable adjunct to cleaning procedures.[2] The use of household bleach has been suggested for contaminated surfaces. Following clean-up, appropriate procedures should be in place for disposal of contaminated materials (see Appendix C).

Duct cleaning is frequently accomplished by vacuuming contaminated surfaces. Conventional vacuuming equipment should not be used in occupied areas because of the possibility of transferring the contaminants into the air. Only vacuuming devices equipped with HEPA filters should be used if the cleaning is done inside occupied space.[28] In all cases where remediation has been accomplished, it should be followed up with routine cleaning and maintenance.

In some cases, cleaning is not possible because of the nature of the contaminated material. Carpets, insulation, and porous ceiling or wall panels fall into this category. When these materials become contaminated, there is no way to determine if fungal growth has been eliminated by cleaning, and complete removal of the affected materials is therefore necessary. Precautions taken during removal of contaminated materials should be similar to those followed for cleaning activities.[28] For example, appropriate protective equipment should be used, and removal of contaminated material should be done by trained individuals. As the removal of porous contaminated materials may result in the creation of hazardous aerosols, it may be necessary to isolate the area with plastic sheeting and carry out the remediation procedure under negative pressure. This precaution prevents the transport of aerosols to non-contaminated areas (see Appendix C). Furthermore, following removal of the

contaminants, it is good practice to disinfect hard surfaces in the containment area with chlorine bleach and to vacuum the area with a HEPA filter-equipped vacuum cleaner. One of the more important aspects of this type of remediation is the proper disposal of contaminated materials. This would include appropriate containment in sealed plastic bags during transport to a landfill disposal site. If disinfection or sterilization is necessary, this can be accomplished with biocides or by incineration or high-pressure steam in an autoclave. The replacement of contaminated materials should be done so as to prevent the recurrence of the contaminants by eliminating those conditions that led to their original amplification.

Many of the indoor air problems associated with fungi can be remedied simply by modifying the environment where the problems occur.[35] For instance, moulds do not require standing water to grow. High relative humidity (RH) can provide sufficient moisture for growth on the surface of porous or non-porous materials if the surface temperature is below the dew point. Fungal amplification might be eliminated under these conditions by simply raising the temperature or by dehumidifying those areas known to promote growth. Leaky water pipes linked to fungal growth should be repaired. The absence of available moisture may be sufficient to prevent fungal amplification. In practice, removal of water sources should prevent the growth of fungi.

Preventing condensation on walls or other surfaces can be accomplished by redirecting air flow to eliminate cold spots.[28] Condensation points can also be eliminated by the appropriate application of a vapour barrier or insulation. Moisture control is the best strategy for controlling fungal growth. Therefore, modifying the environment to prevent moisture is the best approach for remediation and for eliminating future indoor air quality problems.

Although remediation is necessary when fungal hazards have been identified, a much better strategy is to prevent the occurrence of problems.[36,37] Contamination can be prevented by thorough routine cleaning and maintenance and by following some of the modification strategies indicated above. Prevention of fungal contamination is one of the most desirable strategies for risk management.

5.2 Preventive Maintenance

The design, construction, and maintenance of public buildings should minimize conditions that allow the accumulation, amplification, and dissemination of micro-organisms in indoor air. A wide-ranging discussion on building preventive maintenance is beyond the scope of this document, but a few general principles should be considered in the development of programs to avoid the development of fungal amplification sites. Detailed instructions on preventive maintenance to control fungal growth in public buildings are available.[36,37]

5.2.1 Background

The foregoing sections have dealt with the recognition and the correction of fungal problems. The context has been that fungi should not be present above acceptable levels. Elevated levels of fungi are generally indicative of amplification sites or poor outside air filtration. This state of affairs denotes a maintenance problem that must be located and alleviated, using the strategies outlined. However, these can be considered secondary strategies, as the primary aim should be to construct buildings that have been designed to avoid the development of

fungal problems. Also, preventive maintenance programs must be defined and regularly implemented in existing buildings, with the objective of safeguarding against the appearance of fungal problems.

Building maintenance personnel and building managers should be aware of the potential health problems associated with contaminated indoor air, including the importance of the proper design, installation, operation, and maintenance of HVAC systems to minimize the accumulation, amplification, and dissemination of micro-organisms. Staff responsible for operating the building systems should receive intensive training in all applicable maintenance procedures. The absence of such knowledgeable staff members and the low priority often allotted to the implementation of maintenance programs are among the most common factors that lead to the appearance of fungal and other environmental problems in buildings. Use of steam is preferred over use of water spray for humidification.

5.2.2 Building Design Considerations

Several aspects of building design can be implemented to minimize the amplification of fungal contaminants in indoor air:

1. *Limiting access of the outdoor aerosol:* These considerations minimize the entry of outdoor fungi into the building air, through the provision of a tight structural envelope, of particle filtration of the intake air, and of climate control to minimize the need for opening windows.

2. *Eliminating sites of water accumulation:* Sites of unavoidable water collection in cooling and humidification systems should be constructed to be completely drained. Areas of potential water condensation on cold surfaces such as external walls, water pipes, and ducts can be eliminated by proper insulation, ventilation, and humidity control.

3. *Maintaining a sufficient humidity level:* Humidity should be regulated at a sufficient level, high enough for the comfort of the occupants but not so high as to promote condensation. These criteria are generally considered to be satisfied at relative humidities in the range of 20–60%.[38] When establishing RH levels in buildings, the procedure should follow good industrial hygiene practices and occupational health and safety legislation and regulations in the area of jurisdiction.

4. *Facilitating preventive maintenance:* Sites of known and potential water accumulation should be constructed to readily allow inspection and service.

5.2.3 Implementation of a Preventive Maintenance Program

The building operating systems include all components of the heating, cooling, and humidification systems and the air handling and distribution units. The preventive maintenance program should be directed towards minimizing fungal amplification sites by ensuring adequate drainage of sumps and drip pans, regular cleaning of dirt and slime from all constituents, and replacement of filters. The frequency of conducting these procedures varies with each component, from monthly to annually. Porous lining materials should not be present in any part of the HVAC system.

The building constituents include all components of the building envelope and interior. Any sources of external and internal leaks and condensation should be promptly and permanently corrected. Water-damaged insulation, ceiling and wall materials, carpets, upholstery, and other porous components may need to be removed.

5.2.4 Communications

Building maintenance personnel and building managers should be aware of the potential health problems associated with contaminated indoor air, including the importance of the proper design, installation, operation, and maintenance of HVAC systems to minimize accumulation, amplification, and dissemination of micro-organisms. Information-sharing networks should be established to assist public health officials and others concerned about the microbiological quality of indoor air to quickly and effectively deal with problems in public buildings. Public education to increase awareness of the possible contribution of microbiologically contaminated indoor air to allergenic, toxic, or infectious illnesses is essential.

VI. Recommendations for Future Actions and Research

During the course of its deliberations, the Working Group on Mycological Air Quality in Public Buildings identified the following issues that should be addressed before health-based guidelines for the mycological quality of indoor air of public buildings can be developed:

1. Detection Methods for Exposure Assessment

Sampling protocols and analytical methods for fungi should be standardized. Analytical methods based on immunological (e.g., monoclonal antibodies) and molecular (e.g., polymerase chain reaction and gene probes) techniques should be developed. Standard methods for the measurement of mycotoxins and assessment of their toxicity should be developed, including in vitro screening using various biological indicators. The limitations and biases of the selected methods must be well recognized.

Studies indicate that allergenic effects cannot always be correlated with the presence of fungi in indoor air. The use of allergen extract standards, prepared from fungi associated with indoor amplification, should be explored to determine if hypersensitivity reactions can be related to the presence of these fungi in indoor air.

2. Reference Values for Exposure Assessment

Using standard sampling protocols and mycological methods, bioaerosol data from public buildings and adjacent outdoor locations in all regions of the country should be collected and published. These should include baseline quantitative and qualitative data on fungal spores and other propagules, mycotoxins, and other volatile metabolic compounds (e.g., ergosterol and ß-1,3-glucan).

3. Health Effects Assessment

Dose–response data on quantities of airborne fungal propagules, toxins, and volatile compounds required to initiate infection, illness, or allergenic response are required. This information, along with epidemiological studies, is essential to establish meaningful health-based mycological guidelines for public buildings. Health Canada, the Canada Mortgage and Housing Corporation, and Agriculture and Agri-Food Canada are currently conducting such a study.

4. Interactive Computer Model

The development of an expert system, based on the Protocols for Investigation of Indoor Fungal Amplifiers (see Section 4.4), should be considered to simplify the investigation and interpretation of indoor fungal contamination.

The Working Group also strongly recommends that the Federal-Provincial Committee on Environmental and Occupational Health consider the establishment of new working groups to address, individually, other indoor microbiological contaminants of concern to public health, such as viruses, bacteria (including mycobacteria and actinomycetes), protozoans, and dust mites.

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Glossary

ACGIH
American Conference of Governmental Industrial Hygienists.

Actinomycetes
Slow-growing branching filamentous gram-positive procaryotic organisms. Some thermophilic species may cause respiratory problems or allergic pneumonitis.

Allergen
An agent that induces an allergic reaction.

Allergy
A type of sensitivity to chemical, physical, or biological agents.

Amplification
The process of indoor growth leading to an increased indoor fungal concentration compared with the immediate outdoor environment.

Amplifier
An indoor substrate where fungal growth is occurring.

Andersen sampler
A sieve-type air sampling device that uses a vacuum pump to draw air through a radial pattern of 300 small pores, impacting particles in each of the small streams of air onto the surface of microbial growth medium. Andersen samplers with two or six stages separate particles by size and deposit each size class onto a separate plate.

Antigen
A complex chemical substance, such as a cell wall protein or glycoprotein, that is a constituent of one organism and is recognized and attacked by the immune system of another organism. The exposed or invaded organism forms antibodies that bind to the antigens of the circumambient or invading organism.

