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Interim Letter VIII

Mike Winkler, First Vice-President
Administrative and Residual Employees Union Local 4200
705 North Mountain Road, Suite A211
Newington, Connecticut 06111-1411

Dear Mr. Winkler:

Included with this memo are hard copies of a paper submitted to a scientific journal 'Environmental Health Perspectives.' The paper was recently accepted for online publication on October 9th, 2007. The results included in the paper are from the 2001 questionnaire survey and the 2002 environmental survey at the 25 Sigourney Street Building.

The major findings described in the paper are as follows:

1. On average, 57% and 45% of total fungal colonies cultured from floor and chair dust samples, respectively, were identified as hydrophilic (water-loving) fungi (Yeasts, *Phoma herbarum, Chaetomium globosum, Mucor plumbeus, Rhizopus stolonifer, and Stachybotrys chartarum* were the hydrophilic fungi identified from the samples).
2. Even though the levels of total culturable fungi were low [Geometric Mean (GM)=7700 cfu/g, 2000 cfu/m^2 in floor dust; GM=11000 cfu/g, 4900 cfu/chair in chair dust], there were significant associations between the levels of total fungi in dusts and various health outcomes (respiratory cases, epidemiologically-defined asthma cases, and physician diagnosed post-occupancy current asthma cases) with odds ratios of 1.32–1.67.
3. The associations between the fungal levels and the health outcomes were mostly driven by hydrophilic fungi, which implies that the hydrophilic fungi played a major role in the associations of health with fungal exposure.
4. Ergosterol (a fungal cell wall component) and endotoxin (a gram-negative bacterial cell wall component) were also significantly associated with respiratory cases and epidemiologically-defined asthma cases.

Associations between environmental measures and health status do not necessarily imply cause. Cross-sectional analyses cannot prove that the environmental correlations preceded health changes. Environmental measurements may be correlated with an unmeasured cause, an indicator of the true cause. Nonetheless our results support that the exposure to microbial agents in the building might have been involved in the process of disease development.

If you have any questions regarding the information provided in this interim letter, please do not hesitate to contact us at 1-800-232-2114.

Sincerely,

Ju-Hyeong Park

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Hydrophilic Fungi and Ergosterol Associated with Respiratory Illness in a Water-damaged Building

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Disclaimers: The findings and conclusions in this paper have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be construed to represent any agency determination or policy.

Abbreviations:

95% CI	95% Confidence interval
Aw	Water activity
CFU	Colony forming unit
ECRHS	European Community Respiratory Health Survey
Epi-asthma	Epidemiologically defined asthma or asthma-like symptoms
EU	Endotoxin unit
GAM	Generalized additive model
GSD	Geometric standard deviation
GM	Geometric mean
HP	Hypersensitivity pneumonitis
IQR	Inter-quartile range
LOD	Limit of detection
NIOSH	National Institute for Occupational Safety and Health
OR	Odds ratio
spp.	Species

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Abstract

Background: Damp building-related respiratory illnesses are an important public health issue.

Objective: We compared three respiratory case groups defined by questionnaire responses [200 respiratory cases, 123 of these who met the epidemiological asthma definition, and 49 employees who had current physician-diagnosed asthma with post-occupancy onset] to a comparison group of 152 asymptomatic employees in an office building with a history of water damage.

Methods: We analyzed dust samples collected from 323 cases and comparisons' floors and chairs for culturable fungi, ergosterol, endotoxin, and cat and dog allergens. We examined associations of total fungi, hydrophilic fungi (requiring water activity≥0.9) and ergosterol with the health outcomes using logistic regression models.

Results: In models adjusted for demographics, respiratory illnesses showed significant linear exposure-response relationships to total culturable fungi [interquartile range odds ratios (IQR-ORs)=1.37–1.72], hydrophilic fungi (IQR-ORs=1.45–2.19), and ergosterol (IQR-ORs=1.54–1.60) in floor and chair dusts. Of three outcomes analyzed, current asthma with post-occupancy physician diagnosis was most strongly associated with exposure to hydrophilic fungi in models adjusted for ergosterol, endotoxin and demographics (IQR-OR=2.09 for floor and 1.79 for chair dusts). Ergosterol levels in floor dust were significantly associated with epidemiological asthma independent of culturable fungi (IQR-ORs=1.54–1.55).

