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## Review article

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# Health risk assessment of fungi in home environments

Arnoud P Verhoeff, PhD\* and Harriet A Burge, PhD†

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**Learning Objectives:** Reading this article will enable the readers to recognize the public health importance of fungi in the home environment. In view of the recognized impact of fungi on human health, the large population being exposed to fungi, and the large population at risk for developing allergic diseases, there is a need to establish guidelines for allowable exposure to fungi based on a health risk assessment. The aim of this study was to evaluate the status of the data on the relationship between exposure to fungi in the home environment and allergic health effects with respect to the development of such guidelines.

**Data Sources:** The past 10 years of peer-reviewed literature focused on the relationships between respiratory disease and exposure to fungi in indoor environments was examined. Indexing terms included mold, fungi, allergy, asthma, and indoor environment, among others. Each study was evaluated on the following criteria: aim and design of the study, methods for assessing exposure and health effects, and data analysis.

**Study Selection:** Nine population based studies were identified that examined the relationship between allergy and the presence of fungi in the home environment. These studies included quantitative measures of fungal presence in either air or dust.

**Results:** One or more positive associations were found between fungal levels and health outcomes in seven of the nine cross-sectional studies identified.

**Conclusions:** Despite these positive associations it remains impossible to set guidelines for fungi in home environments based on health risk assessment. This is in part because of the cross-sectional study designs, and inconsistency and inadequate validation of the measures used to evaluate exposure and health effects. Future research designed to generate data that can be used for the development of health risk assessment based guidelines for fungi in home environments should focus on susceptible populations, and use measures that accurately represent exposure and adverse health effects.

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## INTRODUCTION

Since the late 1970s increasing attention has been given to the quality of air

in residences, offices, and other public buildings, in part because it is now clear that most people spend a majority of their time indoors and that the levels of some air pollutants are higher indoors than outdoors.<sup>1</sup> Much attention has been focused on chemical pollutants derived from combustion processes, building materials, and consumer products<sup>1-3</sup> and on allergens

derived from house dust mites, pets, and vermin.<sup>4-7</sup> The importance of microorganisms, including viruses, bacteria, and fungi, is increasingly recognized.

Several recent epidemiologic studies reported positive associations between home dampness and respiratory morbidity of the occupants.<sup>8-14</sup> These studies also indicated that dampness and fungal (mold) problems are present in 20% to 50% of modern homes. Fungi are regarded as one of the causal factors in the relationship between home dampness and respiratory symptoms, and homes classified as damp tend to have higher levels of fungi than those not so classified.<sup>15-18</sup> In addition, poorly maintained heating, ventilation, and air-conditioning (HVAC) systems have been recognized as sources of microorganisms, including fungi.<sup>1,7</sup>

Fungi are well known as sources for allergens that cause allergic rhinitis, allergic asthma, and extrinsic allergic alveolitis (hypersensitivity pneumonitis).<sup>19-23</sup> Probably all fungi that may be abundant in indoor environments produce allergens.<sup>21</sup> Up to 10% of the general population is skin test positive to fungal extracts,<sup>24,25</sup> and among patients with respiratory allergy, 2% to 80% are reported to be sensitized to fungi.<sup>19,26-28</sup> Niemeijer and De Monchy reported an age dependency of sensitization to aeroallergens, including *Cladosporium herbarum*, in asthmatic patients.<sup>29</sup> The highest prevalence of sensitization to this fungus was at age 4 years (42%), declining to 10% or less in patients more than 15 years of age. This finding was confirmed in a more recent study.<sup>30</sup> Patients often show

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\* Department of Epidemiology, Municipal Health Service Amsterdam, Amsterdam, the Netherlands.

† Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts.

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multiple positive reactions to different fungal extracts. It is not yet clear whether these patients have independent sensitivities to many fungi or are sensitive to cross-reacting allergens produced by many fungi.<sup>31</sup> The majority of individuals sensitized to fungi are also sensitized to other inhalant allergens.

The cell walls of most fungi contain  $\beta$ -1,3-D-glucan, an inflammatory agent that may act as an adjuvant. The glucans have been suggested as a causal factor in mucous membrane irritation, dry cough, and itching skin reported by occupants of buildings about which indoor air quality complaints had been made.<sup>32</sup>

Fungi may also produce toxic metabolites (eg, mycotoxins). The best known are the aflatoxins, which are human carcinogens.<sup>33</sup> Respiratory exposure to toxins produced by *Stachybotrys atra* has been a focus of attention with respect to nonallergic respiratory symptoms.<sup>34,35</sup> Fungi also produce a variety of volatiles, including alcohols, aldehydes, and ketones, which are often evident as "moldy odors," and which may produce symptoms such as headache, eye, nose and throat irritation, or fatigue.<sup>20,36</sup> Some fungi that are found in indoor environments are also known to be opportunistic infectious agents in man, especially in the case of immunocompromised patients.<sup>22,37</sup>

In view of the recognized impact of fungi on human health, the large population being exposed to fungi both indoors and outdoors, and the large population at risk for developing allergic diseases, there is a need to develop guidelines for acceptable exposure to these bioaerosols. Ideally, these guidelines should be based on dose-response relationships between exposure and disease; however, dose-response data for fungal agents of disease are unavailable.<sup>4,6,7</sup> Instead, guidelines for acceptable exposure have been proposed by several authors. These have been based on surveys designed to characterize normal levels of fungi in indoor environments rather than on

dose or exposure-response relationships.<sup>38</sup>

We have evaluated studies reported over the past 10 years in the peer-reviewed literature that focused on relationships between exposure to fungi in indoor environments and respiratory disease with respect to the development of risk assessment-based guidelines. Each study was evaluated on the following criteria: aim and design of the study, method of exposure assessment (including type of samples collected, sampling and analytical procedures, and validity of the measurements), the assessment of health effects, and data analysis.