ASHRAE
American Society of Heating, Refrigerating and Air-Conditioning Engineers.

Aspergillus
A genus of mould fungi containing over 100 species, approximately 15 of which are commonly encountered in Canadian dwellings. All naturally occurring aspergilli are toxigenic.

Aspergillus fumigatus
A fast-growing thermotolerant mould that may cause opportunistic infection in immunocompromised persons or long-time asthmatics. Toxigenic and allergenic, it may also cause hypersensitivity pneumonitis in chronically heavily exposed individuals and non-specific respiratory symptoms in moderately exposed individuals. It is innocuous to the immunocompetent person under conditions of low exposure. Its habitats are compost, dung, and other organic matter, especially in warm areas with relatively high nitrogen content.

ß-1,3-glucan
A constituent of fungal cell wall suggested as one of the possible causative agents of adverse effects in buildings with a history of water damage.

Bacterium
(pl. Bacteria) A procaryotic organism, typically single-celled. Some of them can have pathogenic potential, others may cause problems in indoor air quality, especially when their mode of transmission is through the respiratory route (e.g., *Mycobacterium tuberculosis*, *Legionella pneumophila*).

Bioaerosol
An airborne dispersion of particles containing whole or parts of biological entities, such as bacteria, viruses, rickettsia, protozoans, actinomycetes, fungi, fecal elements or body parts of dust mites and other arthropods such as cockroaches, and animal fur, skin scales, dander, hair, saliva, and urine.

Biocide
A chemical agent that kills a biological entity. Some common biocides are sodium hypochlorite, quaternary ammonium compounds, formaldehyde, hydrogen peroxide, alcohols, phenolics, and glutaraldehydes.

Biofilm
A thin layer of micro-organisms growing across moist surfaces such as moist duct interiors and producing an adherent organic matrix.

BRI
Building-related illness. Recognized diseases with a defined pathophysiology that can be attributed to airborne building bioaerosols or chemical pollutants (e.g., Legionnaire's disease, systemic mycoses, carbon monoxide poisoning, lung cancer).

Building envelope
The outermost enclosure element of a building.

CFU
Colony-forming units. Small units of biological material (spores, conidia, hyphal fragments) capable of giving rise to individual colonies on growth medium.

Conidia
A term referring to the asexual spores of most types of mould (Hyphomycetes and Coelomycetes). These are much more common in air than are the more specialized sexual spores released by some of the same mould organisms.

Conidiophore
The specialized branch producing the conidia of a mould fungus. Seeing conidiophores in direct microscopy of indoor materials indicates the mould is actually growing and reproducing in the site sampled.

Contact plate
A plate of microbiological growth medium (e.g., fungal or bacterial medium) designed to be pressed directly onto a surface in order to detect microbial inoculum, which, ideally, will grow on the medium in proportion to the degree of surface contamination.

Containment
A safe condition brought about by effecting a barrier to microbial transmission. A containment device may consist of a stand-alone piece of equipment, a facility within a building, or a whole building that has the necessary engineering controls to keep biohazardous agents from escape.

Cryptococcus neoformans
A pathogenic yeast growing in accumulated bird (usually pigeon) or bat dung and causing cryptococcosis in heavily exposed or immunocompromised individuals.

Culture
Cultivation of a micro-organism in a confined vessel: a technique employed to allow the sample material to multiply in number in order to facilitate its identification and assessment.

Dimorphic systemic fungal pathogen
One of a small number of specialized, virulent fungal pathogens growing in a mould form at room temperature and as a particulate phase (budding yeast, fission yeast, or spherule) at 37°C in the host or on specialized growth media. *Blastomyces dermatitidis*, causal agent of blastomycosis, and *Histoplasma capsulatum*, agent of histoplasmosis, are native to Canada.

Direct slide
A slide for microscopic analyses: prepared from materials removed directly from contaminated surfaces without being subjected to interim culture or other amplification techniques.

Dust mites
Microscopic arthropod species common in the indoor environment. An important indoor allergen (e.g., *Dermatophagoides pteronyssinus, Glycophagus* spp.).

Endotoxin
A lipopolysaccharide (LPS) component of the membrane of gram-negative bacteria and algae that is heat stable (resists autoclave conditions) and toxic. A secreted toxin will be an exotoxin.

Ergosterol
A membrane sterol specific for most fungi and not significantly produced by higher animals or plants.

FEV1
Forced expiratory volume in one second. The volume of air expelled in one second during a forced expiration from a full inspiration.

Filamentous
A tubular, apically extending, branched growth form characteristic of certain micro-organisms.

Fungal spores
In vernacular usage, differentiated fungal

reproductive structures such as conidia, chlamydospores, ascospores, etc., which facilitate dissemination and propagation. These are generally more resistant to adverse conditions than their corresponding vegetative state. A more restrictive definition of the term is adhered to in mycological technical literature: it refers only to propagules formed by a process of "free cell formation," i.e., from a nucleus initiating an entirely new cell wall from cytoplasmic material.

Fungus
(pl. Fungi) A kingdom of organisms (equal in rank to the Plant Kingdom or the Animal Kingdom), defined technically as a parasitic or saprobic, filamentous or single-celled eucaryotic organism, devoid of chlorophyll and characterized by heterotrophic growth, production of extracellular enzymes, and a distinctive L-lysine biosynthesis pathway. Fungi (e.g., moulds, yeasts, mushrooms) may cause indoor air quality problems through the dissemination of conidia, spores, toxins, or cell wall constituents.

HEPA
High-efficiency particulate air filters that have been tested to assure removal of 99.97% of particles 0.3 μm in size.

HVAC
Heating, ventilation, and air conditioning.

Hypersensitivity pneumonitis
A chronic respiratory distress syndrome characterized by a type III (delayed type) allergic response to an immunosensitizing substance. Usually prolonged, heavy exposure to the allergic stimulus precedes development of the syndrome in susceptible individuals. Other equally heavily exposed individuals may fail to develop the syndrome, despite manifesting a strong immunological response to the prevalent antigens.

Hypha
(pl. Hyphae) A branching tubular structure that forms the vegetative body of a growing filamentous fungus.

Hypochlorite
The ion that is the active ingredient in bleach, $HOCl^-$. It is a powerful antifungal disinfectant but also bleaches many dyes and strongly corrodes metal.

IgE
Immunoglobulin type E are antibodies that mediate certain acute allergic reactions.

Impactor
A sampling instrument employing impactor plates to collect inspirable, thoracic, or respirable particulate matter using the principle that different-sized particles have different acceleration and removal rates. The impactor plates are located downstream of a sharp bend just below sharp edge slits.

MMEF
Maximum mid-expiratory flow rate. The mean rate of expiratory air flow between 25 and 75% of the forced expiratory vital capacity.

Monoclonal antibody
An antibody secreted by a single clone of antibody-producing cells. Such antibodies have the same combining site, the same light chain, and the same immunoglobulin class, subclass, and allotype.

Mould
(American spelling: mold) Normally refers to fungi with a filamentous growth form, often giving rise to "fuzzy," cottony, woolly, or powdery textured colonies. Moulds produce conidia or spores that are poorly visible or not visible at all to the naked eye and that in many species are specialized to become airborne.

Mycelium
Mass of hyphae derived either from a single fungal colony or from a group of associated fungal colonies.

Mycotoxigenic
Producing mycotoxins, specialized fungal toxins. Many different types of toxins are included in this broad category. Most are small, non-volatile molecules such as polyketides, amino acid derivatives (e.g., penicillin), alkaloids, trichothecenes, and so on. The production of mycotoxins is usually stressed in indoor air mycology only when toxins known to adversely affect humans and other mammals are produced. Each individual toxin has its own spectrum of noxious effects, which may be incompletely known.

Mycotoxin
A class of fungal metabolites that have a toxic effect on animals and humans (e.g., moniliformin; T-2 toxin, a *Fusarium* trichothecene).

OH&S Act
Provincial occupational health & safety acts and the Canada Labour Code for Federal Workplaces, which demand that the employers ensure that the workplace is safe for the employees and that the workers ensure the same for fellow workers and building occupants.

Polymerase chain reaction
A technique for amplifying material from a small amount of DNA segments using denaturation, annealing to primers, and DNA polymerase-directed DNA synthesis.

Propagule
Any disseminable fungal element that can give rise to a new fungal growth.

Public buildings
Offices, schools, etc., but excluding hospitals and industrial workplaces.

RCS
Reuter Centrifugal Sampler. A centrifugal air impaction device for quantitatively sampling microbial propagules. It uses an impeller fan to impact propagules onto the agar-filled wells of a moulded plastic strip lining the inside of the sampler. It is highly portable and hence may be easier to use than other equivalent vacuum/culture devices. Blank strips can be purchased to accommodate any special mycological media as required. Some newer models allow for quantity calibration of the air samples.

Respirator
A personal protection device designed to protect the wearer from inhalation of

hazardous substances in the atmosphere.

Sampling
A process of taking an aliquot for representative analyses.

SAS
Surface Air System sampler.

SBS
Sick building syndrome. According to World Health Organization's definition, consists of a group of non-specific symptoms such as eye, nose, or throat irritation; a sensation of dry mucous membranes; dry skin, rash; mental fatigue; headache; nausea; dizziness; coughing; hoarseness; wheezing; or itching and non-specific hypersensitivity reactions.

Settle plate
A petri plate filled with microbial growth medium and left open for a prescribed period of time so that bioaerosols can settle on it. It is then closed up and incubated to allow the growth of colonies of fungi or bacteria.

Slit-to-agar sampler
A vacuum/culture air sampling device. The Bourdillon slit-to-agar sampler draws air through a narrow slit onto the surface of a rotating petri dish filled with agar. As only one position on the agar surface is under the slit at one time, some idea of the temporal variation in propagule intake can be obtained by examining the plate. This in turn gives an idea of the degree of homogeneity of propagule distribution in the air itself.