Conclusions: Our findings extend the 2004 conclusions of the Institute of Medicine by showing that mold levels in dust were associated with new-onset asthma in this damp indoor environment. Hydrophilic fungi and ergosterol as measures of fungal biomass may have promise as markers of risk of building-related respiratory diseases in damp indoor environments.

Introduction

Mold is ubiquitous in normal indoor and outdoor environments and thus, some exposure is inevitable in everyday life. However, exposure to increased levels of mold and other microbial agents has been implicated in diseases associated with damp indoor environments (Menzies and Kreiss 2006; Park et al. 2006). In the absence of indoor amplification, the fungal profiles inside buildings should be similar to those outdoors (Flannigan and Miller 2001). Increased moisture levels due to water intrusion can support mold growth and may change the profile of fungal populations in a building. Hydrophilic (water-loving) fungi requiring 0.9 or higher water activity (Aw, the amount of free or available water in substrates) will overgrow mesophilic ($0.8 \leq Aw < 0.9$) and xerophilic fungi ($Aw < 0.8$) in damp conditions (Flannigan and Miller 2001; Grant et al. 1989). The presence of hydrophilic fungi is considered an indicator of building dampness (Flannigan and Miller 2001), yet quantitative measures of hydrophilic fungi in damp buildings have not had been studied in relation to health effects.

The analytical method most frequently applied for fungi, a culture technique, is not likely to measure the relevant microbial exposures accurately, because any selected medium grows only a small proportion of the viable spores and because culture counts do not account for non-viable spores and fungal fragments. However, ergosterol, a principal sterol in the fungal membrane, has been suggested as a good measure for fungal biomass (Newell 1994; Sebastian and Larsson 2003; Szponar and Larsson 2000) since it is analyzed by a chemical technique which measures viable and non-viable spores and fungal fragments. However, only a few researchers have attempted to measure ergosterol in environmental samples for assessing exposure to fungi in epidemiological studies (Dales 1998; Dales et al. 1999; Dharmage et al. 2002; Dharmage et al. 2001; Matheson et al. 2005; Mendell et al. 2002). More research using

ergosterol measurements for fungal exposure assessment would be useful to better understand the association of fungal exposure with health.

This report focuses on examining associations of hydrophilic fungi and ergosterol with respiratory health outcomes among employees in a 20-story office building in the northeastern United States. Within a few months of building occupancy in 1994, employees perceived new-onset respiratory conditions to be building-related and complained of a further increase in symptom severity and frequency beginning in the fall of 2000. Sentinel cases of post-occupancy-onset asthma, hypersensitivity pneumonitis (HP), and sarcoidosis had been diagnosed. A building-wide self-administered questionnaire survey in September 2001 [67% participation rate (888 out of 1,327)] documented an excess of respiratory symptoms and asthma prevalence, and a 7.5-fold increased incidence density of adult post-hire-onset asthma (Cox-Ganser et al. 2005; Park et al. 2006), in comparison with pre-occupancy incidence.

Materials and methods

Case and comparison group definitions

We nested a case-comparison study within the participants in the 2001 cross-sectional questionnaire survey. We defined respiratory cases (n=200) as those who had occupied the building at least one year and reported 1) current asthma with post-occupancy physician diagnosis, physician-diagnosed HP or sarcoidosis (potential building-related respiratory disease diagnoses); or 2) three or more of 5 asthma-like symptoms (wheeze or whistling in the chest, chest tightness, shortness of breath, coughing, and awakened by breathing difficulty) occurring weekly over the past 4 weeks; or 3) two or more of 3 symptoms consistent with HP (shortness of breath when hurrying on level ground or walking up a slight hill, fever and chills, or flu-like achiness or achy joints) occurring weekly over the past 4 weeks. Since we were interested in