## OVERVIEW OF QUANTITATIVE CROSS-SECTIONAL STUDIES

In recent years nine population-based studies have been reported that address the association between exposure to fungi in the home environment and health effects of the occupants: Platt,<sup>16</sup> Waegemaekers,<sup>17</sup> Björnsson,<sup>39</sup> Wood,<sup>40</sup> Strachan,<sup>41</sup> Li,<sup>42</sup> Su,<sup>43</sup> Verhoeff,<sup>44</sup> and Wickmann.<sup>45</sup> An overview of these studies is presented in Table 1.

### Study Design

All these quantitative studies were cross-sectional and compared levels of exposure in homes of people with and without atopic diseases and/or reported symptoms by means of simple univariate analyses or more complex linear or logistic regression modeling.

Su<sup>43</sup> randomly selected 150 households from a larger cohort of 350 homes participating in a detailed indoor air quality and respiratory health study. Waegemaekers<sup>17</sup> studied 36 homes (housing 54 children) with reported dampness from 226 initially surveyed. Platt<sup>16</sup> selected areas in three cities in the United Kingdom in which families with young children predominated, the prevalence of damp housing was thought to be high, and the socioeconomic state was likely to be homogenous. In total, of 1220 eligible households 597 (including 597 adults and 1169 children) were willing to participate. Strachan<sup>41</sup> included the homes of 88 children, selected from a larger

population-based survey on home dampness and respiratory symptoms. Four groups were selected: children with reported wheeze and mold in their homes (n = 11), children with reported wheeze but without mold (n = 23), children without wheeze but with mold (n = 29), and children with neither wheeze or mold (n = 25). Verhoeff<sup>44</sup> selected 60 households from a larger population of 281 who participated in a case-control study on home dampness and respiratory symptoms among children aged 6 to 12 years. Presence/absence of respiratory symptoms (chronic wheeze, chronic cough, attacks of shortness of breath with wheezing, or doctor-diagnosed asthma) and dampness were used to define four groups of 15 homes each. These groupings included 31 children with symptoms, of whom 65% were atopic, and 29 asymptomatic children, of whom 55% were atopic. Atopy was defined as having an increased total IgE serum level, adjusted for age. From a random population of 660 adults, Björnsson<sup>39</sup> selected all individuals reporting asthma in the last 12 months, nocturnal breathlessness in the last 12 months or current use of asthma medication (n = 74). A control group was randomly selected from those not reporting these symptoms. Data were obtained from 47 asthmatics, of whom 48% were atopic, and from 41 controls of whom 38% were atopic. Atopy was defined as a positive skin prick reaction to at least one of ten inhalant allergens. Li,<sup>42</sup> Wickmann,<sup>45</sup> and Wood<sup>40</sup> recruited their study groups from patients attending allergy clinics. Li<sup>42</sup> selected 66 children (46 with bronchial asthma, and 20 without) aged 7 to 15 years, who were sensitized to at least one of 13 inhalant allergens, and 26 children without a history of atopic diseases. Wickmann<sup>45</sup> selected 64 children sensitized to house dust mites, 64 children sensitized to other inhalant allergens, denoted as atopics, and 58 children without a history of atopic diseases, living in the neighborhood of the house dust mite sensitized children. Finally, Wood<sup>40</sup> recruited 106 patients of whom 55 had a history of asthma, 68 a

Table 1. Summary of the Cross-Sectional Studies on the Relationship Between the Exposure to Fungi in the Home Environment and Health Effects

Reference	Study Design	Exposure Assessment
Björnsson <sup>39</sup> Uppsala, Sweden October–April	cross-sectional, adults (n = 88)	Total and viable airborne fungal particles: Nuclepore filter sampler bedroom Co-pollutants: total and viable airborne bacteria guanine in house dust
Li <sup>42</sup> Taipei, Taiwan July–September	cross-sectional, case-control children (n = 93)	Viable airborne fungal particles Andersen-one stage sampler living room, bedroom, outdoors Co-pollutants: none
Verhoeff <sup>44</sup> Noord-Holland, the Netherlands Oct–Nov	cross-sectional, case-control children (n = 60)	Viable dust borne fungal particles settled dust, bedroom floor, mattress Co-pollutants: none
Su <sup>43</sup> Topeka, US winter	cross-sectional, children (n = 150)	Viable airborne fungal particles Andersen-one stage sampler living room or family room Co-pollutants: none
Wickmann <sup>45</sup> Stockholm, Sweden February–March	cross-sectional, case-control children (n = 175)	Viable dust borne fungal particles settled dust, living room floor Co-pollutants: none
Strachan <sup>41</sup> Edinburgh, UK October–March	cross-sectional, case-control children (n = 88)	Viable airborne particles Andersen-six stage sampler living room, bedroom, kitchen or room with visible mold growth. Co-pollutants: none
Platt <sup>16</sup> Edinburgh, Glasgow, London, UK February–April	cross-sectional, children (n = 1169) adults (n = 597)	Viable airborne fungal particles Surface Air System sampler kitchen, living room, bedroom (in part of the homes only) Co-pollutants: none
Waegemaekers <sup>17</sup> Katwijk, the Netherlands April–May	cross-sectional, children (n = 190) adults (n = 328)	Viable airborne fungal particles Andersen-one stage sampler living room (in part of the homes only) Co-pollutants: none
Wood <sup>40</sup> Baltimore, US January–March	cross-sectional, children and adults (n = 106)	Viable dust borne fungal particles settled dust, mixed sample of bedroom, bathroom, TV area, basement. Co-pollutants: housedust mite and pet allergens.