Stachybotrys
A genus of toxigenic moulds (Hyphomycetes) characterized by producing slimy heads of warted, ellipsoidal, usually black conidia from clusters of inflated phialides (flask-shaped fertile cells). The toxins produced by *Stachybotrys chartarum* (= *atra*) are extremely potent macrocyclic trichothecenes. *Stachybotrys* lives primarily on damp cellulose.

Substrate
Substance on or in which a fungus is living.

Thermophilic
Organisms that prefer a temperature above 37°C for growth and survival. Such agents can grow in temperatures up to 58°C and are isolated in hot springs, soil, compost piles, gas furnace humidifiers, etc. This may be an important group causing indoor air problems in buildings.

Toxigenic
A substance or biological entity that has the property itself or can produce one or more compounds that have the property to harm humans or other animals.

Trichothecene
A class of toxins produced by certain fungal species such as *Fusarium sporotrichoides* and *Stachybotrys chartarum* (= *atra*). These mycotoxins cause severe health effects in humans and other animals (e.g., deoxynivalenol, or DON; vomitoxin; nivalenol; T-2; HT-2; diacetoxyscirpenol, or DAS).

Vacuum/culture technique
Air samplers that sample mould propagules by means of a vacuum drawing airborne matter onto culture media.

VOC

Volatile organic compounds. Some VOCs are of industrial origin, such as compounds that evaporate from housekeeping or maintenance products used or in storage. Others are produced by certain micro-organisms, including 1-octen-3-ol, 2-pentanol, and 3-methyl-2-butanol.

Water activity
The molecular availability of water. Water becomes less available (lower in free energy) when molecules are ordered by interactions with solute molecules or capillary surfaces.

WHMIS
Workplace Hazardous Materials Information System. The legislation ensuring the worker's right-to-know using three key elements: labels, Material Safety Data Sheets, and worker training.

Xerophilic
Refers to fungi that prefer to grow in substrata with low water activity.

Yeast
Yeast is vernacular for unicellular fungal organisms that reproduce mostly by budding, although some fission yeasts do exist. This term is of no taxonomic significance and is useful only to describe a certain morphological form of a fungus. Most true yeasts belong to one of the phyla of true fungi called Ascomycota (some others in Basidiomycota and those not yet shown to have a sexual stage in Deuteromycota). The characteristic sexual spores of Ascomycota occur in even numbers (normally eight) in a sac called an ascus. The asexual spore is a budding cell called a blastoconidium. Yeasts may be pathogenic, such as *Candida albicans*, *C. tropicalis*, and *Cryptococcus neoformans*. Other yeasts are of industrial importance, such as baker's yeast for household or brewing use.

APPENDIX A

Human Health Effects of Indoor Fungi

Human Health Effects of Indoor Fungi

A.1 Introduction

Several excellent reviews have compiled the myriad illnesses caused by fungi.[1–5] The list includes the major categories of cancer, infection, toxic diseases, and immunologic diseases. Mechanisms of pathogenesis are equally eclectic. Fungi produce potent mycotoxins, allergens, and biologically active cell wall components, and spores can induce polyclonal cell activation in vitro.[1] In most public and private buildings, indoor concentrations of fungi are lower than outdoor concentrations, and the species mix is similar. Thus, the health risks of fungal exposure should not be increased indoors. However, in some buildings there are environments that promote the growth of fungi, resulting in higher indoor concentrations and a different species mix. This situation has been associated with adverse health effects in several case reports, usually of extremely high exposure to toxigenic species.

Apart from these isolated case reports, the population health significance of indoor fungal contamination is unknown. Mould amplifiers are frequently reported by occupants of homes in North America and Europe. In a large questionnaire study of Canadian homes, reported indoor dampness and/or mould growth were positively associated with reported asthma and respiratory and non-specific symptoms. If this association were causally related, indoor dampness and/or moulds would be responsible for a significant amount of respiratory illness in the general population. Improved building structure and function would effectively reduce dampness and improve the health of our society. Because of its potential significance to public health, research continues into clarifying the nature of the epidemiological association. This review summarizes the currently available information concerning the population health effects of indoor dampness and moulds and assesses the strength of evidence for causality.

A.2 Methods Used to Search the Literature

Data bases searched included Medline (1988–1993), Embase (1988–1993), and BIOS (1991–1993). Keywords used were [(fungi or mould or mycotoxin) and (respiration or pulmonary or breath or inhalation) and (indoor air or public housing or public area or public building or workplace or work place)]. To verify completeness of the search, the keyword “indoor air pollution” was used by itself for Medline, but this did not contribute further relevant articles. Several articles and abstracts were obtained by personal communication with investigators active in the area of indoor mould and dampness. The following types of literature were included: journal articles, reviews, conference proceedings, technical reports, monographs, and meeting abstracts. The references of the reviews were also searched for further relevant articles. No studies of human health effects of indoor moulds and dampness were excluded. All are discussed with their strengths and weaknesses. Many poor quality studies may still provide useful pieces of information unaffected by the overall methodological weaknesses. The evidence used to assess causality was taken from the published series of McMaster University Clinical Epidemiology Rounds.[6]

A.3 Fungi in Residential Buildings

Epidemiological studies from several countries have demonstrated health effects associated with home dampness and moulds. The findings are remarkably consistent across different climates, societies, housing characteristics, and scientific investigators. To allow the reader to appreciate this consonancy, studies have been grouped by geographic region.

A.3.1 United Kingdom

Melia et al. administered questionnaires to mothers of 191 schoolchildren 5–6 years of age in northern England and measured relative humidity in the children's bedrooms and living rooms.[7] The prevalence of at least one respiratory symptom (cough, chest colds, wheeze, asthma, bronchitis) was 85% among boys and 64% among girls whose bedroom weekly mean relative humidity was at least 75%. Corresponding prevalences were approximately 58% and 45% where humidity was lower ($p<0.05$, boys only).

Strachan et al. randomly sampled one in three Edinburgh primary schools.[8–11] Questionnaire information obtained for 1000 children 6–7 years of age demonstrated associations between interior bedroom wall dampness and wheeze, cough, and chest colds ($p<0.01$). The odds ratio between wheeze and bedroom mould was 3.00 after adjusting for housing tenure, household smokers, crowding, and gas cooking.[8] However, exercise-induced one-second forced expiratory volume (FEV1) decline, measured for 873 children, was not related to reported mould growth.[8] Measured bedroom humidity number (n) (n=778) and viable airspora (n=88) were not associated with respiratory symptoms or with exercise-induced FEV1 decline. Thus, the strong relations between questionnaire-reported respiratory symptoms and questionnaire-reported home dampness/mould could not be validated by objective measures of asthma, humidity, and mould growth. Either the questionnaire ascertainment of mould growth and symptoms created an artifactual relationship or the objective measures of asthma were less sensitive than the questionnaire: the within-subject coefficient of FEV1 variation was 8.5%, and only 40 of 873 children demonstrated an FEV1 decline of at least 20%. Relative humidity in a room may not accurately reflect damp (and mouldy) micro-environments. Airspora measurements are highly variable, as physical activity within the room will render settled dust and spores airborne. If species identification is not performed, the total spore count will represent a variable combination of toxigenic and non-toxigenic species. Using total spore counts with varying toxigenicity will obscure any existing association between toxigenic fungi and health.

Martin and colleagues studied 358 tenants of Edinburgh flats constructed in the 1930s and 1960s.[12] Children's and adults' respiratory symptoms were obtained by questionnaire completed by an adult woman tenant. Inspectors measured the humidity inside the home and recorded signs of dampness, mould, and condensation. Damp homes were associated with more respiratory problems, aches and pains, diarrhea, and headaches among children but not among adults. At least one respiratory problem over the previous two months was reported in 85% of children living in damp homes as opposed to 60% in homes not considered damp. A subsequent and larger study (n=597 households) by the same investigators found associations for adults between home dampness and moulds and the following symptoms: bad nerves,

aching joints, nausea and vomiting, backache, blocked nose, fainting spells, constipation, and breathlessness ($p<0.05$).[13] The last symptom was also associated with indoor total air spore counts ($p=0.019$). No significant differences in psychological distress measured by questionnaire were found between residents living in "damp" and "dry" homes.

Hyndman studied 345 British Bengalis living in 60 publicly owned flats.[14] Visible mould growth was present in one-half of the centrally heated homes and all of those non-centrally heated. Generally, respiratory and other non-specific symptoms, but not peak flows, were associated with average weekly humidity, temperature, and percent mould cover in the most severely affected room, with odds ratios approximating 2. Total spore counts, taken over 15 minutes, were associated only with reported depression.

A.3.2 The Netherlands

Waegemaekers and colleagues studied 328 adults and 190 children from 185 homes in a Dutch coastal town.[15] Mean measured spore concentrations were 192 colony-forming units per cubic metre (CFU/m^3) (geometric mean 2.8) in homes classified by questionnaire as damp and 107 CFU/m^3 (geometric mean 2.1) in homes classified as dry. When homes were classified as damp or dry, adjusted odds ratios for adults were greater than 1 for most respiratory symptoms and were statistically significant at $p<0.01$ for reported wheezing and allergy. No non-respiratory complaints were investigated, and the presence of allergy was not considered a potential confounder but simply an outcome variable.

