Review of Methods Applicable to the Assessment of Mold Exposure to Children

H. Kenneth Dillon,† J. David Miller,‡ W.G. Sorenson,§ Jeroen Douwes,¶ and Robert R. Jacobs†

†Department of Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, Alabama USA; ‡Department of Chemistry, Carleton University, Ottawa, Ontario, Canada; §Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia USA; ¶Department of Environmental Sciences, Wageningen Agricultural University, Wageningen, The Netherlands

This article presents discussion of the assessment of the exposure of children to fungi, substances derived from fungi, and the environmental conditions that may lead to exposure. The principles driving investigations of fungal contamination and subsequent exposure are presented as well as guidelines for conducting these investigations. A comprehensive description of available research sampling and analysis techniques is also presented. Key words: (1→3)-β-D-glucans, children, ergosterol, exposure assessment, fungi, mold extracellular polysaccharides, mycotoxins, tricothecenes, water damage.

Respiratory diseases and their symptoms in children have been associated with dampness and the amplification of fungi in homes (1–5). Several investigators have found evidence supporting the possibility that airborne secondary metabolites produced by fungi, i.e., mycotoxins, and (1→3)-β-D-glucans, present in the cell wall of most filamentous fungi, may contribute to respiratory symptoms (6–9). Opportunistic fungal infections are rare in children but may occur in the immunocompromised (10–13).

The public health professional must be able to recognize and define hazards associated with fungal amplification in buildings, including the residential environment. Confounding these tasks, however, is the fact that the current body of knowledge is inadequate to identify hazards definitively. The lack of good quantitative methods to assess fungal exposure is one of the primary reasons that knowledge about fungal-related respiratory health risks is poor. Consensus has been reached by several professional groups on the following principles: fungal growth in homes or buildings is unacceptable, such growth should be removed and further contamination prevented, and the intrusion or accumulation of moisture in a home or building is the primary factor inducing fungal amplification (14–16). These principles stress the importance of the prevention or removal of the potential for exposure to fungi or agents derived from fungi.

Planning and Conducting Exposure Assessment

Guidance has been given for exposure assessment involving building-related illnesses and symptoms (i.e., sick building syndrome) (14,16,17). Many of the same concepts apply to the residential environment and to assessment of exposure of children to fungi and substances produced by fungi.

Defining the Objective

Prior to the planning or execution of exposure assessment, the objective should be formulated. The possibilities are numerous; a few examples are offered:

- To determine the precise nature and extent of proliferation and transport of fungal contaminants
- To judge the success of remedial and behavioral changes
- To establish the cause of diagnosed disease
- To link environmental conditions or activities with symptoms

The format of the objective may be a simple specific question, e.g., Are fungi growing in a home? The question may be much more complex, e.g., Does fungal contamination in the residential environment increase the severity and frequency of asthmatic attacks? The former question might be answered by visual evidence of growth. The latter would require an epidemiologic investigation including not only documentation of fungal contamination but also documentation of the following: the presence of fungal antigen(s) to which children have developed sensitivity, evidence of the presence of inflammatory agents that could aggravate asthma, evidence of a means by which exposure to the antigen(s) and inflammatory agents could occur, and documentation of the severity and frequency of attacks over the course of the study. An epidemiologic study requires a formal statement of a statistical hypothesis, e.g., the severity and frequency of attacks in children with asthma in homes contaminated with fungi are equal to the severity and frequency of attacks in children with asthma in homes that are not contaminated with fungi. It should be recognized, however, that disproving this hypothesis, and thereby demonstrating an association, does not prove causality.