Table 1. *Continued*

Health Outcomes	Results
<p>Reported respiratory symptoms last 12 months: asthma, wheezing, daytime and nocturnal attacks of breathlessness.</p> <p>Skin prick testing.</p> <p>Lung function: FEV<sub>1</sub> and daily variation in PEF for one week.</p> <p>Bronchial hyperresponsiveness</p> <p>Total serum IgE and serum ECP, blood eosinophil count.</p>	<p><i>Univariate analyses:</i> Total fungal counts (GM) significantly higher for reported asthma, daytime attacks or nocturnal attacks of breathlessness</p> <p><i>Multivariate analyses:</i> Total or viable fungal counts not associated with reported symptoms; total bacterial counts associated with reported asthma and wheezing.</p> <p>Total or viable fungal counts not associated with lung function parameters, or bronchial hyperresponsiveness.</p> <p>Serum IgE and serum ECP associated with total fungal counts.</p>
<p>Three groups of children: bronchial asthma (n = 47) non-asthmatic atopic (n = 20) control (n = 26) (clinical diagnosis)</p>	<p><i>Univariate analyses:</i> Highest total counts for controls, followed by asthmatics and non-asthmatic atopics. No significant differences.</p>
<p>Reported respiratory symptoms: cases (n = 31) controls (n = 29)</p>	<p><i>Univariate analyses:</i> Total fungal counts (GM) were comparable for cases and controls. Counts of <i>Aureobasidium pullulans</i> in floor dust and of yeasts in mattress dust significantly higher for controls, and of <i>Wallemia sebi</i> in mattress dust significantly higher for cases.</p>
<p>Reported respiratory symptoms: lower respiratory illness wheezing and/or asthma hay fever</p>	<p>Fungal counts not presented.</p> <p><i>Univariate analyses:</i> Higher than average counts of Cladosporium, Alternaria, Aureobasidium, Epicoccum, yeasts in homes of children with reported wheezing and/or asthma, or hay fever.</p>
<p>Three groups of children: house dust mite sensitized (n = 61) atopic, not sensitized to house dust mite (n = 57) control (n = 57) Skin prick testing. (clinical diagnosis)</p>	<p><i>Univariate analyses:</i> Total fungal counts significantly higher for controls compared with atopic and house dust mite sensitized children. No association between fungal counts, species isolated, and positive skin prick tests.</p>
<p>Reported respiratory symptoms: wheeze past year (n = 34) controls (n = 54)</p> <p>Lung function: FEV<sub>1</sub>, bronchial lability</p>	<p><i>Univariate analyses:</i> Total fungal counts (GM) were comparable for wheezing children and controls. Counts of mycelia sterilia significantly higher for wheezing children than controls. Total fungal counts (GM) were higher for children with a &gt;10% reduction in FEV<sub>1</sub></p>
<p>Reported respiratory and other symptoms for the last two weeks for children and adults.</p>	<p>Fungal counts not presented.</p> <p><i>Univariate analyses:</i> Exposure-response relationship between fungal counts and following reported symptoms in adults: high blood pressure, persistent cough, bad nerves, backache, palpitations, breathlessness. Exposure-response relationship between fungal counts and following reported symptoms in children: wheezing, irritability, fever.</p>
<p>Reported respiratory symptoms: cough wheeze or shortness of breath or asthma</p>	<p>Total fungal counts higher in homes classified as damp (n = 24) than in dry homes (n = 8).</p> <p><i>Multivariate analyses:</i> For children, the prevalence of reported respiratory symptoms increased with increasing total fungal counts. No data presented for adults.</p>
<p>History of asthma. Skin prick testing Symptoms on mold exposure. (clinical diagnosis)</p>	<p><i>Univariate analyses:</i> Total counts (median) were comparable for those with and without history of asthma. Higher total counts (median) for those with positive skin prick test to molds than for those without, and for those with symptoms on mold exposure than those without.</p>

history of rhinitis and 8 a history of eczema.

#### *Measures of Health Impact*

Six studies used randomly selected populations and health effects were assessed by self administered questionnaires.<sup>16,17,39,41,43,44</sup> Symptoms addressed included asthma, cough, wheeze, shortness of breath, hay fever, and bronchitis. Björnsson,<sup>39</sup> Strachan,<sup>41</sup> and Verhoeff<sup>44</sup> validated respiratory symptoms using pulmonary function measurements. Björnsson<sup>39</sup> also measured daily variations in peak expiratory flow rate over one week and performed methacholine challenges, skin prick tests, and collected blood samples for measurement of serum concentrations of eosinophil cationic protein (ECP), blood eosinophil count, and total serum IgE. The panel used for skin-prick testing included only *Cladosporium* and *Alternaria*. Verhoeff<sup>44</sup> used RAST to measure total IgE and IgE specific for a mixture of fungi (*Penicillium*, *Alternaria*, *Aspergillus*, *Cladosporium*). Wood,<sup>40</sup> Li,<sup>42</sup> and Wickmann<sup>45</sup> used clinic populations and performed skin tests using a panel of common inhalant allergens that included *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* (Wood<sup>40</sup>), *Cladosporium*, *Penicillium* and *Alternaria* (Li<sup>42</sup>), *Cladosporium herbarum* and *Alternaria alternata* (Wickmann<sup>45</sup>). All groups used unstandardized, commercially available allergen extracts.

#### *Exposure Assessment*

##### **Air Sampling**

In six studies, exposure was estimated by air sampling of culturable fungal particles. In four of these studies, Malt Extract Agar (MEA) was used as the collection medium in either the Andersen single stage<sup>17,42,43</sup> or 6-stage sampler.<sup>41</sup> Platt<sup>16</sup> used the Surface Air System sampler (SAS) and the collection medium was not noted. Björnsson<sup>39</sup> estimated exposure to both total spores and culturable fungi by sampling on polycarbonate filters, according to the method described by Palmgren et al.<sup>46</sup> The type of medium

used to enumerate culturable fungi was not indicated.