restricting analyses to asthma as the outcome, we defined epidemiological asthma (epi-asthma) cases (n=123) as having current asthma with post-occupancy physician diagnosis, or three or more of 5 asthma-like symptoms. Finally, to increase the specificity of our asthma definition, we defined post-occupancy asthma cases (n=49) as those with current asthma with post-occupancy physician diagnosis. The epi-asthma cases were a subset of the respiratory cases, and the post-occupancy asthma cases were a subset of the epi-asthma cases. We defined the comparison group (n=152) as those who reported none of the lower respiratory or HP-like symptoms in the past year, as well as no physician-diagnosed HP, sarcoidosis, or post-occupancy asthma. An informed consent procedure was followed before participants completed the questionnaire (approved by the National Institute for Occupational Safety and Health Human Subjects Review Board).

Environmental sampling and analysis

In April 2002, we collected floor and chair dust samples from workstations of 323 case and comparison group employees. We sent dust samples for analyses of culturable fungi, ergosterol, endotoxin, cat allergen, and dog allergen. Fungal colonies were cultured with malt extract (selective for a broad spectrum of fungi), cellulose (selective for cellulolytic fungi), and dichloran 18% glycerol (selective for xerophilic fungi) agars at room temperature for 7-10 days, identified to species level, and enumerated. If a species grew on more than one medium, a standardized laboratory protocol was used to select which medium would be the basis for the reported colony count results. Ergosterol was analyzed with gas chromatography-mass spectrometry (Sebastian and Larsson 2003). Endotoxin was analyzed using the kinetic quantitative chromogenic Limulus amoebocyte lysate (KQCL) method (Chun et al. 2002). Cat (Fel d 1) and dog (Can f 1) allergens were analyzed with an enzyme-linked immunosorbent assay

(Chapman 1988; de Groot 1991). For epidemiological analyses, we used units per m^2 or per chair based on our previous findings (Park et al. 2006). Details of the methods are described elsewhere (Park et al. 2006).

Data analysis

Due to right-skewed distributions, we transformed all the environmental measurements using natural logarithms and reported geometric means (GM) and geometric standard deviations (GSD) by exponentiating the means and standard deviations of the log transformed data. We assigned a value of half the limit of detection (LOD) to samples below the LOD due to the large GSD (Hornung and Reed 1990). We grouped fungal species into mesophilic ($0.8 \leq Aw < 0.9$) and hydrophilic ($Aw \geq 0.9$) categories based on Aw (Burge and Otten 1999; Flannigan and Miller 2001; Grant et al. 1989), and created a combined group of mesophilic and hydrophilic fungi ($Aw \geq 0.8$). We created two additional groups: fungi not classified as having an $Aw \geq 0.8$ and fungi not classified as hydrophilic.

We used analysis of variance (ANOVA) to compare the levels of microbial agents in floor and chair dust. We estimated odds ratios (ORs) for each case definition in relation to various microbial indices using multivariate linear logistic regression models (SAS 9.1, SAS Institute, Cary, NC, USA). Single environmental variable models included one environmental variable and demographics (age, gender, race, smoking status, and building occupancy time) which are potential confounding factors and were also adjusted for in the models used in our previous publication (Park et al. 2006). Multiple environmental variable models included demographics and three environmental variables [ergosterol, endotoxin, and total fungi (total fungi models) or hydrophilic fungi (hydrophilic fungi models)]. We performed additional analyses to examine the effects of fungi which were not classified as having an $Aw \geq 0.8$ or fungi which were not

classified as hydrophilic on health outcomes using the single environmental variable models. Since the interactions among these environmental variables were not significant for all three outcomes, we did not include interactions in the final models. We examined possible non-linear relationships between exposure and health outcomes using generalized additive models with a smoothing spline function (degrees of freedom=4; S-Plus 6.1, Insightful Corporation, Seattle, WA, USA). We reported adjusted ORs and 95% confidence intervals based on increase in exposure by interquartile range (IQR=75th-percentile minus 25th-percentile).