Inspection of the Building or Residence

Inspection of the building or residence is required before a comprehensive exposure assessment strategy can be formulated and may, in fact, preclude the need for a more comprehensive investigation. A thorough inspection of a school, a daycare center, or residential housing for dampness, water leaks, and signs of past flooding is an important component of an estimation of fungal contamination of the building. An estimation of the area of visible mold is an important measure of the degree of exposure to mold. Inspections must be performed by people familiar with building science and residential construction. It should also be emphasized that occupant behavior is important. Storing of firewood inside, not venting dryers to the outside, not maintaining plumbing or heating, ventilation, and air/conditioning systems, and attaching greenhouses to the living space are examples of bad practices. Concomitant with the presence of moisture, poor housekeeping favors the cultivation of fungi (as

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The specific features of a building that lead to mold contamination vary according to country and even regions of countries with strong climate differences such as the United States. In the northeast, leaky basements, faulty window framing, and lack of ventilation are the key sources of water problems. More fundamental problems, such as inappropriate location of the vapor barrier in the wall cavity or lack of ventilation in attics or crawl spaces, can also be important reasons for mold problems. Lack of ventilation in closets and behind drawers is a frequent source of mold. In subtropical and desert areas of the United States, condensation in wall cavities or in wallboard is an important cause of mold. In the Pacific northwest and the southeastern U.S., water leaks from rain are important. In areas prone to flooding, failure to clean up properly afterwards can lead to problems.

Examination of total visible mold requires a powerful flashlight, a basic plan of the building to record the observations, a magnifying glass, and a means of testing whether signs of mold are actually mold. This can include materials for taking a sample and an eyeprober with bleach. The sample can be examined microbiologically or cultured. Stains, rather than mold, are sometimes decolorized upon application of bleach. The appearance of mold on an inside surface usually means that there is more mold behind the wall or ceiling. Destructive sampling, that is, the removal of wallboard, is often required to estimate the extent of contamination. Finally, on wallboard, mold growth sufficient to influence the air quality of the adjacent space extends for as much as 0.5–1 m beyond visible heavy mold (18).

The investigator(s) should pay attention to odors. The musty odors of microbial volatile organic compounds (MVOCs) may be an indication of hidden fungi. Chemical odors, e.g., volatiles from cleaning agents or pesticides, indicate the presence of compounds in the air that may contribute, along with fungi or fungal agents, to respiratory symptoms.

**Sampling Strategy**

Air, surface, and bulk materials sampling during inspection or at a later time may be justified. The visual observation of active or past microbial growth indicates the potential for exposure. The likelihood of exposure may be established by air sampling in the living spaces. Sampling may support a possible association between airborne fungal spores or substances derived from fungi and symptoms consistent with exposure. Air sampling may also help to locate hidden growth. Surface samples or bulk material samples, including settled dust, increase the likelihood that sources will be found. It is not recommended that aggressive air sampling for viable fungi or fungal agents be conducted. Aggressive sampling involves activities intended to encourage the generation of biologic aerosols during air sampling, e.g., the rapping of ventilation ducts. If it is possible without compromising the health of the building occupants, air sampling should be conducted during normal indoor activities.

Designing a sampling strategy involves deciding the agent(s) to be sampled, where and when to sample them, the number of samples, and the appropriate sampling and analysis techniques. An analysis laboratory should be selected prior to sampling and can often offer advice in the choice of the appropriate sampling methods. Laboratory selection criteria have been specified (14); the American Industrial Hygiene Association (AIHA) of Fairfax, Virginia, plans to accredit laboratories for the determination of environmental microbiologic samples. Sampling should be conducted in areas suspected of contamination, in areas not suspected of contamination, and outdoors near air intakes. Sampling outside should be conducted as high off the ground as possible to avoid the sampling of soil fungi.

The question of how many samples to take is complex, and recommendations should be interpreted with caution. Eudey et al. present a thorough discussion of hypothesis testing with samples involving biologic aerosols (19). In an epidemiologic investigation, one is often attempting to demonstrate the presence or augmentation of a physiologic effect in the test population of children that is not present or is diminished in the control population. Exposure assessment may be included in an epidemiologic study to search for associations or dose–response relationships that may explain why a particular effect is observed. The investigator can estimate sample size given some preliminary evidence of the expected effect size and the variability in measuring the outcome. Generally, the numbers of children in each of the test and control populations must be high (n > 30) if an effect has a reasonable chance of being detected. The numbers of air, surface, or bulk samples used to demonstrate an association between exposure and health effect are generally much higher than subject test and control population size because the environmental variability of exposures must also be considered.