Brunekreef reported results from two studies of 6- to 12-year-old children carried out in 1987 ($n=1051$) and 1989 ($n=3344$).[16] Damp stains were reported in 15–24% of homes, and mould growth was reported in 9–15%. Odds ratios ranged from 1.5 to 3.0 between cough and wheeze and dampness and mould growth after adjusting for parental education, household nitrogen dioxide level, and smoking. Maximum-mid expiratory flow (MMEF) was reduced 5.4% in homes with reported mould ($p<0.1$) compared with 1.0% in those without mould. Among the adult respondents of the 1991 survey, the odds ratio for the association between cough and either dampness or moulds approximated 2.0 ($p<0.001$), an effect size similar to that of current smoking.[17]

Verhoeff provided evidence that the respiratory symptoms associated with home dampness may be mediated through allergic sensitization to dust mites and possibly moulds.[18] From a random sample of 7632 schoolchildren 6–12 years of age, 259 cases (defined by the presence of chronic cough or wheeze, or dyspnea and wheeze, or physician-diagnosed asthma) were compared with 257 controls (those without respiratory symptoms) on residential mould and dampness (as judged by both the tenant and an independent inspector). Odds ratios for dampness and mould approximated 1–2 for all cases and were approximately 0.5 higher for cases with elevated immunoglobulin type E (IgE) against dust mites and common moulds. However, between cough and reported mould, odds ratios increased to 6.4 (95% CI 0.8–49.3) when restricted to those with elevated IgE to mites and/or moulds. To validate their methods, the investigators correlated the presence of respiratory symptoms with diminished FEV1, and the kappa statistics for agreement between reported and measured mould and/or dampness were 0.4–0.7.

A.3.4 Sweden

Holmberg studied 33 adults in 13 Swedish residences selected by mould and moisture problems. Increased levels of airborne *Aspergillus* (>50 CFU/m^3) were associated with increased reports of eye and skin irritation, cough, phlegm, and common colds.[19] A larger questionnaire study of 4990 Swedish children whose parents were non-smokers found dampness to be associated with cough, as indicated by an odds ratio of 1.9 ($p<0.05$).[20] Rylander et al. reported an association between β-1,3-glucan and cough and pruritus.[21] Five glucan measures were available, one from each of five buildings. Thus, the association may be confounded by other differences between the buildings or their occupants.

A.3.5 United States

Brunekreef et al. studied 4625 children from six U.S. cities.[22] Home dampness was indicated by a positive response to at least one of the following questions: (1) Does water ever collect on the basement floor? (2) Has there ever been water damage to the building? and (3) Has there ever been mould or mildew on any surface inside the home? The odds ratios between dampness and respiratory symptoms ranged from 1.23 (95% CI 1.10–1.39) for persistent wheeze to 2.16 (95% CI 1.64–2.84) for persistent cough after adjusting for maternal smoking, age, sex, city of residence, and parental education. The adjusted odds ratios between mould (question 3) and respiratory symptoms ranged from 1.40 (95% CI 1.13–1.74) for non-chest illness to 2.12 (95% CI 1.64–2.73) for cough. There was no statistically significant ($p<0.05$) association between spirometric measures and the dampness indicators.

A.3.6 Canada

In 1988, Health and Welfare Canada conducted a questionnaire-based study of 30 Canadian communities.[23–25] In total, 17 962 parents or guardians of schoolchildren received a questionnaire, and 14 948 (83%) questionnaires were returned. The reported prevalence of home dampness or moulds in Canadian homes was approximately 38%. The prevalence of lower respiratory symptoms (any of cough, phlegm, wheeze, wheeze with dyspnea) was increased among those who reported dampness/moulds. Among the 12 569 children between five and eight years of age, the prevalences of lower respiratory symptoms were 19.5% and 13.2% in homes with and without reported dampness/moulds, respectively. The corresponding adjusted odds ratio was 1.50 (95% CI 1.35–1.67). Among the adult non-smoking questionnaire respondents, lower respiratory symptoms were reported in 19% and 11% of those with and without reported exposure to dampness/mould. The odds ratio for all adults adjusted for smoking was 1.62 (95% CI 1.48–1.78).

A.4 Fungi in Office Buildings

Compared with residential settings, there are very few studies of exposure to fungi in office buildings. Although several buildings may contain thousands of employees, the number of buildings available for analysis is small. If no buildings with fungal contamination were included, health effects from fungi would not be expected.

Skov and colleagues carried out a comprehensive examination of 14 municipal buildings in Denmark.[26] Questionnaire-reported symptoms were related to several factors, such as floor dust, floor coverings, crowding, building age, and ventilation, but not airborne micro-fungi. Generally, measurements were taken from one site in each of the buildings and generalized to the entire building and its occupants. The duration of sampling and growth media were not stated. Levels of airborne fungi were unremarkable, between 0 and 111 CFU/m^3.

Harrison and colleagues reported a study of 15 office buildings in Great Britain.[27] Using a six-stage Andersen sampler, the following median fungal counts were obtained: approximately 277 CFU/m^3 in the four naturally ventilated buildings, and about 30 CFU/m^3 in the remainder. Although symptom prevalences were lower in naturally ventilated buildings, a positive association was reported between symptom prevalences and counts of fungi and bacteria.

Tamblyn et al. studied four mechanically ventilated high-rise office buildings in Montreal during the spring or fall of 1990.[28] Within each building, the ventilation was randomized six times to either 20 or 50 cubic feet per minute per person for one week. Increased ventilation sometimes increased and sometimes decreased the spore counts. The resulting changes in viable spore counts explained 36–64% of the variation in symptoms ($p=0.06$ for the Pearson and $p=0.20$ for the Spearman correlation coefficient). This association was seen in only one building, and varying the ventilation rate also influences many other air quality characteristics, making any change difficult to attribute to any one cause. In this population, a second study design was employed, comparing employees reporting work-related symptoms with the remainder of the employees. Fungal spore counts were 24 CFU/m^3 higher in the latter than the former group.

Recently, 43 of 49 New York employees exposed to satratoxin H.-producing *Stachybotrys atra* in an office building (>100 000 viable spores per cubic centimetre in sheetrock walls) underwent a health examination.[29] Complaints involved the general areas of respiratory, fatigue, central nervous system, and skin and eye irritation. IgE specific for *Stachybotrys atra* was detected in four subjects. No control subjects were mentioned, making it impossible to determine if there was an exposure–health association in this descriptive study.

A.5 Summary of the Literature

There were 23 publications in the English-language literature from the United Kingdom,[7–14,26,27] the Netherlands,[15–18] Sweden,[19–21] the United States,[22,29] Canada,[23–25,28] and Denmark.[27] These represented 17 unique populations; in several cases there was more than one paper per population.[9–10,16–17,23–25] Twelve studies included children,[7–11,16,18,20–22,24,25] and 11 included adults.[12–15,17,19,23,26–29] The majority of studies identified an association between self-reported respiratory symptoms and exposure, whether assessed by the resident, building inspector, or spore count. In contrast, objective measures of respiratory illness have not been associated with exposure.

A.5.1 Exposure Assessment

In the majority of studies, exposure took place in private dwellings. Much less published information was available from public buildings.[21,26–28] Eight studies assessed exposure to home dampness and moulds by questioning the home resident.[8,9,15,16,18,20,22,23] Questions used included signs of dampness or mould, damp spots, evidence of water damage, a history of flooding, stale odour, and the presence of a wet crawl space, silver-fish, or sow-bugs. Six studies used independent inspectors,[12–14,16,18,20] who assessed homes for the characteristics just listed. Three studies measured relative humidity,[7,10,14] and nine studies measured airspora.[11,13–15,19,21,26–28] In Edinburgh, approximately 50 species or genera were isolated, with 50% of total CFU/m^3 being *Penicillium* and *Cladosporium*. Total spore counts ranged from 0 to 40 000, with the median being about 200 CFU/m^3.[11] Another study identified the common fungi as *Penicillium*, *Aspergillus*, *Botrytis*, and *Cladosporium*, with counts ranging between 34 and 2000 CFU/m^3.[15] One other study identified *Aspergillus*, *Cladosporium*, and *Penicillium*, also with median total spore counts of approximately 230 CFU/m^3.[19]

A.5.2 Health Outcomes

In all studies, self-reported respiratory symptoms were ascertained. These generally included cough, wheeze, asthma, bronchitis, and colds going to the chest. Occasionally, hay fever and sore throat were included. Four studies attempted objective confirmation of respiratory illness, either by resting spirometry or peak flows[14–16,22] or by exercise-induced FEV reduction.[9] Eight studies assessed self-reported non-respiratory symptoms,[12–15,19,22,23,28] which included aches and pains, vomiting, diarrhea, bad nerves, headache, and fever.

A.5.3 Results

Among children, all studies detected associations between self-reported symptoms and self-reported mould/dampness. Among adults, similar associations were found in six of seven residential studies[13–15,17,19,23] and one of three office studies.[26–28] Sample sizes ranged between 33[18] and 14 799,[23] with at least five studies having more than 1000 subjects. When exposure was self-reported, associations were always found with respiratory symptoms. The same was

true for the presence of "other" symptoms. In contrast to self-reported symptoms, objective indicators of respiratory illness have never been shown to be associated with self-reported mould and dampness. Although no one respiratory symptom was outstanding in its strength of association with self-reported exposure, cough and wheeze were consistently related and often had the strongest odds ratios, usually ranging between 1.5 and 3. In one study, the odds ratios increased in cases with increased serum-specific IgE to the house dust mite *Dermatophagoides pteronyssinus* and the moulds *Penicillium*, *Alternaria*, *Aspergillus*, or *Cladosporium*.[18] Similar to self-reported exposures, studies assessing exposure by a building inspector showed associations with respiratory symptoms.[12,13,14,16,18] Two of three studies using relative humidity demonstrated associations with respiratory symptoms.[7,14] Four[13,15,19,21] of five residential studies and one of three office studies[26–28] measuring airspora demonstrated associations with respiratory symptoms.