In all studies, samples were collected in the living or family room. In addition, Platt,<sup>16</sup> Björnsson,<sup>39</sup> Strachan<sup>41</sup> and Li<sup>42</sup> collected samples in the bedroom, and Platt<sup>16</sup> and Strachan<sup>41</sup> collected samples in the kitchen and/or room(s) affected by mold growth. The amount of air/sample varied considerably. Su<sup>43</sup> collected 14 or 28 L, Li<sup>42</sup> 14 or 85 L, Strachan<sup>41</sup> 85 L, Björnsson<sup>39</sup> 225 L, and Waegemaekers<sup>17</sup> 284 or 426 L. Platt<sup>16</sup> did not provide information on the duration of sampling. The sampling height varied between 0.45 m and 1.50 m. Parallel outdoor samples were collected by Li<sup>42</sup> and Su.<sup>43</sup> Strachan<sup>41</sup> did repeated sampling four times at monthly intervals, but did not present information on the variation with time of fungal counts. Su<sup>43</sup> sampled in summer and winter. Viable counts were clearly higher in summer than in winter, both indoors and outdoors. Björnsson<sup>39</sup> reported total fungal counts; others identified colonies at least to genus. Waegemaekers<sup>17</sup> measured reproducibility of air samples using sequential replicates. They reported a coefficient of variation of 22.4%. Li<sup>42</sup> and Su<sup>43</sup> used sequential and simultaneous replicates respectively, but did not report on reproducibility. None of these provided information on the reproducibility of the results in terms of fungal taxa isolated.

##### **Dust Sampling**

The remaining three groups (Wood<sup>40</sup>; Verhoeff<sup>44</sup>; Wickmann<sup>45</sup>) used the presence of cultural fungal particles in settled house dust to estimate exposure to fungi. In these studies, 30 mg of unsieved dust was plated onto DG18 agar (Verhoeff<sup>44</sup>) or V-8 agar (Wood<sup>40</sup>; Wickmann<sup>45</sup>). Verhoeff<sup>44</sup> collected dust by vacuuming 2 m<sup>2</sup> of the floor of the child's bedroom for four minutes, and the entire upper mattress surface for two minutes after the bedding had been removed. Wickmann<sup>45</sup> sampled 3 m<sup>2</sup> of the living room floor for one minute. Wood<sup>40</sup> obtained a mixed dust sample from the floors of the bedroom, bathroom, TV area, and basement. The area sampled

was not recorded. Verhoeff<sup>44</sup> and Wickmann<sup>45</sup> identified colonies to species and Wood<sup>40</sup> to genus.

Verhoeff<sup>44</sup> analyzed samples in duplicate, and sampling was repeated after a period of 6 weeks in some of the homes. The coefficient of variation for the duplicate samples in terms of colony-forming units per gram of dust (CFU/g of dust) was approximately 15%, and the agreement rate in terms of species isolated approximately 60%. The reliability coefficient for repeated measurements was 0.19 for mattress dust and 0.21 for floor dust. The agreement rate for species isolated was 45% for mattress dust and 40% for floor dust. These results indicated that a single sample of house dust had a low predictive value for the presence of culturable fungal particles over time.

#### *Summary of Results*

Reported associations with quantitative exposure measures and asthma or asthma-like symptoms are summarized in Table 2. One or more positive associations were found between fungal levels and health outcomes in seven of the nine cross-sectional studies described above. In the Björnsson<sup>39</sup> study, the geometric mean levels of total countable airborne fungi were significantly higher in homes of asthmatic patients (35,000 versus 25,000/m<sup>3</sup>), patients with daytime attacks of breathlessness (40,000 versus 27,000/m<sup>3</sup>), and nocturnal attacks of breathlessness or chest tightness (figures not given) compared with homes of those without these symptoms. Also, total serum IgE and serum-ECP levels were positively associated with total fungal count ( $r = 0.24$  and  $0.26$ , respectively). On the other hand, levels of cultural fungi were not associated with symptoms, and no correlations were found between lung function parameters and any fungal exposure measure.

The Su<sup>43</sup> studies revealed positive relationships between hay fever symptoms and winter airborne cultural counts of *Cladosporium*, *Aureobasidium*, and yeasts. Odds ratios for one unit increase in the natural long-concentrations of fungi were 1.44 (95% CI

Table 2. Overview of Reported Associations for Fungi and Asthma or Asthma-Like Symptoms

Study	Positive Association	Negative or No Association	Study Population
Björnsson <sup>39</sup>	Total airborne	Total cultural airborne	Adults
Su <sup>43</sup>	Aspergillus	Total cultural airborne, other taxa, total countable airborne	Children
Strachan <sup>41</sup>	Mycelia sterilia	Total cultural airborne, other taxa	Children
Platt <sup>16</sup>	Cultural airborne		Children and adults
Waegemaekers <sup>17</sup>	Cultural airborne		Children
Verhoeff <sup>44</sup>	<i>Wallemia sebi</i> , mattress dust	Total cultural dust, other taxa	Children
Lj <sup>42</sup>		Total cultural airborne	Children
Wood <sup>40</sup>		Total cultural dust	Children and adults
Wickmann <sup>45</sup>		Total cultural dust	Children

1.04 to 1.98), 1.29 (95% CI 1.00 to 1.67) and 1.33 (95% CI 1.04 to 1.71), respectively. Winter counts of *Aspergillus* were significantly associated with reported wheeze (odds ratio not given). The odds ratios were not adjusted for possible confounding variables like age, sex and passive smoking, although a recent reanalysis controlling for these variables produced an increased odds ratio for *Cladosporium*, and confirmed results for the other taxa. An analysis of these same data using factor analysis revealed higher than average counts of a group of fungi including *Cladosporium*, *Alternaria*, *Aureobasidium*, *Epicoecum*, and yeast in homes of patients reporting wheeze and/or asthma, and hay fever. No significant symptom associations were found for any summer measure.

The Strachan<sup>41</sup> culture-based data revealed significantly higher levels of mycelia sterilia (non-sporulating fungi) in homes of wheezing children than in other homes (2.1 versus 0.7 CFU/m<sup>3</sup>). Total fungal counts were consistently highest for children with bronchial lability (n = 26, defined as a reduction in FEV<sub>1</sub> of more than 10% of pre-exercise value, at either five or ten minutes free running exercise challenge). No major differences were revealed between total fungal counts in homes of children with and without wheeze.