Going beyond the demonstration of an association of an exposure variable with the outcome to establish causality requires a more rigorous approach. Hodgson has reviewed other criteria necessary to support causality (20):

- similar findings across multiple cohorts
- high odds ratio
- cause precedes the effect
- data from different kinds of studies converge
- fewer other causes for the syndrome
- dose–response relationships
- mechanistic considerations make the effect likely

At the present time, no target or threshold levels for fungi or substances derived from fungi have been sanctioned by professional organizations. When reasonable causality has been demonstrated between some fungal agent and disease and an exposure guide can be recommended, then exposure assessment may be conducted for prevention of disease or for the recognition of the established hazard. Guidance in comparing observed means to occupational health limits has been given (21). Time-integrated samples that may be used to characterize exposure levels are likely to approximate a lognormal distribution and to have statistical population variances (geometric standard deviations [GSDs]) that are comparable to those experienced in industrial hygiene studies, i.e., 1.5–3.5. For example, in a study of 60 homes in the Netherlands, counts of colony-forming units (CFU) in settled dust demonstrated GSDs ranging from 1.8 to 2.3 (22). Measures that are likely to be related to numbers of culturable propagules integrated over time, such as ergosterol or (1→3)-β-D-glucans as described below, might be expected to exhibit comparable variability. However, the actual environmental variability of most fungal agents has not been characterized definitively.

Practical recommendations for the number of samples required for a qualitative assessment of the biodiversity of fungi have been given (14). Samples should be taken in duplicate at each test and control location and at each sampling time. Comparative indoor and outdoor sampling should be conducted in the morning and in the afternoon. Such sampling should be separated by as much time as possible...
because outdoor levels are expected to change during the day and indoor levels will vary with work activity and changes in temperature. Some investigators take outdoor samples in the morning, at noon, and in the afternoon that bracket in time the samples taken indoors. For a contaminated indoor environment, a difference in rank order of species inside when compared to those outside is often obvious without statistical analysis. As stated in more detail in the section on culture methods, the ratio of the sum of the concentrations of soil fungi to the sum of the concentrations of phylloplane fungi should be near 1 if fungi are not being amplified indoors because it is primarily the soil fungi that grow on building materials. If many samples have been taken at many locations in a structure, cluster analysis may demonstrate differences in species distribution (16). The collection of many samples may also allow the determination of the frequency with which an organism is found in samples taken at different times and locations. Frequency of occurrence over various environmental conditions can be useful in identifying those conditions that lead to increased exposure.

**Description of Methods for Exposure Measurement**

Sampling methods should be consistent with the intended purpose of the investigation. Methods that are likely to be applicable to the determination of fungi and fungal agents in environments where children spend most of their time are summarized below. Primary emphasis is given to the description of air-sampling methods, but most of the analysis techniques presented can be used for determinations in bulk and surface samples. The methods have been selected according to their ability to provide measures or estimates of exposure in residential and school environments but not in agricultural or industrial environments where fungal contamination can be much heavier.

**Culturable Fungi**

Traditional methods for determining microbial contaminants involve culturing on nutrient agar. Air-sampling methods for bioaerosols involve impaction of culturable organisms onto nutrient agar or impingement of organisms into a suitable liquid medium for subsequent culture on agar. Multihole impactors (such as the Andersen cascade impactor, two-stage impactor, and the N6 single stage impactor (Andersen Instruments, Inc., Smyrna, GA) or the surface-to-air sampler (Pool Bioanalysis Italiana, Milan, Italy) and the Reuter centrifugal sampler (Biotest Diagnostics Corp., Denville, NJ) have been used extensively for the sampling and determination of airborne fungi. Details of their operation, advantages and disadvantages, and interpretation of results have been presented (14,16). Results are measured as CFUs, and individual colonies can be identified by experienced mycologists. Culturable fungi in surface swab samples and bulk materials can also be plated and identified. The high sensitivity of culture methods allows determinations of typically <10 CFU/m² in an air sample, 0.05 CFU/cm² for a 100-cm² surface area, and 5 CFU/g of settled dust or other bulk material.