A.6 Arguments Supporting a Causal Association Between Indoor Air Fungi and Population Health

A.6.1 Fungi Cause Disease

Mediators of disease include mycotoxins, allergens, biologically active cell wall components, and polyclonal cell activators. Antigenic properties of fungi have been implicated in asthma, allergic bronchopulmonary aspergillosis, extrinsic allergic alveolitis, and humidifier fever.[30–34] *Stachybotrys atra*, a hydrophilic mould that can produce highly toxic macrocyclic trichothecenes,[33] was thought to be responsible for chronic health problems in a family dwelling.[34] The disease symptoms were consistent with a toxin etiology, toxins were isolated from the air, and workers became ill while removing contaminated materials. Other adverse human health effects from mycotoxin inhalation, documented in uncontrolled case reports, include renal failure,[35] tremorgenic encephalopathy,[36] organic dust toxic syndrome,[37] and non-specific complaints including headache, sore throat, alopecia, flu symptoms, diarrhea, fatigue, dermatitis, malaise, cough, rhinitis, epistaxis, and fever.[12,13,34] Fungal volatiles, typically short-chained alcohols and aldehydes, account for mouldy odour: 1-octen-3-ol has a mushroom odour, 2-octen-1-ol smells musty, and geosmin has an earthy smell.[1] Reaction to exposure is variable, from none to illness,[38] but data on their health effects are scarce. β-1,3-glucan, a constituent of fungal cell walls, may be related to dry cough and irritation of the skin, eye, and throat.[21] Relevant to the residential setting, Tarlo et al. reported that 14 of 26 allergic subjects (rhinitis, asthma) tested positive, in skin prick tests, to fungi in their homes.[32]

A.6.2 Toxigenic Fungi Are Present in Indoor Air

Mycotoxin-producing fungi are not uncommon in residential buildings. Smith et al. employed the MRC-5 monolayer cultures to assay mycotoxins in 83 fungal isolates from damp public housing in Edinburgh.[39] Forty-seven percent of isolates were considered toxigenic, causing 12–51% cell mortality. In a study of 52 Canadian homes, evidence of mycotoxin production was found in three homes containing *Aspergillus fumigatus*.[40]

Trichothecene mycotoxins have also been isolated from the ventilation system of three “sick” buildings in Montreal.[41]

Epidemiological findings are consistent across geographic regions. Similar health effects have been consistently found in both children and adults and by different investigators in different countries using different questionnaires. This association has usually been found whether exposure to dampness and moulds is ascertained by questioning a resident, building inspection, or enumerating spores. The Canadian study also showed a dose–response effect.[23,24] The odds ratio for cough was 1.61 (95% CI 1.36–1.89) for the presence of one versus no mould sites and 2.26 (95% CI 1.80–2.83) for two versus no mould sites.

A.7 Arguments Against a Causal Association Between Indoor Air Fungi and Population Health

Study designs have been cross-sectional in nature, with no evidence for temporality or reversibility. Home dampness and moulds have usually been assessed by the occupant's response to questions concerning flooding, water damage, and visible moulds in the house. Although dampness promotes mould growth, it may also indicate inadequate home ventilation and increased levels of several indoor air contaminants, from allergens to combustion products. Dampness also promotes dust mites, which can cause respiratory disease. Bacterial endotoxin has been implicated in several cases of building-related illness (BRI) involving humidifier reservoirs contaminated with gram-negative bacteria.[31] The majority of studies have ascertained symptoms by questionnaire, which poses doubts. First, the existence of a group of respondents who generally over-report and another group of respondents who generally under-report could result in an artifactual relationship if those who report more respiratory symptoms also report more home dampness and moulds, and vice versa. Second, subjects ill for other reasons may be more likely than those who are symptom-free to report the presence of home dampness and moulds in an attempt to explain their health problems. This could also result in an artifactual relationship. However, Brunekreef, contrasting symptom reporting with objective measures of lung function, concluded that bias or confounding was unlikely.[16] The Canadian study, however, did not find evidence for (but cannot exclude) this bias; whether or not subjects had been diagnosed as having allergies or asthma, the observed association between symptoms and dampness was not influenced. Finally, the measured relative risks were usually small, less than 2, and therefore relatively susceptible to confounding.

A.8 Summary and Conclusion

Fungi can and do cause a myriad of diseases. Potentially pathogenic fungi are not uncommon in the indoor environment, and diseases caused by indoor fungi have been documented in case reports. The burden of illness in the population attributable to fungi in private homes and public buildings is not yet known. Epidemiological studies have consistently detected an association between respiratory symptoms and home dampness and mould growth, but causality in these studies has not been established. Until the magnitude of the population risk is known, it would be prudent, based on current evidence, to remediate indoor sources conducive to fungal growth.

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APPENDIX B

Indoor Air Quality in Office Buildings:
A Technical Guide, Health Canada, 1993 (Pages 48–49)

Indoor Air Quality in Office Buildings:
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5.2.8.4 Interpretation of Results. Since 1989, the ACGIH Bioaerosols Committee has recommended rank order assessment as a means of interpreting air sampling data. This interpretation has been part of the practice in Government of Canada investigations since 1986. The presence of one or more species of fungi indoors, but not outdoors, suggests the presence of an amplifier in the building. Species identification is critical to the analysis. Because of the problems noted above, numerical guidelines cannot be used as the primary determinant of whether there is a problem. However, numerical data are useful under defined circumstances.

Information from a large data set obtained by experienced individuals using the same instrument has practical value. Investigations of more than 50 federal government buildings over several years has resulted in the creation of such a data set. Fungal data from about 600 samples taken between 1986 and 1991 with a Reuter centrifugal sampler with a 4-minute sampling time have been used to prepare the interpretation notes shown below. Data acquired with other samplers require similar analysis. However, if a 4-minute sampling time is used, the numerical data from any proprietary sampler will probably be comparable.

- Significant numbers of certain pathogenic fungi should not be present in indoor air (e.g., *Aspergillus fumigatus, Histoplasma,* and *Cryptococcus*). Bird or bat droppings in air intakes, ducts or rooms should be assumed to contain these pathogens. Action should be taken accordingly. Some of these species cannot be measured by air sampling techniques.

- The persistent presence of significant numbers of toxigenic fungi (e.g., *Stachybotrys atra, toxigenic Aspergillus*, *Penicillium,* and *Fusarium* species) indicates that further investigation and action should be taken accordingly.

- The confirmed presence of one or more fungal species occurring as a significant percentage of a sample in indoor air samples and not similarly present in concurrent outdoor samples is evidence of a fungal amplifier.

- The “normal” air mycoflora is qualitatively similar and quantitatively lower than that of outdoor air. In federal government buildings, the 3-year average has been approximately 40 CFU/m^3 for *Cladosporium*, *Alternaria,* and non-sporulating basidiomycetes.

- More than 50 CFU/m^3 may be reason for concern if there is only one species other than *Cladosporium* or *Alternaria* present. Further investigation is necessary.

- Up to 150 CFU/m^3 is acceptable if there is a mixture of species reflective of the outdoor air spores. Higher counts suggest dirty air filters or other problems.

- Up to 500 CFU/m^3 is acceptable in summer if the species present are primarily *Cladosporium* or other tree and leaf fungi. Values higher than this may indicate failure of the filters or contamination in the building.

- The significant presence of fungi in humidifiers and diffuser ducts and on mouldy ceiling tiles and other surfaces requires investigation and remedial action regardless of the airborne spore load.

- There are certain kinds of fungal contamination not readily detectable by the methods discussed in this report. If unexplained SBS [sick building syndrome] symptoms persist, consideration should be given to collecting dust samples with a vacuum cleaner and having them analysed for fungal species.

APPENDIX C

Standard Operating Procedure:
An Example for the Investigation and Remediation of Indoor Air Quality Contamination by Mycological Agents

Standard Operating Procedure:
An Example for the Investigation and Remediation of
Indoor Air Quality Contamination by Mycological Agents

Any procedures involving direct handling and manipulating of potentially contaminated materials should be assigned only to trained personnel as required by WHMIS [Workplace Hazardous Materials Information System] and the provincial Occupational Health and Safety Acts. These individuals must have received information on the possible hazards as well as effective strategies to protect building occupants and themselves. They must also be reminded to wear appropriate personal protective equipment prior to entering contaminated areas. In small scale operations, such as 0.3 square metres or less, gloves and masks with good fit and proper seal may be used. The affected material should be decontaminated prior to removal. For intermediate scale operations such as 3 square metres or less, gloves and half-face respirators are advised. It should be noted that half-face respirators and gloves are strongly recommended for remediation at any scale if the presence of toxigenic fungi is known or suspected. Procedures that involve large scale operations, such as 10 square metres, will result in the disturbance of contaminated materials. Redistribution of these contaminants by the HVAC (heating, ventilation, and air conditioning) system could also follow. Under such circumstances, the area must be physically isolated (especially with respect to ventilation) by sheets of plastic and taping. Engineering controls such as a negative pressure air unit equipped with a HEPA (high-efficiency particulate air) filter and vacuum cleaners with HEPA filters are required especially when remediation is on a large scale. Evacuation of building occupants during such procedures must be considered. A full disposable suit with head to toe coverage, including a full face respirator equipped with HEPA cartridges, is acceptable. It is important to note that a chlorine removal cartridge is required with the use of high concentrations of bleach. When dealing with highly toxic materials, extra precautions must be exercised during removal of the personal protective equipment to avoid exposure. All equipment and other surfaces (such as the floor, ceiling, or the plastic drapes) within the segregated areas must, prior to removal, be surface decontaminated by treating with a 10% solution of household bleach (laundry or household bleach contains approximately 5.25% available chlorine before dilution) with an optional addition of 0.1-0.7% non-ionic detergent for an enhancement of cleansing and penetrating actions. Contaminated materials should be double bagged and tied for disposal by incineration. A thorough cleaning and decontamination of the areas immediately adjacent are also recommended. Sampling attachments and tools must be decontaminated between each assay in order to avoid cross contamination of samples, rooms and/or buildings.

If there is a necessity, during the investigative stage, to transport a sample of the suspected contaminant to another location for diagnostic or consultational purposes, it must first be rendered innocuous. Alternatively, when one is not certain that the sample is

dangerous, it should be packaged and transported as a diagnostic specimen under the TDG [Transportation of Dangerous Goods] Act and Regulations. However, if the sample has been identified as dangerous to human health and safety, then it must be packaged and transported by a TDG certified individual under the classification of 6.2.