Platt<sup>16</sup> categorized mean levels of culturable fungal counts into five groups (group 1: low <100 CFU/m<sup>3</sup>; group 2: medium 101 to 300 CFU/m<sup>3</sup>; group 3: high 301 to 1000 CFU/m<sup>3</sup>; group 4: very high 1001 to 5000 CFU/

m<sup>3</sup>; group 5: extremely high >5000 CFU/m<sup>3</sup>). These category scores were then divided into three exposure categories: low (1), medium (1.01 to 2), and high (>2). For adults, significant positive relationships were found between exposure category and persistent cough, breathlessness, high blood pressure, bad nerves, backache, and palpitations. For children, significant positive relationships were found for wheezing, irritability, and fever/high temperature.

In the Waegemaekers<sup>17</sup> study, linear multivariate regression analyses were applied to assess associations between reported respiratory symptoms in children and culturable fungal counts. Adjustment was performed for age, sex, parental smoking, heating system, closed or open kitchen, presence of pets, and type of flooring in living room and bedroom. Adjusted odds ratios for one unit increase in the ln-concentrations were 1.98 (P < .05) for reported cough, and 1.28 (P > .05) for reported wheeze, shortness of breath, or asthma.

Verhoeff<sup>44</sup> used *t* tests to compare the presence of culturable fungi in settled house dust in bedrooms of cases (children reporting respiratory symptoms) and controls. Counts of *Wallemia sebi* in mattress dust were significantly higher for cases than for controls. The geometric means of total culturable fungi per gram of dust were similar for both groups. Counts of some fungal groups (*Aureobasidium pullulans*, yeasts) were higher for controls than for cases. Only one child included in this study had detectable

IgE to the fungal mixture used in the RAST assay.

Wood<sup>40</sup> found higher median numbers of CFU/g of dust in homes of those with a positive skin prick tests to fungi and for those with symptoms on fungal exposure than in other homes, but no significant differences for patients with and without asthma.

The Wickmann<sup>45</sup> and Lj<sup>42</sup> studies produced no positive correlations between their exposure measures and any of the health outcomes. Wickmann<sup>45</sup> reported significantly higher fungal counts in homes of nonatopic children compared to those for children sensitized to house dust or other allergens; however, the distributions overlapped considerably. Skin prick test responses to *Cladosporium herbarum* and *Alternaria alternata* were not related to the frequency of isolation of these species. Lj<sup>42</sup> also found higher mean counts for controls compared with those for asthmatics and non-asthmatic atopics, although the distributions overlapped considerably.

In most of the studies many comparisons were made so that the changes of randomly occurring correlations were significant. It should also be noted that, as discussed below, the potential for exposure misclassification is significant for all studies, and would tend to obscure positive associations.

## OTHER STUDIES

Several cross-sectional studies have reported qualitative measures of fungal presence using settle or gravity sampling,<sup>47,48</sup> swab samples,<sup>49</sup> or frequency of recovery of taxa from volumetric samples.<sup>50</sup> In most of these, study de-

sign precludes assessment of associations between exposure and health outcomes.

Tarlo et al<sup>50</sup> compared the frequency of isolation of fungal taxa in air, using an Andersen-two stage sampler with Rose Bengal agar, with the frequency of positive skin reactions to the fungi isolated from each patient's home and adjacent outdoor air. Fourteen of the 26 patients (54%) had positive skin tests to one or more of the fungal extracts prepared from fungi identified in the homes. The highest frequency was found for *Cladosporium cladosporioides* followed by *Alternaria tenuis* (*alternata*), *C. sphaerospermum*, and *Fusarium* spp. The most frequently isolated fungus from both indoor and outdoor air was *C. cladosporioides*, followed by *C. sphaerospermum*, *C. herbarum*, and *A. tenuis*.

Yoshida et al<sup>48</sup> isolated *Trichosporon cutaneum* by settle plate sampling from 11 of 22 homes of patients with summer type hypersensitivity pneumonitis, but in none of 195 homes of control subjects. Antibodies to this fungus were found in 80% of the patients. This assay was not conducted for the controls.

Zwick et al<sup>51</sup> measured total fungal particles using a Burkard slide personal sampler for ten minutes in the homes of six patients with hypersensitivity pneumonitis, eight patients with idiopathic lung fibrosis, and six healthy controls, primarily to obtain fungal material to demonstrate precipitating antibodies in the patients serum. Spore counts in the homes of patients with hypersensitivity pneumonitis tended to be higher than in homes of controls and patients without hypersensitivity pneumonitis.

Two longitudinal studies reported on the relationship between acute symptoms and fungal exposure. Beaumont et al<sup>52</sup> investigated the association between the prevalence of airborne culturable fungi indoors and outdoors and the course of obstructive lung disease in eight adult patients with partially reversible chronic air-flow obstructions and strong positive skin reactions and RAST tests to one

or more fungi. Samples were collected at four indoor and one outdoor site once every month for 22 months. Peak flow measurements were taken 3 times each day, and respiratory symptoms (shortness of breath, cough, and phlegm) were recorded by the patients. Spirometry, eosinophil counts, total and specific IgE measurements were made and a chest radiograph taken every 3 months. An increase in pulmonary complaints (the outcome variable) was defined as a subjective increase in bronchial complaints with a decrease in peak flow values of more than 15%, or an increased cough with expectoration with or without a rise in body temperature. Three patients had allergic bronchopulmonary aspergillosis. A rise in total IgE and eosinophils with new infiltrates was considered an exacerbation of this syndrome. Mean peak flow values on the day with the highest fungal counts outdoors for each patient were significantly lower than those on the day with the lowest fungal count outdoors. These relationships were not seen for indoor fungal counts. Most episodes with increased pulmonary complaints occurred in the months July through November along with the majority of the peak fungal recordings. Thirteen episodes corresponded with fungal peaks indoors, and ten with an outdoor fungal peak.