The primary strength of culture techniques is the ability to identify fungal species. Although it requires extensive pure culture study, determination of fungal species is important in assessing risk because not all fungi pose the same potential hazard. For example, some xerophilic species such as *Eurotium* spp. are of health importance because they have been associated with hypersensitivity pneumonitis (29). Certain fungi, such as *Stachybotrys chartarum*, are of concern because they produce potent toxins. Others, such as *Aspergillus fumigatus*, are opportunistically pathogenic and may also have the potential to produce toxins. Most fungi, including *Alternaria* spp., are potentially allergenic because of their protein content.

Speciation is also important in the evaluation of homes or buildings involving the ecology of molds that grow indoors. In settled dust, normal buildings contain outdoor molds, including the phylloplane molds such as *Cladosporium* and *Alternaria* spp. and the spores of the dominant mushrooms (*basidiomycetes*) for the region. Much is known about the prevalence of the spores of these species outdoors (24). In July, spore burdens in outdoor air approach 20,000/m³. *Aspergillus/Penicillium* spp. (Asp/Pen) are present in outdoor air at a relatively constant low rate associated with windblown soil particles. The prevalence of Asp/Pen varies from 10% (of very low numbers) in the winter to <0.1% in the summer.

A relatively small number of species grow commonly in water-affected building materials. Fungi are restricted by water activity (available water) and to a lesser extent by substrate type (wood, wallboard, carpet, insulation, furnishings). *S. chartarum, Chaetomium globosum*, and *Memnoniella echinata* are associated with water-saturated building materials containing cellulose, most often the paper layers of wallboard. These organisms are highly competitive on these surfaces and are common after leaks and floods that take a long time to dry out. In North America, *S. chartarum, C. globosum, Trichoderma harzianum*, some *Penicillium* such as *Penicillium thomii*, *Penicillium decumbens*, and *Penicillium fellutanum*, as well as wood rot fungi occur in water-saturated wood including particleboard and plywood. Also in North America, wetted or damp wallboard is contaminated by salt-tolerant xerophilic molds such as *Aspergillus versicolor, Penicillium aurantiogriseum, Penicillium viridicatum, Penicillium brevicompactum*, and *Paeclomyces variotii*, depending on the amount of available water in the material. An additional 20 or so species of molds occur as important contaminants of housing. These may grow in carpets, insulation, materials, clothing, and shoes. Detection of these species in air samples can direct investigators to sources of hidden mold.

Several shortcomings of methods based on culturing, however, place limitations on their utility, especially limitations in the determination of CFU-count concentrations in air that are representative of time-weighted exposures. Such samples represent very short-term collections (<5 min). For several reasons, including that there can be order of magnitude variation in spore concentrations in minutes, the numerical values are of limited use (8). Different species of fungi have different growth requirements, so the use of any medium produces different recoveries. The spores of different species decline in viability with time; some spores remain viable for years and others for months. In general, the numbers of fungal propagules determined by culture are substantially less (1–50%) than those determined by methods that determine total propagule counts, but this varies between species. Finally, some species are very aggressive in culture and produce antifungal agents that affect the growth of others, e.g., *Trichoderma* species (25). A longer review of these issues can be found elsewhere (8).

To illustrate this point, Table 1 illustrates the putative response of placing 1,000 spores of *S. chartarum, 40 spores of P. aurantiogriseum, 20 spores of Wallemia sebi, and 40 spores of T. harzianum on three media: malt extract agar, corn meal
agar, and DG18 agar (14). From the 1,000 spores plated, 10 of S. chartarum grew on corn meal agar, the best medium of the three for this species, or 1% of the total spores plated. Recoveries on the medium for moderate xerophiles (DG18) were very low. In contrast, a high percentage of the moderate xerophile, P. aurantiogriseum spores were cultivatable on DG18, its optimum medium. For the xerophile W. sebi, good recoveries were obtained on DG18. Colonies of the hydrophilic, wood-soft rot species T. harzianum are difficult to count under ideal circumstances, and the organism was poorly recovered on DG18.

Table 2 illustrates the putative response of plating the mixture of species used to form the above example. S. chartarum recoveries, even in the optimal corn meal agar medium used, are much reduced because of the presence of the Trichoderma. W. sebi is not reported at all, and only the Penicillium species emerges unsathed.