Reference

New York City Department of Health, New York City Human Resources Administration, and Mount Sinai–Irving J. Selikoff Occupational Health Clinical Center. Guidelines on assessment and remediation of *Stachybotrys atra* in indoor environments. New York, NY (1993).

APPENDIX D

Sampling Methodology for Fungal Bioaerosols and Amplifiers in Cases of Suspected Indoor Mould Proliferation

Sampling Methodology for Fungal Bioaerosols and Amplifiers in Cases of Suspected Indoor Mould Proliferation

Problems attributed to moulds in indoor air are usually associated with on-site proliferation of the moulds in the affected building. Only occasionally are mould conidia from an outdoor source suspected of being potential sources of symptoms. Moulds proliferating within a building usually do so in discrete locations where moisture and substrate conditions are conducive. Typical sites of indoor mould proliferation are damp cellulosic materials (e.g., wallboard, wallpaper, carpet backing, damp papers); debris in ventilation ducts, in carpets, or in mattresses or upholstered furniture; poorly maintained humidifiers; insulation on which organic film has accumulated; constantly humid painted, caulked, or plastic surfaces (e.g., windowsills, shower stalls, cold air return vents); and potted plant soils. The ultimate goals of diagnostic indoor air mould studies are:

- to determine if sufficient mould propagules, particularly those bearing irritating or immunosensitizing chemical components, are being produced and dispersed within the building to account for (or predict) symptomatology; and

- if a connection between moulds and symptoms is likely, to find and eliminate sites of mould amplification within the building.

These goals are usually addressed through one or both of the following strategies:

- air sampling (or sometimes dust sampling) to determine the level and types of viable fungal propagules within building trouble spots, and to suggest the presence of significant indoor mould growth sites ("amplifiers"); and

- physical search of likely problem areas to detect conspicuous mould amplifiers.

Further details of these two strategies are outlined below.

D.1 Sampling for Fungal Bioaerosols

D.1.1 Major Types of Mycological Air Sampling Techniques

Air sampling for fungal structures is, at the most fundamental level, divided into techniques based on the culture of live propagules and techniques based on the trapping and visualization of living or dead materials. This appendix deals primarily with the former type of sampling methodology. This is because many situations of suspected indoor air contamination involve toxigenic, allergenic fungal genera with small, nondescript conidia, such as *Penicillium* and *Aspergillus*. These are often difficult to assess accurately with particle-trapping devices such as Rotorod samplers and spore traps, where culturing cannot be done and analysis of samples tends to be biased towards the identification of larger, distinctively shaped, and/or dark-pigmented structures. Common fungi possessing such large

or dark propagules, such as *Cladosporium*, *Alternaria*, *Pithomyces*, and *Bipolaris*, are often relatively innocuous fungi, predominantly coming from outdoor sources. Other conspicuous allergens, such as basidiospores of the bracket fungus *Ganoderma applanatum* and ustilaginaceous smut teliospores, may also be counted with unparalleled accuracy, but these are once again from outdoor sources and have little relevance to the major questions assessed by the Working Group. It should be noted, however, that Kozak et al. found a Rotorod sampler to be very useful for visualizing non-viable *Stachybotrys*, *Ulocladium*, and *Alternaria* conidia emanating from contaminated carpets in homes.[1] Although these authors actually located and identified the problem moulds by a combination of inquiries about water damage, site inspection, and direct sampling from suspect surfaces, they felt that the Rotorod could be an important component of a detailed evaluation. They recommended its use in combination with an Andersen sampler for viable propagules, plus rigorous site examination, history taking, and direct sampling.

The air sampling techniques elucidating viable propagules can be grouped into two categories: those relying on gravity to effect sedimentation of the mould propagules onto growth medium, and those based on pumping a measured amount of air onto or through a propagule collecting device. The sedimentation plate is archetypical of the former category, whereas the latter category contains a variety of sampling devices. The former will be dealt with first.

D.1.2 Settle (Sedimentation) Plates

A large number of publications substantiate the fact that settle plates elucidate a biased sample of the viable airborne mould propagules.[2] The reasons for this are twofold. First, propagules have differential settling rates according to their weight and aerodynamic form. Settle plates are particularly efficient at detecting large conidia in indoor air, whereas the proportion of conidia belonging to important small-spored genera such as *Aspergillus* and *Penicillium* is underestimated. Second, whereas pumping samplers cause some air disturbance, settle plates are still. Some disturbance is usually necessary in air sampling to resuspend settled conidia, which would ordinarily become airborne under conditions of normal human activity in the rooms being investigated.[3] Actual normal human activity substantially improves fungal isolation, even where pump samplers are used.

Despite these limitations, settle plates are still widely used, at least in preliminary studies, in remote or impoverished areas, or as an adjunct to physical searching for amplifiers. As semi-quantitative samplers, when adequately exposed (a commonly used protocol is for one hour at tabletop level under conditions of ordinary room activity),[4] they can readily be used to discern the likely presence of significant indoor mould amplifiers (except in special cases, e.g., *Stachybotrys* amplifiers). A problematic indoor mould amplifier, if it consists of small-spored fungi such as *Aspergillus* or *Penicillium*, produces a sufficiently large quantity of airborne propagules that these species show up as a significant proportion of the isolates occurring on a settle plate. The gravitational bias against these smaller conidia is compensated for by the high numbers of conidia produced by any significant amplifier (subject to normal disturbance).

Some specific sampling deficiencies have been attributed to settle plates by researchers investigating parameters not relevant to the detection of significant indoor amplifiers. The strength of this technique lies in the fact that epidemiologically important toxigenic or allergenic fungi, unlike invasively pathogenic fungi, must be present in large quantities. The quantitative species distribution of fungi growing in any habitat tends to have an inverse exponential distribution, in which there are very large numbers of individuals representing a few predominant taxa and very small numbers of individuals representing each of an indefinitely large number of minor taxa.[5,6] Hence, to point out that settle plates tend to grow fewer taxa than impacted air plates[2] or that they detect members of a particular fungal group in fewer sites than do impacted air samplers[7] is not necessarily of practical significance. With adequate exposure times, especially under conditions of typically low air turbulence indoors, only members of the asymptotic "tail" of minor taxa are strongly likely to be missed in any given habitat. The best general definition of an adequate exposure time is that necessary to obtain an adequate representation of the smallest spore type of practical interest. In indoor studies, this spore type is often the *Aspergillus/Penicillium* conidium; the present author finds that the majority of one-hour, indoor settle plates he receives from putatively mould-affected buildings have members of these taxa as predominant species. This ineluctable but anecdotal finding needs to be followed up by more definitive comparative studies.

Published comparative studies between volumetric and gravitational techniques often have inadequacies. Sayer et al. compared 15-minute samples taken by means of a vacuum sampler drawing 28.3 L of air per minute with gravity plates exposed for an entirely inadequate, and only desultorily parallel, 15 minutes.[2] Solomon, in a better-designed study, found that 30-minute settle plate samples showed very little correlation with 1- to 10-minute Andersen volumetric samples, and that numerically predominant, small-spored taxa were sometimes missed or very poorly represented.[8] Verhoeff et al., however, found a strong correlation between Andersen samples and duplicate 60-minute settle plate samples in two different studies.[4,9] The earlier of these two studies[9] showed that the number of species isolated on settle plates on conventional high water activity medium (malt extract agar) was not significantly different from that obtained with four major types of volumetric air samplers. The later study[4] showed that settle plates isolated significantly fewer species than the Andersen sampler but did not comment on whether these species were relatively abundant or uncommon. No study to date has deviated from the prevailing focus on abundance and commented on the extent to which different types of samples allowed the recognition of synecological patterns signifying the presence and types of indoor fungal amplifiers. Such patterns (e.g., *Penicillium brevicompactum* and *Aspergillus versicolor* usually signifying moist but not currently saturated wall covering paper) can be seen even in a relatively light deposition of smaller-spored types on a settle plate (or in a light outgrowth of low-viability spore types in either gravitational or volumetric sampling) and are sufficient to direct further on-site investigation. Either a heavy or a light deposition of such a pattern indicates the likely presence of a larger or smaller, closer or more distant, exposed or more concealed, but in any case undesirable mould proliferation site. Notwithstanding the serviceability of longer settle plate exposures in detecting these patterns, however, settle plates are best used in combination with a thorough initial site inspection to detect any macroscopically visible

mould growth. (Because of low-viability propagule types like *Stachybotrys*, the same caution holds for volumetric samples.) Volumetric air sampling should be regarded as superior and used whenever it can be made available.

In Canada, the species predominant in indoor mould amplifiers ordinarily form a small or insignificant proportion of spora in outdoor air samples (except near large compost sites such as municipal leaf dumps or where abundant dust from stored crops or wood is encountered). Common examples of fungi strongly associated with indoor proliferation are *Aspergillus versicolor*, *A. fumigatus*, *A. niger*, members of *Penicillium* subgenus *Penicillium* (with a few exceptions), and black-spored *Scopulariopsis* species. Receipt of settle plates predominantly colonized by significant numbers of such fungi is an excellent indicator of potentially problematic indoor mould amplification. Accompanying outdoor air control plates, exposed sufficiently far away from the building studied to avoid outflow of building bioaerosols, are strongly recommended: they are characteristically negative for these fungi.