Results

On average, the cases and comparisons in the study were 46-years-old and had occupied the building for 6 years (Table 1). More than half of them were white (69%), never smokers (61%), and female (59%). There were fewer white employees and never smokers but more females in the case groups than in the comparison group. The proportion of current smokers was lowest (6.1%) in the post-occupancy asthma cases.

We collected 338 floor and 327 chair dust samples from 323 employees' workstations among the 352 case and comparison group employees. We could not locate workstations for 29 participants. For those who had multiple samples due to changes in their workstations between September 2001 and April 2002, we assigned measurements of microbial agents from the workstation they occupied during the 2001 questionnaire survey. Due to the limited amount of dust collected for some samples, we prioritized sample analysis by endotoxin, culturable fungi, ergosterol, and allergens. We recovered a total of 67 fungal species from floor dust samples and 69 species from chair dust samples. In addition, unidentifiable species of *Penicillium*, yeasts (*Rhodotorula* and *Sporobolomyces*), and non-sporulating fungi were cultured. The GM of total culturable fungi was 7,700 colony forming units per gram (cfu/g) in floor dust which was

significantly ($p<0.005$) lower than that (11,000 cfu/g) in chair dust (Table 2). In the floor dust, on average, 57% of total fungal colonies were identified as hydrophilic fungi and 19% as mesophilic. In the chair dust, on average, 45% of total fungal colonies were identified as hydrophilic and 28% as mesophilic. Eighty-seven percent of the hydrophilic fungi in floor dust and 74% of those in chair dust were yeasts. GMs of the ergosterol (0.5 ng/mg) and endotoxin (10.9 EU/mg) levels in floor dust were significantly (p-values<0.002) higher than those in chair dust (0.4 ng/mg and 2.1 EU/mg, respectively). The levels of cat (GM=2.5 μg/g) and dog (2.1 μg/g) allergens were significantly (p-values<0.0001) lower in floor dust than those in chair dust (GMs 12.5 μg/g and 5.7 μg/g, respectively) (Table 2).

Aureobasidium pullulans and *Epicoccum nigrum* were the most prevalent of the mesophilic fungi (Table 3). Yeasts and *Phoma herbarum* were the most prevalent hydrophilic fungi recovered and their median concentrations (2,400 cfu/g in floor dust and 2,340 cfu/g in chair dust for yeasts; 2,850 cfu/g in floor dust and 3,600 cfu/g in chair dust for *Phoma herbarum*) were among the highest found for the mesophilic and hydrophilic fungi. *Rhodotorula* was the predominant genus of yeast identified from floor (49.4%) and chair (46.0%) dust samples. Among those which were not classified as having an Aw of 0.8 or higher, *Penicillium chrysogenum* (12.5%) and *Pithomyces chartarum* (10.4%) were the most frequently found species in floor dust samples, and *Pithomyces chartarum* (23.3%) and *Phoma glomerata* (11.7%) in chair dust samples. *Penicillium chrysogenum* and *Aspergillus niger* were the most prevalent *Penicillium/Aspergillus* species identified in both floor and chair dust. Non-identifiable *Penicillium* species were found from 17.1% and 13.5% of the floor and chair dust samples, respectively. We found *Stachybotrys chartarum* in 4 floor samples and 5 chair samples.

The levels of ergosterol per m^2 or chair were significantly (p-values < 0.0001) but poorly correlated with total fungi, fungi requiring Aw≥0.8, hydrophilic fungi, and yeasts both in floor and chair dust samples (r=0.15–0.27). Ergosterol had a higher correlation with endotoxin in floor dust (r=0.47) and chair dust (r=0.34). Ergosterol and culturable fungi were also significantly but weakly correlated with cat and dog allergens in both floor and chair dust (r=0.12–0.31). Endotoxin had a higher correlation with cat (r=0.39) and dog allergens (r=0.36) in chair dust than in floor dust (r=0.25 for cat and 0.21 for dog allergens).