These examples demonstrate the point that counting CFUs in air or dust samples overrepresents the long-lived tolerant Penicillium species. It also illustrates why the application of descriptive statistics to concentrations of CFU/m³ does not necessarily give useful information because each sample can be quite different from another and incomparable from a numerical perspective (8). Thus, CFU data either from air samples or from dilution plating of bulk samples may have little inherent quantitative value. Furthermore, a count of CFUs does not provide a measure of exposure to bioactive substances derived from microbes, including mycotoxins, glucans, or allergens. These substances are also present in nonculturable organisms, which generally outnumber cultivable organisms by orders of magnitude.

A similar phenomenon occurs when dilution plating bulk samples such as soil or building materials, especially wallboard, except there is an additional difficulty. It has long been understood in soil mycology that dilution plating does not give a reliable indication of the species active in the ecosystem. Kjoller and Struwe (26) have reviewed data from dilution-plating soil samples over about a 1-year period in comparison to measurements of myphal growth in the soil. Typically, they found no statistically significant correlation between the two such measures, the latter being a direct measure of fungal activity. Fully 85% of the cultures found by dilution plating are of species not active in the ecosystem (27).

On the other hand, the direct measurement and plating of bulk samples (soil crumb method) is the most useful nonbiochemical method of measuring fungal activity in samples. This technique minimizes emphasis on species that are inactive in the system. Using this technique, the analysis of settled dust samples for cultivable fungi may provide evidence of exposure integrated over time. In one study, measures of CFU in house dust were associated with greater surface area of visible mold growth and higher moisture source strength (2). Some investigators caution that measures of biocontamination in settled dust may not represent concentrations in air (28). Because settled dust contains nutrients, settled-out fungal spores may grow at high humidity (29).

Dilution plating has considerable value in determining the total diversity of species present in a sample, and there is some evidence that the data from many replicates produce data that are related to the absolute value (30). When species grown from air samples taken indoors are compared to the species grown from samples taken outdoors, a difference in rank order remains a meaningful indicator of biologic contamination within the building. It is generally true in North America that in any given sample taken indoors, the ratio of the sum of colonies of typical soil fungi, e.g., Aspergillus, Penicillium, and Eurotium spp., compared to the sum of phyloplane fungi, e.g., Alternaria, Cladosporium, and Epicoccum spp., provides evidence of amplification. In a building that is not contaminated, the ratio should be about 1.

A new study of these issues has been made by Miller et al. (18) in which the data from extensive air sampling inside and outside an apartment building were compared to studies of mold damage after massive destructive inspection to examine wall cavities. The CFU/m³ values between the two data sets were not significantly different using descriptive statistics. However, the proportion of samples that failed the AIHA guidelines for comparing rank order of species inside and outside was different (p < 0.005). When the data were compared to the area of visible mold revealed by the extensive destructive testing, there was no correlation with CFU/m³ values. The proportion of "AIHA fails" was fairly well correlated with measured mold area with p-values of 0.03–0.10, depending on the stringency of the test.

**Total Fungal Propagule Counts**

Fungal propagules collected on a filter or on a sticky surface such as that incorporated into the Air-O-Cel cassette (Zefon, Inc., St. Petersburg, FL) can be counted microscopically to yield a measure of total (culturable and nonculturable) fungal mass in an air sample. Propagules in a surface sample taken with sticky tape or in a bulk sample can also be counted microscopically. These techniques have been described in detail in other publications (14, 16, 31–33). Some fungi have distinctive spores, but many that grow indoors, e.g., Penicillium and Aspergillus spp., are small spherical spores with few distinctive features that can be viewed with a light microscope. A scanning electron microscope may increase qualitative information for some spore types, but its use is not practical for the routine identification of species. Fluorescent staining with dyes such as acidine orange have been useful, but some fungal spores will not absorb the dyes, and dark spores often mask the fluorescence. Immunofluorescent staining can, however, be used to distinguish propagules of specific species.