False negative or ambiguous settle plates may be obtained from buildings with very restricted mould amplifiers, with very still air in undisturbed rooms, with amplifiers of poorly culturable species (e.g., *Stachybotrys chartarum* [= *atra*]), or with amplifiers consisting of species with poor airborne dissemination (e.g., *Aureobasidium* on windowsills, *Cladosporium* on painted cold air vents, *Fusarium* and many other wet-spored fungi from indoor plants, and possibly *Chaetomium*). The health effects of the species with low aerial dispersal have been suggested to be insignificant,[10] but, as intermittent or cumulative airborne dispersal of desiccated material may occur, some wet-spored species may be quite significant. *Stachybotrys* is an example of a wet-spored fungus for which significant airborne dissemination and health effects are well substantiated. Also, noxious volatiles may be produced by some wet-spored fungi. Little is known, however, about the health effects of the volatiles of wet-spored indoor fungi; many such species are not odoriferous to ordinary olfaction.

With settle plates, as with any other culturing of airborne moulds, the most informative level of interpretation usually requires that the analyst be able to distinguish among the predominant species and species-groups of the genus *Penicillium*, *Aspergillus*, and other relatively complex fungal groups. Also, if outdoor air controls are inadequate, the analyst must be sufficiently familiar with the local ecology of moulds and yeasts to detect deviations from their ordinary seasonal frequency in outdoor air. In Toronto, for example, a high number of *Penicillium* subgenus *Aspergilloides* colonies on a household settle plate in winter is an excellent indicator of mould proliferation indoors; the same finding in September might be non-diagnostic. Such interpretation of uncontrolled samples requires a mycologist with some aerobiological baseline data.

As meaningful analysis of settle plates is based primarily on recognizing the synecological assemblage of isolates consistent with the presence of indoor mould amplifiers and is only secondarily concerned with the actual numbers of colonies detected, the problem of establishing acceptable and unacceptable numbers of colonies in indoor samples cannot be addressed. Any actions taken against indoor mould proliferation must therefore be triggered by factors other than the demonstration of a threshold count. Locating and examining any mould amplifiers not detected in preliminary inspection are logical follow-up steps once settle plates have revealed that these amplifiers are present. The substrate nature of the amplifier

can usually be read from the settle plate. For example, *Stachybotrys* indicates very moist cellulose, often in previously flooded or soaked material, not uncommon in sheltered areas behind wallpaper or wallboard paper growing in contact with the glue. *Penicillium chrysogenum* suggests crumbs; *Eurotium* suggests, among other things, carpets with accumulations of dry skin scales and dust; *Aspergillus versicolor* suggests humid wallboard or other humid cellulose, including cellulosic dust within ducts; and so on.

In practice, common indications for characterization and remediation of the discovered amplifiers are occurrence of symptoms consistent with adverse reaction to indoor moulds and/or building management or administrative concerns that such amplifiers might cause symptoms in future, might indicate or exacerbate degradation of materials, or might cause offence owing to noxious odours or to the cosmetic, aesthetic, psychological, or political disadvantages of harbouring conspicuous decay. Once established mould amplifiers have been demonstrated, managers usually find themselves under strong pressure to clean them up.

D.1.3 Vacuum/Culture (Pump) Samplers

Pump samplers for viable propagules can be broken down into (1) samplers impacting a stream of air onto a fungal medium surface; (2) samplers trapping propagules from an airstream in a viscous fluid, which can then be plated on growth medium; and (3) samplers trapping propagules on a membrane filter, which can be eluted onto growth medium. In category #1 are slit samplers such as the New Brunswick slit-to-agar sampler, sieve impactors such as the Andersen and SAS samplers, and centrifugal impactors such as the RCS (details of sampling with these devices are outlined by Muilenberg; also, see Glossary for information about Andersen, RCS, and slit-to-agar samplers; see Verhoeff et al. for a chart showing the air intake rates, usual sampling times, and particle size biases of volumetric and non-volumetric sampling devices and techniques).[9,11] In category #2 are liquid impingers and modifications of slit samplers to deposit propagules in easily melted glycerol/gelatin gels.[12] In category #3 are various assemblages of pumps and filter cassettes drawing measured quantities of air through membrane filters impervious to fungal conidia.

A considerable literature exists comparing the efficacy of these samplers. Indeed, until recently, such studies have greatly predominated over other kinds of studies that might have predictive value in the analysis of indoor mould problems — for example, studies of the biological effects of exposure to mould conidia, or studies of the composition of fungal communities associated with indoor proliferation and related symptoms. Even though much useful information has been gathered by the analysis of sampling devices and accurate sampling is important, an overemphasis on this essentially non-biological topic is deleterious. At present, even if propagule concentrations in room atmospheres were known with absolute accuracy, we would be little further ahead in understanding the association (if any) between these numbers and symptoms, and we would not be assisted in the location or remediation of mould proliferation sites. In any case, correlative statistics should allow any moderately imperfect but reasonably consistent sampler to yield numbers that could be meaningfully gauged against symptoms, toxin levels, and a variety of related topics. These numbers must be broken down by fungal group, not given as total colony-forming units (CFU), as the

totality of spora includes varying proportions of potentially irritating and relatively benign particles. (Imagine, as a comparison, trying to gauge the chance of acquiring malaria simply by counting "total mosquitoes" in any habitat.)

Several recent studies have been performed comparing the sampling efficiencies of different pump samplers. Most of these studies have embodied some uncontrolled variables: for example, some fail to standardize the sampling durations and volumes, and many generate data by sampling in unpredictably non-homogeneous room air. They must therefore be interpreted with considerable caution, and those interested in this topic are advised to do a more detailed review than can be accomplished here. A few recent studies are worthy of mention, but the conclusions mentioned below should not necessarily be taken at face value. Buttner and Stetzenbach analysed the efficiencies of Andersen six-stage, SAS, and Burkard (suction slit impactor for direct examination of particles) samplers and settle plates in a controlled room with known concentrations of *Penicillium chrysogenum* conidia.[3] The Andersen sampler gave the most accurate and consistent results, but differences between it and the other volumetric samplers were marginal. Verhoeff et al. did comparative field trials in houses with the slit-to-agar, single-stage (N6) Andersen, RCS, and SAS samplers.[9] The slit-to-agar sampler and the Andersen were concluded to be the most precise. This study, however, was criticized[13] because it did not take into account the variation attributable to air mass discontinuity over the different sampling times used. (Total volume of air sampled was standardized at the expense of varying sampling duration.) Smid et al. similarly compared the single-stage Andersen, slit, RCS, and SAS samplers.[14] Once again, the Andersen and slit samplers were reported to give the best results, with RCS reasonably comparable; SAS underestimated CFU counts by about 50%. These authors concluded that the RCS remained useful because of its convenience of use and acceptable accuracy.

In heavily contaminated environments (e.g., barns), Andersen samplers may suffer from overexposure, with multiple propagules being counted as one after impaction via the same sieve hole and with subsequent colony overgrowth. Correction factors have been published for moderately overexposed plates but are inadequate for heavily overexposed plates. Diminishing the sampling time is a possible response but has the disadvantage that spatial/temporal discontinuities in airborne mould propagule distribution may skew results. For example, a 30-second exposure may fortuitously sample a current of relatively clean air from a window draft not generally representative of a contaminated room; or, likewise, a short exposure may sample the peak of a burst of conidia from a disturbed mould amplifier or reservoir and may show numbers well above those typically found in the room. For this reason, devices trapping propagules in liquid may be more accurate in heavily contaminated environments. With such samplers, sampling times can be longer without overwhelming the analytical capabilities of the system. Thorne et al. found that both impinger samples and eluted Nucleopore filters were more accurate than Andersen samples in barns housing swine.[15] Blomquist et al. modified a slit sampler to deposit spores on agar or glycerol/gelatin gels and then homogenized or liquefied these gels and plated them out in a classic dilution series.[12] When glycerol/gelatin gels were used, results were comparable to those obtained using eluted Nucleopore filters.

Impingers have not been widely accepted in ordinary indoor mould sampling work. Most potentially problematic airborne moulds have highly water-repellent conidia that, in contact with aqueous media, tend to adhere to surface films and hydrophobic surfaces and to clump together in minute air pockets. Trapping of such hydrophobic particles in impingers is not efficient.[11] A comparative study of Andersen and impinger samplers from a hospital under renovation showed that impingers underestimated CFU by 90% or more (R.C. Summerbell, Ontario Ministry of Health, pers. commun.).

In conclusion, for public buildings, various slit, sieve, and centrifugal samplers should give comparable results. Absolute propagule count is not a realistic sole criterion for building remediation, as large counts from outdoor air are possible at some times of year, particularly in buildings with openable windows or with air filters that do not exclude smaller fungal conidia. The most efficient use of samplers, arguably, should be to detect conidial shedding by indoor mould amplifiers. Although such shedding may very well result in high CFU counts, and although high counts in general will be more significant than low counts, certain factors may cause a truly problematic amplifier to yield low to moderate counts — for example, sampling at a distance from the amplifier, misleading air distribution patterns, and low conidial viability. Pasanen et al. found that viable spore counts were sometimes less than 25% of the total spores detected by scanning electron microscopy in farm and urban homes.[16] Other difficulties are outlined by Miller.[17] Such information strongly argues on behalf of using air sampling as a detector of amplifiers and a semi-quantitative indicator of approximate bioaerosol density rather than as an absolute arbiter of indoor air standards.

The relevance of spora counts is greatest where there is a diffuse and difficult-to-access amplifier present in a building. Two recurrent examples are mould growth in ventilation ducts in buildings with self-contained air recirculation systems and moulds apparently associated with a multiplicity of lightly and sporadically contaminated books in a library. In these cases, the idea of finding a discrete amplifier and eliminating it, the practical solution for the great majority of indoor mould problems, may be problematic. Although heavily contaminated ducts or books must clearly be cleaned up or otherwise dealt with (as must ducts or books with confirmed *Stachybotrys* colonization), the possibility of lightly contaminated environments is evident. The traditional question of determining an air contamination level requiring action is relevant in these instances.