The number of subjects in each of the statistical models varied, depending on the number of subjects with information missing in any of the demographic variables or the exposure variables. We found significant linear exposure-response relationships between various microbial measurements (total fungi, fungi requiring Aw≥0.8, hydrophilic fungi, ergosterol, and endotoxin) in dust and health outcomes (respiratory cases, epi-asthma cases, and post-occupancy asthma cases) in the single environmental variable models. We found no significant associations (at α=0.05) among health outcomes and fungi which were not classified as having an Aw≥0.8, or fungi which were not classified as hydrophilic (Table 4). In generalized additive models, test p-values of these environmental variables for a null hypothesis of linearity were greater than 0.05, which indicates no evidence of non-linear relationships between exposure and health outcomes. The associations between health outcomes and fungi were mostly driven by exposure to fungi requiring Aw≥0.8 and specifically hydrophilic fungi in both floor and chair dust.

In single environmental variable models, we found more than 65% increases in the odds of being a respiratory case for increasing interquartile range (IQR) exposure [IQR-odds ratio (OR)=1.66–1.73; p-values<0.05] for total fungi, fungi requiring Aw≥0.8, and hydrophilic fungi in floor dust (IQR for each of the microbial measurements for all dust samples analyzed are

presented in Table 2). Yeasts in chair dust and non-yeast hydrophilic fungi in floor dust were significantly associated with increases in the odds of being a respiratory case of 46% and 50%, respectively (Table 4). Ergosterol and endotoxin in floor dust were associated with significantly increased odds of being a respiratory case (IQR-OR=1.56 and 1.60, respectively). Cat and dog allergens in floor dust were not significantly associated with respiratory cases at α=0.05. In general, the odds of being a respiratory case for chair dust were lower than those for floor dust, except for the yeasts.

When compared to respiratory case outcome models, we found slightly larger magnitudes of IQR-ORs (range: 1.46–1.80) for epi-asthma cases associated with total culturable fungi, fungi requiring Aw≥0.8, hydrophilic fungi, and ergosterol in both floor and chair dust (Table 4). When we examined the associations of those who reported physician-diagnosed HP or having two or more HP-like symptoms with microbial exposures, we found similar magnitudes of ORs for fungi but stronger associations with ergosterol (IQR-OR=1.93) and endotoxin (IQR-OR=1.80) in floor dust, and yeasts in chair dust (IQR-OR=1.65) (Data not shown).

In the models with post-occupancy asthma cases as an outcome, the associations with fungi requiring Aw≥0.8, hydrophilic fungi, and yeasts in both floor and chair dust were stronger than those in the models with respiratory and epi-asthma cases as outcomes (Table 4). Of all the environmental variables, hydrophilic fungi in floor dust (IQR-OR =2.19) were most strongly associated with post-occupancy asthma cases. Increased exposure to yeasts in floor dust and hydrophilic fungi without yeasts in chair dust by IQR significantly increased the odds of being a post-occupancy asthma case by 77% and 44%, respectively.

When we ran models with culturable fungi, ergosterol, endotoxin and demographic variables simultaneously, the ORs of the respiratory and epi-asthma cases for total fungi and