Holst et al<sup>53</sup> reported a comparable study among 35 asthmatic patients and 11 nonasthmatic controls, followed for 9 months. Culturable fungi were sampled monthly using settle plates using Malt Extract Agar with Rose Bengal exposed for one hour at five indoor and one outdoor locations. Diaries were used to collect information for 1 week each month on medication use and peak expiratory flow rates. Skin tests were performed using commercially available extracts of fungi, including *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Mucor*, *Phoma* and *Rhizopus*, and total serum IgE was measured. For six patients there was a positive significant correlation between reported symptoms, peak expiratory flow rates and counts of *Aspergillus* colonies. Positive correlations were

also found between these outcome variables and counts of *Cladosporium* in three patients, *Penicillium* in three patients, *Basidiomycetes* in two patients, and *Alternaria* in one patient. None of these patients showed a positive skin test to the fungal extracts. Eight patients had positive reactions to fungal extracts but showed no apparent variation in asthma symptoms with changes in fungal counts.

Delfino et al<sup>54</sup> reported a study among 12 asthmatic subjects aged 9 to 16 years, followed daily over a period of 2 months. Total outdoor fungal spores were sampled daily with a Burkard 7-day recording volumetric spore collector. Diaries were used to collect information on asthma symptoms and medication use. Skin tests were performed using commercially available extracts of *Alternaria tenuis*, *Cladosporium cladosporioides*, *Helminthosporium interseminatum*, *Aspergillus* spp. and *Penicillium* spp. Ten patients had at least one positive skin test to one of the fungi. A formal time-series analysis was used to investigate the association between exposure and health outcomes. Asthma severity was positively associated with total fungal spore levels measured during a period of 0 to 12 hours prior to the 24-hour period encompassed by the diary responses. The use of inhalers was also positively associated with total fungal counts. The strongest associations were found for the total spore counts minus the counts of the five genera used for skin testing.

## DISCUSSION

### *Overall Status*

It is clear that fungi do cause allergic disease, both in terms of eliciting a specific IgE response as measured by skin and in vitro testing, and in terms of symptoms related to exposure on a case by case basis. In addition, in seven of nine epidemiologic studies where fungal exposure was quantitatively assessed, some relationship was seen between one or more of the fungal exposure indices and symptoms of respiratory disease. Nevertheless, of the

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multiple comparisons made between exposure measures and symptoms in each of these studies, many were not significant. Factors that might contribute to this problem include inadequate study design, inappropriate exposure measures, problems associated with assessing symptoms and specific sensitization, and approaches to data analysis.

#### *Study Design*

The allergic diseases are, at least in part, mediated by genetic factors that are present in approximately 35% of the population.<sup>55</sup> This means that only about one-third of randomly selected subjects are likely to be at risk for development of the disease and symptoms associated with allergen exposure. For example, in the Su<sup>43</sup> and Waegemaekers<sup>17</sup> studies, which included 150 and 54 children respectively, only about 50 and 20 were likely to be at risk. Exposure to allergens in the remainder of this population is probably irrelevant. The highest number of children and adults likely to be at risk were included in the Platt<sup>16</sup> study. Björnsson,<sup>39</sup> Strachan,<sup>41</sup> and Verhoeff,<sup>44</sup> selected subgroups of individuals on the basis of reported respiratory symptoms. An adequate test of the hypothesis that fungi play a role in allergen-induced disease requires the use of an at risk population, ie, a population with a family history of allergic disease and/or demonstrated atopy.

The design of all studies was cross-sectional, comparing levels of fungi at one point in time in homes of individuals with a history of disease (ie, respiratory symptoms or atopy) previous to the sampling event, and without disease. Patients with respiratory allergy regularly take allergen avoidance measures in their homes,<sup>56</sup> hampering the estimation of the association between past exposures, sensitization, and development of respiratory symptoms.

#### *Measurement of Exposure to Fungi*

Measurement of exposure is an essential part of environmental epidemiologic studies, including those investigating the relationship between

exposure to fungi and health effects. The most valid and accurate methods for representing exposure should therefore be used. Several major problems are associated with virtually all large epidemiologic studies that have evaluated the role of fungal exposure in the development and exacerbation of allergic disease. Most have focused on indoor home-based exposures. Contrary to the other indoor allergens, it is likely that significant exposure to fungi occurs outdoors and in schools and other settings. Ignoring these alternate sources is likely to obscure relationships between fungal exposure and disease.

Likewise, exposure assessment protocols used to date have had serious limitations. Impaction of fungal particles onto a solid surface (eg, culture medium) is the most widely used approach in epidemiologic studies for assessing exposure to fungi. All commonly used culture plate impactors use short sampling periods, typically 30 seconds to several minutes. Unfortunately, the dynamics of indoor airborne spore populations over time has not been studied. Preliminary data (Burge, unpublished) indicate that levels of some fungal spore types (eg, *Penicillium*) can vary by orders of magnitude over a 24-hour period. This variability is reflected, in part, by the only moderate reproducibility of parallel and sequential duplicate samples.<sup>57</sup> In addition, repeated sampling within weeks has demonstrated that overall variation over time within homes is much higher than variation between homes.<sup>18</sup> This means that a single-grab air sample has only a low predictive value for exposure over time.

Air samples have been collected by filtration, a process that enables both cultural and microscopic analysis of samples and allows collection of sample over longer periods of time than can be used for the culture plate impactors.<sup>39,46</sup> Use of filtration for cultural analysis may result in underestimation due to death of cells on the filter. Microscopic analysis requires relatively high concentrations of particles for accuracy.

Finally, air samples have also been collected using slide or tape impactors for microscopic analysis.<sup>43</sup> Samples are usually collected over roughly a 10-minute period, although devices are available that allow collection of continuous time-discriminated samples over periods as long as a week.<sup>58</sup> Although analysis is labor intensive, these devices allow mapping of changing concentrations over time, and may provide the best available exposure measure for fungi.

No sampler collects all particles with equal efficiency and it is, therefore, not surprising that different quantitative and qualitative results are obtained using different air sampling devices.<sup>59</sup> The choice of the collection (culture) medium also controls the kinds and levels of fungi recovered.<sup>57</sup> So far, few published data are available on the validity of the measurement of airborne fungal particles as estimate of the exposure.