Sequential filter sampling (31) and moving-tape or slide samplers such as the Burkard Volumetric Spore Trap (Burkard

Table 1. Model data from plating four fungal taxa on agar: pure cultures.

| Malt extract agar | Corn meal agar | DG18 | Spores plated |
|-------------------|----------------|------|---------------|
| Stachybotrys chartarum | 1 | 10 | 1 | 1,000 |
| Penicillium aurantiogriseum | 10 | 20 | 25 | 40 |
| Wallemia sebi | 1 | 1 | 10 | 20 |
| Trichoderma harzianum | 10 | 10 | 1 | 40 |

Table 2. Model data from plating four fungal taxa on agar: mixture of four species.

| Malt extract agar | Corn meal agar | DG18 | Spores in mixture plated |
|-------------------|----------------|------|--------------------------|
| Stachybotrys chartarum | 5 | 5 | 0 | 1,000 |
| Penicillium aurantiogriseum | 20 | 10 | 20 | 40 |
| Wallemia sebi | 0 | 0 | 0 | 20 |
| Trichoderma harzianum | 10 | 10 | 1 | 40 |
burden in personal samples. However, (1→3)-β-D-glucan is not a specific marker of fungal mass and originates from a large variety of sources, including most fungi and yeasts, some bacteria, most higher plants, and many lower plants. Sensitive determinations of (1→3)-β-D-glucans with membrane filter sampling can be performed using the Limulus amebocyte lysate (LAL) technique with Factor G as the analysis technique (37). These glucans have variable molecular weight and degree of branching that may appear in various conformations, e.g., triple helix, single helix, and random coil structures of which the triple helix appears to be the preferred form in the environment. Environmental samples are denatured in alkaline solution (0.3 M NaOH) (37) or by hot water extraction (38) prior to determination. However, a standardized protocol for extraction and storage of environmental (1→3)-β-D-glucan samples does not yet exist. The method is highly sensitive; picogram quantities can be detected, allowing concentrations in the nanogram per cubic meter range to be detected. There is also a specific enzyme inhibition assay (EIA) for (1→3)-β-D-glucan measurements, although sensitivity is not as good as with the LAL method (38). The EIA technique has been used for the determination of (1→3)-β-D-glucans in settled dust in the home and in occupational environments.

Mold extracellular polysaccharides (EPS) also offer potential for the measurement of fungal mass and have been related to culturable fungi in settled house dust (39). Mold EPS are heat-stable and water-soluble nonbranched glycoproteins with variable molecular weight that are an essential part of the mycelial cell wall of practically all molds. During growth of molds these polysaccharides are released in the environment. Antibody responses against EPS from Aspergillus/Penicillium are directed to the galactomannans in EPS, of which β(1→5)-linked D-galactofuranoside residues present in the galactomannans are immunodominant (40). EPS usually have an antigenic specificity at the genus level, while EPS from Aspergillus and Penicillium spp. are cross-reactive. The quantity of antigens produced by molds is fairly related to the quantity of mycelium, and antigens are produced under almost all growth conditions (41). This demonstrates the potential usefulness of EPS as quantitative markers for mold biomass, not only in food products but also in the general and occupational environment. A highly specific sandwich EIA has been described by Kamphuis et al. (42) with which EPS of Asp/Pen can be measured. This method recently has been applied successfully in an indoor study (39). Three other sandwich EIAs have recently been developed for the specific detection of mold spp. from three other mold genera: Alternaria, Mucor, and Cladosporium. Application of these assays on environmental samples would thus allow partial classification of the mold genera present. The determination of EPS is an experimental method and has not yet been routinely applied in indoor studies.

Volatile organic compounds produced by fungi may also be suitable markers of visible or hidden fungal growth because the compounds may permeate porous walls in buildings (14). It has been suggested that these compounds, which have been termed fungal MVOCs, may also cause respiratory symptoms, but no supporting evidence has been offered (43). They are collected on a solid sorbent (Anasorb 747; SKC Inc., Eighty Four, PA), extracted with methylene chloride, and determined by GC–MS (14). The method is sensitive, allowing determination of a concentration of about 10 ng/m³ in 25-L air samples. About 15 MVOCs are emitted by fungi, although some are emitted by bacteria as well. 3-Methylfuran has been used as a measure of active fungal growth, 1-octene-3-ol as a measure of inactive growth, and geosmin as an indicator of either active or inactive growth.