hydrophilic fungi in both floor and chair dust were slightly smaller (IQR-OR: 1.46–1.62 for floor dust and IQR-OR: 1.36–1.57 for chair dust) but generally remained significant at α=0.05. The exception was the modeling of respiratory cases with total chair fungi (IQR-OR =1.36, p=0.06) adjusted for ergosterol, endotoxin, and demographic variables (Table 5). In the total fungi models of the multiple environmental variable models, the magnitude of the IQR-ORs for the respiratory and epi-asthma cases associated with total culturable fungi was smaller than that for hydrophilic fungi in the hydrophilic fungi models. Exposure to hydrophilic fungi in floor and chair dust were associated with about a two-fold increase in the odds of being a post-occupancy asthma case (IQR-ORs: 2.09 for floor dust and 1.79 for chair dust) (Table 5). Generalized additive models (GAM) with non-linear spline smoothing functions did not provide evidence of non-linear relationships - that is, the log odds (logit) of respiratory illnesses in all of these multiple environmental variable models increased linearly with increase of exposure. A sensitivity analysis was performed by re-running all statistical models after assigning floor-specific mean values (new models) of microbial measurements to the respiratory cases and comparisons with no individual measurements. For each exposure variable we calculated the ratio (expressed as percentage) of the odds ratios for the new model to the original model. The results showed that the models were not substantially sensitive since the odds ratio from the new models ranged from 84.8% to 102.8% of the original odds ratios presented in Tables 4 and 5.

Discussion

Among employees of a water-damaged office building, we found linear associations between respiratory illnesses and the levels of fungi, which were largely explained by hydrophilic (water-loving or tertiary) fungi including yeasts. These linear exposure-response relationships based on individual exposure measurements extend our previous findings based on

exposure assigned as floor-specific mean values of fungal measurements (Park et al. 2006). Our current study indicates that exposure assessment using individual dust samples has an advantage over assigning exposure using floor-specific mean values because we found associations between physician-diagnosed post-occupancy onset asthma and fungal exposure which were not demonstrated in the previous study. The enhanced findings are likely to be explained by minimization of exposure misclassification by using individual samples in exposure assessment. Of the three respiratory health outcomes studied, asthma diagnosed after building occupancy had the strongest association with the levels of hydrophilic fungi in dust. This finding implies that a more specific (less sensitive) definition of outcome based on physician diagnosis, which likely represents more severe disease, probably minimized health outcome misclassification in relation to building exposures. We previously reported that the incidence of adult-onset asthma in this population was 7.5 times higher after building occupancy (Cox-Ganser et al. 2005). Taken together, these findings are consistent with the involvement of building-related fungal exposure in the causal chain of adult-onset asthma, although we cannot rule out that fungi, specifically hydrophilic species, may be simply markers of other causative agents in damp environments.

Among hydrophilic fungi, yeasts in both floor and chair dust played an important role in associations we found for increased odds of respiratory illnesses. Yeasts have been reported to be among the most abundant fungi found in indoor air (Cheong et al. 2004; Rantio-Lehtimaki 1988), and in house dust (Flannigan et al. 1993; Verhoeff et al. 1994). Similarly, in our study, the *Rhodotorula* genus of yeasts was one of the most prevalent and abundant fungi recovered in floor and chair dust. *Rhodotorula* species have been shown to be implicated in IgE-mediated allergy responses (Day and Ellis 2001), as well as being potential causative agents of HP in case studies (Hodges et al. 1974; Siersted and Gravesen 1993). Our study also showed that yeasts in

chair dust were significantly associated with an epidemiological definition of HP (those who reported physician-diagnosed HP or having two or more HP-like symptoms). In occupational environments such as bakeries, breweries, and distilleries, the yeast *Saccharomyces cerevisiae* is a major allergen source for allergic diseases (Day and Ellis 2001). Without yeasts, the hydrophilic fungi as a group (*Phoma herbarum*, *Chaetomium globosum*, *Mucor plumbeus*, *Rhizopus stolonifer,* and *Stachybotrys chartarum*) were also strongly associated with odds of respiratory illnesses. In these models, we assigned a value of half the LOD (200 cfu/g) to a large proportion of samples below the LOD (Table 2), and these assigned values were multiplied by the amount of dust collected for each subject to obtain cfu per m^2 or chair. This assignment might have produced non-differential misclassification in exposure, resulting in the underestimation of odds ratios. Even with this potential misclassification, we still found significant associations between hydrophilic fungi without yeast and respiratory illnesses. *Phoma* spp., *Mucor* spp., *Rhizopus* spp. have been implicated in IgE-mediated allergy (Day and Ellis 2001). However, we are not aware of epidemiological studies demonstrating increased risk of building-related asthma or other respiratory illnesses associated with yeasts and other hydrophilic fungi in floor and chair dusts in water-damaged non-industrial buildings.