Exposure to fungi has also been estimated by culturing fungi in settled house dust samples. Dust is plated directly onto a culture medium, or suspended and diluted prior to plating. The results, both quantitatively and qualitatively, depend on the method of inoculation and on the culture medium used.<sup>60</sup> Repeated sampling within weeks has demonstrated that variation in time within homes is higher than the variation between homes, both quantitatively and qualitatively; therefore, a single dust sample has only a low predictive value for exposure over time.<sup>60</sup> In addition, some types of fungi that are common and abundant in dust are not common in air.<sup>61</sup>

#### *Evaluation of Symptoms*

In six population-based studies, self-reported respiratory symptoms, which are nonspecific as predictors of fungal related respiratory allergy, were used as the outcome variables. Most of these nonspecific symptoms can result from both allergic and nonallergic responses to different fungal components, or to completely different agents.



Some of the evidence for (and against) fungi as important allergen sources is based on skin prick testing using commercially available extracts derived from fungal cultures. Serum IgE specific to a few fungal allergens can also be measured by means of commercially available RAST kits. Bronchial provocation tests are also used to diagnose fungal allergy, mainly in suspected cases of allergic alveolitis. So far, allergens have been purified from only a few fungi.<sup>21</sup> The isolation, purification, and standardization of fungal allergens are major problems related, in part, to the tendency of fungi to vary allergen production depending on the growth substrate, the strain, the length of time in culture, and other factor; therefore, individual lots of commercial fungal antigen preparations may or may not contain the antigens relevant to inhalant allergic reactions. The inadequacy of commercially fungal extracts has been clearly demonstrated in skin test studies where reactivity to a specific fungus varied from 10% to 60% depending on the extract used.<sup>62</sup> Yunginger et al<sup>63</sup> demonstrated similar variability for *Alternaria* extracts using RAST. The absence of standardized fungal allergen preparations from the common environmental fungi tends to promote underestimation of the extent of fungal sensitization in the atopic population.

#### *Data Analysis*

It is becoming clear that fungal allergen exposures are complex, and that simply measuring exposure to "total fungi" may not reveal important relationships between specific taxa and human disease. Interpreting air sample data requires an understanding of the dynamics of fungal populations, and a recognition that grab samples may measure transient, relatively unimportant exposures. Careful comparisons of samples collected both indoors and outdoors may alleviate this problem to some extent, since much of the variability of indoor exposures may be associated with rapidly changing prevalence patterns outdoors. Likewise, consideration of the kinds of fungi that

are likely to be released from indoor reservoirs may allow focusing of data analysis procedures.

In most of the studies reviewed here, only univariate analyses were presented and possible confounding factors in the relationship(s) under study were not evaluated. Björnsson<sup>39</sup> and Wood<sup>40</sup> considered microorganisms other than fungi as well, but they reported no multivariate analyses including both fungi and other microorganisms.

### CONCLUSIONS

Fungi do contribute to allergic disease, and the extent of their involvement is probably greater than is indicated by the available clinical and epidemiologic studies. This review of epidemiologic studies where fungal exposure was quantitatively assessed did not reveal data to allow the setting of guidelines for fungi in home environments based on health risk assessment.

Future studies designed to quantitate the role of fungi in allergic disease should address the following four factors.

1. Studies should focus on susceptible populations. The nature of sensitive populations depends on the hypothesis under consideration. To investigate the hypothesis that exposure to fungi plays a sensitizing role in the development of inhalation allergies and asthma, newborns with a family history of allergy/asthma are appropriate. To investigate the hypothesis that exposure to fungi plays an important role in the exacerbation of respiratory allergies in atopic people, the study population should consist of atopic children (>5 years old) or adults. Finally, to investigate the hypothesis that exposure to specific fungi is a risk for acute exacerbation of symptoms in fungal allergic patients, the study population should include atopic people with a clinical history consistent with fungal allergy.
2. Measured health outcomes should be specific for allergic disease. Daily incidence of respiratory

symptoms (using validated questionnaires), medication use, and variation in lung function (peak expiratory flow rate) can be used. Sensitization to fungi can be assessed by skin prick testing and/or RAST assays, preferably using extracts specially prepared from fungi recovered in the subjects' homes.

3. Optimal use of available exposure methodologies should be used. Exposure assessment protocols should include accurate representations of exposures at home, outdoors, and in other indoor settings. The traditional sampling methods for culturable fungi in air or dust should be collected in duplicate and should be repeated over time within one season and in different seasons. As culturable fungal particles may only comprise a small fraction of the total, a method for measuring non-culturable particles should be included. Microscopic identification of particles is readily applied to air samples. Measurements of indicator substances such as  $\beta$ -1,3-D-glucan, ergosterol, or extracellular polysaccharides have been successfully applied to dust samples.<sup>36,64-66</sup>  $\beta$ -1,3-D-Glucans are components of the cell wall of most fungi, and ergosterol is the principal fungal sterol so that these measures provide no information on the kinds of fungi present. Extracellular polysaccharides may include genus-specific antigens.<sup>67</sup> The relationships of these measures to more traditional analytical approaches needs additional study.
4. Finally, the role of co-pollutants needs investigation. Both indoor and outdoor exposure to other inhalant allergens (eg, dust mite, cockroach, furry animal, and pollen allergens) as well as inflammatory agents (eg, endotoxin) and irritants (ozone, nitrogen oxides, and volatiles) may exacerbate responses to fungal allergens.

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*Request for reprints should be addressed to:*  
 Arnoud P Verhoeff, PhD  
 Municipal Health Service  
 Department of Epidemiology  
 PO Box 20244  
 1000 HE Amsterdam  
 The Netherlands

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## CME Examination

Identification No. 007-006

Questions 1-24, Verhoeff AP and HA Burge. 1997;18:544-56.