Mycotoxins

Instrumental methods, particularly high-performance liquid chromatography (HPLC) and GC, and immunoassays exist that may be adapted to determinations of mycotoxins by collection on membrane filters in long-term air sampling, corresponding to air volumes greatly in excess of a cubic meter or to determinations in settled dust. However, many of these methods were developed specifically for the determination of mycotoxins in food stuffs, e.g., aflatoxins, that rarely amplify in indoor environments (44). Recent and ongoing research has provided methods for mycotoxins produced by fungi that grow in water-damaged buildings. For example, methods utilizing HPLC–thermospray mass spectrometric analysis and GC–negative ionization mass spectrometric analysis have been developed for the determination of macrocyclic tricothecenes (45,46). HPLC and thin-layer chromatographic methods have been developed for determinations of several mycotoxins produced by Aspergillus and Penicillium spp.,
including sterigmatocystin produced by *A. versicolor* (47).

Cytotoxicity assays based on application of membrane filter extracts to cell cultures have been useful in studies involving potential exposure to mycotoxins (48), and demonstrate adequate sensitivity for fixed-point air sampling or for determinations in settled dust. The method detects the presence of many cytoxins, including endotoxin. However, the lack of specificity does not preclude the use of the method as a screening tool.

A recently developed method employing filter sampling has demonstrated the ability to quantify the toxicity of tricotheccenes in personal samples using a sensitive *in vitro* protein translation assay (49). This technique may be the first to allow personal sampling that is a quantitative measure of the primary biologic effect of a mycoxin. The method is 400 times more sensitive than a cell culture-based cytotoxicity assay. Also, the protein translation assay is a more specific measure of toxicity because the primary effect of tricotheccenes is inhibition of protein synthesis. Initial field testing has indicated a strong correlation between mycotoxin activity and the presence of toxigenic fungi determined by culture methods.

**Fungal Allergens**

Only a few major fungal allergens can be measured in house dust (50). Few have been characterized, and allergen stability and production may be highly variable (51).

**Other Measures of Fungi**

The polymerase chain reaction (PCR) is a highly specific molecular biology technique that has been used more and more frequently in the diagnosis of mycotic infections, including aspergillosis, and in the identification of mold contaminants in grains and other foodstuffs (52–55). Methods useful for the determination of environmental fungi are under development (56). PCR techniques target a particular gene and are dependent on the availability of suitable probes. These techniques can be developed to be as specific as or as non-specific as desired, i.e., species-, genus-, or even group-specific. Semiquantitation is possible with the use of internal standards.

Lipid signature profiles have been characterized for several fungal species (57–59). The isolation and determination of lipids distinctive of other fungal species is under-way. This technique has the potential to allow the same selectivity in identification of fungi as has been afforded for bacteria by their lipid signatures.

**Measures of Other Respiratory Irritants and Disease Agents — Potential Confounding Factors**

In addition to the measurement of fungi as potential causes of respiratory disease, allergens from animals, insects, and dust mites should be measured. Also, attention should be paid to compounds that potentiate or otherwise attenuate the effects of allergens and toxins such as endotoxin; formaldehyde; ozone; carbon monoxide; sulfur dioxide; nitric oxide and nitrogen dioxide (NO₂); and tobacco smoke. High-water activities in building materials or standing water favor not only the growth of hydrophilic fungi, but also bacteria, including Gram-negative bacteria with its concomitant endotoxin, and peptidolycan, a cell wall component of all bacteria (36,60). Plywood, particleboard, and fibberboard may be significant sources of formaldehyde, a potential carcinogen, respiratory irritant, and allergen for some individuals. In the summer months especially, ozone formed in the ambient air from pollutant precursors may infiltrate a home or building. The use of ozone-generating devices, e.g., electrostatic dust collectors, may cause unhealthy concentrations inside the home and is discouraged. Natural gas space heaters, hot water heaters, and cooking stoves contribute carbon monoxide and NOₓ to indoor air and should be properly vented. Kerosene heaters emit these pollutants and also sulfur dioxide, all of which may accumulate indoors if not properly vented. Smoke from burning wood and tobacco smoke add significantly to the burden of fine aerosols (PM_{2.5}) in the residential environment.