Clearly, it simplifies matters to restrict the analysis only to those fungi associated with the amplifiers and to exclude fungi known to be associated with any incident outdoor air. (It is unlikely that indoor and outdoor types will have an additive effect, as their toxin chemistry and antigenicity will be largely distinct; the possibility of additive glucan effects needs further investigation.) The difficulty is to know what factor to correlate numbers of indoor-generated mould propagules with in order to assign health significance to the findings. Essential dose–response information needed to correlate numbers of fungal propagules of particular chemical composition to health effects in humans is absent for all moulds, and, as tolerance to moulds appears to vary biologically among individuals and appears to relate at least partially to the vagaries of allergic sensitization, acceptable dose information would doubtless be arduous to acquire even if ethical tests could be devised. Surrogate tests such as in vitro tests for the responses of human cells (e.g., alveolar macrophages) are in their infancy, and animals lack the ability to corroborate or disconfirm the persistent, subjective

symptoms commonly reported in cases of indoor mould proliferation. The need for objective measures of adverse responses to mould inhalation is great, and devising such measures would be an important step in coming up with scientific correlates between spore counts and the need for remediation of buildings.

This appendix will not discuss direct air or dust sampling methods for fungal biochemicals. Methods detecting general fungal materials such as chitin, glucan, and ergosterol lack the ability to discriminate between fungal elements from indoor and outdoor sources. Hence, they will tend to give unambiguously interpretable single-case results (as opposed to multi-case statistical trends) only in cases where there is an extreme indoor build-up or where indoor accumulation of outdoor fungal material is otherwise known to be insignificant. Tests detecting specific toxins or volatiles may be very useful, but in their specificity they are beyond the scope of this document. See Miller for a summary of some limitations of sampling for volatiles.[17]

Dust, carpet, or surface swab samples may serve in place of air samples but may contain many normally settled elements (e.g., Mucorales; also *Fusarium* other than predominant species on local agricultural crops), which in many cases are not significantly present in the air. On the other hand, dust samples have the advantage of containing a relatively long-term record of the history of fungal deposition within a building and may thus relieve investigators of problems posed by the bioaerosol variability of different air currents seen in short-duration air samples. Further investigation is needed to give criteria for the interpretation of dust sample results, but results to date show some promise. Swabs may play a useful role in the sampling of patches of mould growth that have been detected visually, but they are inferior to surface scrapings, as they tend to select spores and leave conidiophores/pycnidia/ascomata behind.

D.1.4 Media Used in Sampling

The media used in sampling fungal air and dust spora are diverse. They fall into several distinct categories: generally permissive media of high water activity, designed to allow growth and in situ identification of a wide range of fungi (e.g., Sabouraud, 2% malt extract agar, V-8 agar); generally permissive media with components restricting colony diameter (“restrictive media”), minimizing colony overgrowth and allowing in situ identification of at least some fungi (e.g., rose bengal agar, various high water activity media containing dichloran, Littman oxgall agar); media of low water activity, with or without factors restrictive of colony diameter, for isolation of moderately osmotolerant to xerophilic fungi (e.g., dichloran 18% glycerol agar, Czapek's + 40% sucrose agar, 2% malt extract agar + 10% salt [“malt and salt” agar]); and media selective for particular groups of fungi (“selective media,” e.g., Sabouraud/cycloheximide medium for the majority of human pathogens, Onygenales, Herpotrichiellaceae, and Ophiostomatales; media with benomyl for Basidiomycetes, Zygomycetes, Endomycetes, *Pleospora/Cochliobolus* anamorphs, and Microascaceae). At least two apparently irreconcilable dichotomies must be addressed by the person trying to select a single medium for an indoor fungal study: first, that no one medium will optimize growth of both the significant fungi adapted to high substrate water activity (e.g., *Stachybotrys*) and the significant fungi requiring lowered water activity

(e.g., *Eurotium, Wallemia*); and second, that the best media for identifying organisms in situ are also the most problematical for colony overgrowth and formation of spurious satellite colonies in shipping and handling.

Currently, the two most widely used media for general sampling are malt extract agar (MEA) and dichloran 18% glycerol agar (DG18). The former was recommended by the American Conference of Governmental Industrial Hygienists (Burge et al.),[18] while the latter has been shown to be useful in a variety of recent studies (e.g., Verhoeff et al.).[9] The limitations of MEA are that it allows extensive colony overgrowth and supports osmophilic fungi poorly; DG18 supports osmophiles well and facilitates growth of the moderately osmotolerant fungi that form the majority of indoor species of concern (*Penicillium*, *Aspergillus*), but it causes poor growth in moderately osmointolerant fungi such as *Scopulariopsis* (van Reenen-Hoekstra et al.)[19] and may support *Stachybotrys* and other highly osmointolerant species poorly or not at all (R.A. Samson, pers. commun.). A near-ideal sampling protocol might include both media. "Malt and salt" agar may be a good alternative for DG18: long used for isolation of osmotolerant fungi, it also allows growth of *Stachybotrys chartarum* (J.D. Miller, pers. commun.). The colonies of this fungus are restricted but are readily seen.

In general, workers who, for practical reasons, prefer to use a single medium must be mindful of the types of fungi they will be excluding from their data.

Because most indoor environments contain a variety of osmophilic *Aspergillus* species, DG18 often tends to isolate the greatest number of species in comparison trials (e.g., Verhoeff et al.)[9]; if a single medium must be chosen, DG18 may be optimal, but it should not be used alone except in combination with thorough visual and microscopic visual search to detect excluded fungal types, especially *Stachybotrys*. Such physical searching is recommended for *Stachybotrys* in any case, since *Stachybotrys* may be predominantly represented in the environment by non-viable conidia. Despite this, *Stachybotrys* is not uncommonly obtained in air samples, and any investigator wishing to maximize the likelihood of detecting significant *Stachybotrys* amplifiers is obliged to consider the use of air sampling with an appropriate medium.

The present author uses Littman oxgall agar extensively, primarily because of its tendency to repress sporulation and prevent satellite colony formation and colony overgrowth during shipping and handling in transit from test sites to the laboratory. It grows *Stachybotrys* well, has been observed to grow *Eurotium* (*Aspergillus glaucus* and allies) in high numbers in at least some cases (R.C. Summerbell, unpublished data), grows *Wallemia* occasionally (but probably not in a good representation of its true population predominance), and does not grow *Aspergillus restrictus* and allies. In its only formal comparison test as an indoor mould sampling medium, it showed significantly fewer colonies than three other media, including Sabouraud agar, at the sixth day of incubation (Morring et al.).[20] The authors conceded, however, that this time period was too short for a full evaluation. Littman showed that the eponymous medium outperformed Sabouraud agar over longer incubation periods.[21] Littman oxgall agar, however convenient it may be for shipping, requires much labour, since the majority of colony types must be subcultured for identification. Further, they must be subcultured soon after plates are received since, as nonsporulating colonies, they may become non-viable within 2–3 weeks. This medium would therefore be a

suboptimal choice for anyone doing his/her own sampling and mycology or receiving plates or strips within a day or two of exposure.

Rose bengal agar or its dichloran-supplemented variant are also restrictive of overgrowth and, while delaying or repressing sporulation to a lesser extent than Littman oxgall agar, may be relatively robust in shipping while permitting a relatively high level of in situ identification. It must be borne in mind that rose bengal generates high-energy oxygen species on exposure to light, and illuminated medium may become lethal to fungi. Dichloran rose bengal agar has grown significantly fewer colonies than other media in at least one study,[9] although this effect was not observed in others (e.g., Smid et al.).[14] Unfortunately, Verhoeff et al. did not record the time period allowed for incubation.[9] Restrictive media in general slow colony growth rates, and, for a fair biological (as opposed to purely practical) trial, such media should be observed only after sufficient incubation to ensure maximal colony outgrowth. An in-house trial showed that Littman oxgall agar gave colony numbers equivalent to those obtained with MEA in hospital renovation air samples incubated for 14 days (R.C. Summerbell, unpublished data).

The fact that all existing fungal sampling media have recognized shortcomings is a further blow against the former aerobiological ideal of using a perfected, standardized sampling device with a perfected, standardized growth medium to evaluate potential fungal aerosol problems with reference to standard guidelines for acceptable numbers of CFU. This ideal, which was always predicated on the reduction of all members of the three major terrestrial fungal phyla to a single, alchemical mass substance, clearly must yield to the reality of biological diversity. As more becomes known about the actual hazardous substances associated with airborne fungal materials, methods indicating the occurrence or likely occurrence of these substances will be developed. In the meantime, the investigator engaged in detecting potentially significant amplifiers must simply ensure that an adequate diversity of techniques is used to cover the diversity of possible amplifiers.

D.2 Direct Detection of Amplifiers

Procedures for the direct detection of mould amplifiers may be used either after an air sample has predicted the presence of amplifiers or as a preliminary survey. Common places where significant amplifiers can be visually identified are in water-damaged walls on or under wallpaper or wallboard paper (whether painted over or not), on the backings of water-damaged carpets, on heating, ventilating, and air conditioning (HVAC) coils, pans, vanes, and so on, on damp papers (e.g., after flood, including floods created by fire-fighting operations), within walk-in refrigerators and incubators, and in any moist organic materials, including any moist object composed of cellulose. Amplifiers may also be visible on windowsills, as well as shower stalls and washroom fixtures.[7] These windowsill and washroom amplifiers, if small and not involving cellulosic material (i.e., moulds growing only on paint, ceramic, grouting, or plastic), are seldom problematic. More extended amplifiers in these situations may be problematic, and even hypersensitivity pneumonitis, which normally requires long-term heavy exposure to develop, has on rare occasion been linked to heavy growth of fungi in shower or other washroom amplifiers.

Additional amplifiers may be detected by microscopic sampling. Common practices are the transparent tape sampling of duct interiors, slide examination of humidifier basin materials, and examination of small fibre samples cut or pulled away from filters or carpet backings in which mould growth is suspected. Further direct detection of amplifiers may be performed by culturing — for example, plating out of humidifier fluids and plating out of scrapings or swabs from recognized or suspected mould growth on walls or other surfaces.

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