### CEM Test Questions

- The prevalence of fungal allergy in the general population is approximately
  - 0.1%
  - 1.0%
  - 5.0%
  - 10.0%
  - 20.0%
- The highest prevalence of sensitization to fungi is generally found among asthmatics of age
  - 0-5 yr
  - 6-10 yr
  - 11-20 yr
  - 21-50 yr
  - >50 yr
- Using commercially available fungal extracts for skin prick testing
  - generally over-estimates the prevalence of sensitization to fungi
  - gives a precise estimate of the prevalence of sensitization to fungi
  - gives highly reproducible results, independent of the nature of the extracts used
  - generally under-estimates the prevalence of sensitization to fungi
  - none of the above
- The best approach to assess fungal related respiratory allergy is
  - to use self administered questionnaires regarding respiratory symptoms
  - to perform skin prick testing using commercially available fungal extracts
  - to measure specific IgE to fungal allergens by means of commercially available RAST test kits
  - to perform skin prick testing using extracts of fungi to which patients are actually exposed
  - to perform pulmonary function measurements
- The best available approach to estimate the exposure to fungi is
  - to culture fungi in settled house dust samples
  - to perform gravity sampling
  - to take air samples using culture plate impactors
  - to take air samples by filtration, providing data on both total fungal counts and culturable counts
  - none of the above
- Which of the following statements regarding air sampling for fungi is correct?
  - one air sample provides valid information for exposure over time
  - the reproducibility of simultaneous duplicate samples is high
  - differences between houses are usually greater than differences within houses over time
  - settle or gravity sampling provides valid quantitative information
  - the choice of culture medium is irrelevant with respect to the results from cultural air samples
- Which of the following statements regarding the setting of guidelines for fungi in home environments is correct?
  - the available data do allow the setting of guidelines based on health risk assessment
  - the available data do not allow the setting of guidelines based on health risk assessment
  - published guidelines for acceptable exposure are based on health risk assessment
  - to set guidelines based on health risk assessment exposure-response relationships are irrelevant
  - none of the above
- To investigate the hypothesis that exposure to fungi plays a sensitizing role in the development of inhalation allergies and asthma, the following study design is appropriate:
  - cross-sectional, sample of the general population
  - cross-sectional, atopic children
  - cross-sectional, atopic adults
  - prospective, atopic children
  - prospective, genetically predisposed newborns
- The most commonly used approach to assess the exposure to fungi is
  - gravity sampling
  - culture of fungi in settled house dust samples
  - air sampling using culture plate impactors
  - air sampling using filtration, for total and culturable counts
  - air sampling using filtration, for immunochemical analyses
- Which of the following shortcomings in the available studies is most important regarding the fact that there are no data to allow the setting of guidelines for fungi in home environments based on health risk assessment?
  - study design
  - data analysis
  - lack of validity of methods used to assess the exposure
  - lack of validity of methods used to assess symptoms
  - all of the above are equally important
- Total counts of culturable fungi
  - are probably not the best measure for evaluation the role of fungi in asthma
  - are the easiest way to assess fungal spore exposure
  - provide useful information with respect to immunotherapy decisions
  - overestimate actual exposure to fungi
  - adequately represent fungal allergen exposure

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12. Approximately what percentage of modern homes appear damp or moldy?
    - a. 5%
    - b. 10–15%
    - c. 20–50%
    - d. 40–60%
    - e. about 75%
  13. Outdoor fungal exposures
    - a. can be ignored when studying indoor exposures
    - b. are always higher than exposures indoors
    - c. have been shown to increase pulmonary complaints
    - d. only occur in September and October
    - e. are usually similar to those indoors
  14. Filtration sampling to assess fungal exposure
    - a. is the most accurate method available
    - b. results in underestimates of viable (culturable) fungi
    - c. should be used only in low-spore environments
    - d. requires the use of immunoassays
    - e. is simple and reproducible
  15. Which of the following statements is NOT true regarding time-discriminating spore trapping:
    - a. spore concentrations can be mapped over time
    - b. analysis of spore trap samples requires specialized skills
    - c. most investigators use spore trapping for indoor exposure measurement
    - d. samples can be collected continuously for as long as a week
    - e. spore traps have been used in outdoor fungal spore studies
  16. Fungi that are common in dust
    - a. are also common in air
    - b. have all settled out from the air
    - c. are most important with respect to human exposure
    - d. are not necessarily common in air
    - e. none of the above
  17. Factors controlling fungal allergen production
    - a. are well known
    - b. include many unknown factors
    - c. are primarily genetic
    - d. vary with season
    - e. are the same as those for any other allergen source
  18. In order to interpret fungal air sampling data one must
    - a. be a statistician
    - b. understand how fungal populations vary in time and space
    - c. be a mycologist
    - d. have personally collected samples
    - e. none of the above
  19. Interactions between fungal and other allergens
    - a. are well-known
    - b. have not been studied
    - c. are not important
    - d. are known to depend on the presence of air pollutants
    - e. none of the above
  20. In order to study fungal-related exacerbation of asthma, clinical studies should
    - a. focus only on children
    - b. include all atopic individuals
    - c. include only people with positive skin tests to *Alternaria*
    - d. include all asthmatic patients
    - e. none of the above
  21. To study fungal-related allergic disease, exposure assessment should
    - a. only include fungi for which skin test materials are available
    - b. focus primarily on the indoor environment
    - c. include only fungal assessment
    - d. include measures of exposure at home, outdoors, and in other indoor environments
    - e. include only measures of cultural fungi
  22. Endotoxin
    - a. is a part of fungal cell walls
    - b. is an inflammatory agent and may exacerbate responses to fungal allergens
    - c. is irrelevant with respect to fungal allergen exposure
    - d. is a mycotoxin
    - e. none of the above
  23. (1->3) $\beta$ -D-glucans
    - a. provide the best indicator for the presence of specific fungal allergens
    - b. are a part of most fungal cell walls
    - c. are not associated with building related complaints
    - d. are cancer-causing mycotoxins
    - e. are allergens
  24. Multiple skin tests to different fungal allergens implies
    - a. cross-reactivity among fungal allergens
    - b. independent sensitivities to many different fungi
    - c. non-specific reactivity to fungal extracts
    - d. a and b
    - e. none of the above
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