**Discussion of Exposure Measurement Methods**

The most pressing need in the investigation of the impact of fungal contaminants on child health appears to be the establishment of causal relationships. To prove causality, reliable measures of exposures to candidate agents by individual children are required. Generating quantitative exposure information can be difficult, especially for children. Personal sampling is the most definitive technique to estimate exposure dose. Getting children to wear personal sampling equipment is problematic, but miniaturization and computerization have produced a new generation of pumps that are less burdensome and more rugged (61). Lack of analytical sensitivity precludes personal sampling for many fungal agents, but a few methods among the methods discussed earlier will allow the determination of average concentrations over periods representative of many exposure situations (1–24 hr). These methods include the LAL method with Factor G for (1→3)-β-D-glucans (37), the determination of MVOCs (14); and the protein translational assay for tricotheccenes (49). However, a standardized protocol for the use of the LAL method to determine (1→3)-β-D-glucans has not been established, and the other methods have not yet received wide usage in epidemiologic investigations.

Fixed-point air sampling in areas where children are active can confirm the potential for exposure. With careful preliminary observations, sampling locations can be selected that may represent worst-case exposures; however, fixed-point sampling does not provide a true measure of exposure. These methods generally involve relatively high sampling rates with membrane filters, e.g., 2–20 L/min for extended times such as > 24 hr. The following examples are given, but the methods presented for personal sampling are also applicable: determination of markers of exposure to fungi, including ergosterol, mold EPS; enumeration of fungal propagules with microscopic techniques; determination of mycotoxins by HPLC; and cytotoxicity screening of collected aerosols. Although not an air-sampling technique, the analysis of settled dust samples for culturable fungi and for bioactive substances such as those named above may provide evidence of exposure integrated over time.

Fixed-point sampling over short periods of time, i.e., 1–5 min, can provide evidence of exposure. However, estimating exposures even for one given location requires a large number of samples. Traditional culturable sampling and analysis methods for fungi fit this method classification; however, the cost of applying these methods to the estimation of exposures over an 8- to 24-hr period would generally be prohibitive. Furthermore, the uncertainties inherent in a determination of CFU/m³ would make the results of questionable value.

On the other hand, the ability of culturable sampling and analysis methods to provide qualitative information offers a distinct advantage, especially when compared to methods in which only markers of fungal exposure are determined. The importance of the speciation of the fungi that contaminate homes or buildings has been discussed at length in the previous
section. The use of surrogates to estimate exposure should be accompanied by the use of techniques that can identify the fungi present. Fixed-point sampling to determine culturable fungi in areas where children spend most of their time is, therefore, justified. Furthermore, fewer samples are needed to conduct a rank order assessment than would be required to estimate exposures with short-term fixed-point sampling, and culturable sampling accurately establishes rank order differences between indoors and outdoors.

Conclusions and Recommendations

Without further research or discussion, the public health practitioner can conclude that the prevention of microbial growth in buildings is an effective means of preventing disease and that moisture intrusion and accumulation in a building are the primary causes of biocontamination. Thus the prevention of microbial growth is an attainable goal that can be realized by the proper maintenance of a building or home.

Continued research is necessary to establish firm causal relationships between disease agents resulting from microbial growth and disease. An increasing number of methods are available that can be used to measure or estimate exposure of children to fungi or fungal agents. However, continued methods development is necessary to provide tools that can be used to provide more definitive support of causal relationships between disease and currently recognized and potential fungal disease agents. Some of the methods described here have not been used extensively, e.g., the in vitro protein translation assay for tricothecenes, and need further field evaluation. A better understanding of the advantages, disadvantages, and complementary attributes of the agents that may be used as indices of fungal mass, i.e., ergosterol, (1→3)-β-D-glucan, and mold extracellular polysaccharides, needs to be reached. Finally, much work needs to be done on the further development of promising techniques such as qualitative, and eventually quantitative, PCR, or the determination of fungal lipid signature profiles.

In the absence of statistically definitive causal relationships and in the absence of measurement methods capable of establishing the needed associations to establish causality, decisions regarding the respiratory health of children must be made based on the professional judgment of qualified teams. These professional teams should comprise experts in mycology, exposure assessment, epidemiology, medicine, and building engineering.

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