We found poor correlations ($r<0.3$) between ergosterol levels and culturable fungi in more than 300 dust samples, although Saraf et al. reported a higher correlation ($r=0.65$) in 17 house dust samples (Saraf et al. 1997). This is not a completely unexpected result for the following reasons. First, ergosterol is found in mycelia and fungal fragments, as well as intact spores (Miller 2001). Second, ergosterol can be detected in both viable and non-viable spores. Third, culturable fungi represent only a small portion of the viable spores that can grow on the selected media (Dales et al. 1999; Saraf et al. 1997). Lastly, the proportion of viable and nonviable spores

may differ across the samples, and the proportion of the viable spores that can be cultured may also differ. Hence, ergosterol has the potential to measure fungal biomass more accurately than the culture technique. Since health effects such as allergy and inflammation do not rely on viability of fungal contaminants, measuring ergosterol to estimate total fungal biomass in exposure assessment is warranted (Nielsen and Madsen 2000; Pasanen et al. 1999).

A few research groups have used ergosterol measurements for exposure assessment in epidemiological studies, and the results have been inconsistent. In a cross-sectional analysis of the European Community Respiratory Health Survey (ECRHS) sub-cohort followed up in 1996 (n=485), Dharmage et al. found a significant association of ergosterol levels in bedroom dust with sensitization to fungi and having wheezed in the last year (Dharmage et al. 2001). They also performed a longitudinal analysis on repeated measurements of wheeze and ergosterol in bedroom dust in 1996 and 1998 on the same ECRHS sub-cohort. They found a statistically significant interaction effect in which the effect of increasing ergosterol on the chance of remission of wheeze depended on the initial levels of ergosterol in 1996 (Matheson et al. 2005). From the same ECRHS sub-cohort, 35 young asthmatic adults sensitized to fungi were followed over 4 seasons. No association was found between either culturable fungi or ergosterol levels in bedroom floor dust and peak flow variability. However, in this study, the authors discussed that this lack of association could have been partly explained by misclassification of exposure (Dharmage et al. 2002). In a study of children in Canada, Dale et al. reported a significant association between living in fungal-contaminated homes having higher airborne ergosterol levels and an increased number of CD3+ T cells expressing CD45RO (Dales 1998). In their later study (Dales et al. 1999), they examined the association of airborne ergosterol levels in bedrooms with respiratory symptoms and nocturnal cough among elementary school children but

did not find associations. In this study (Dales et al. 1999), airborne ergosterol levels were estimated from less-than-a-day sampling. This might have misclassified children's exposure in relation to the one and 12 month time periods covered by the symptom questionnaire. Mendell et al. measured airborne ergosterol in a double-blind cross-over study evaluating the effect of replacement of filters on occupants' symptoms and indoor particles in an office building; they found ergosterol levels below LOD for 7 of 8 air samples (Mendell et al. 2002).

In contrast, we found that 230 or 260 ng increases in ergosterol levels per square meter of floor or per chair respectively, elevated the odds of respiratory illnesses (especially epi-asthma cases) by 46% to 55% in models adjusted for demographics, culturable fungi, and endotoxin. It is not known whether ergosterol can directly induce respiratory health effects or if it is a surrogate measure of exposure to fungi or of another unmeasured exposure related to dampness. However, our finding of associations between ergosterol and health outcomes, independent of culturable fungi, suggests that measuring both ergosterol and culturable fungi may be important to fully understand health effects associated with fungal exposure in epidemiological studies.

In this study building which had a long history of water damage, the first major construction activity related to water incursion began in 2000, with repair of roof copings and brick caulking. From 2000 to 2002, cubicle partitions and carpets were cleaned, wetted carpet and stained wallboard replaced, wallpaper and underlying mold removed from bathrooms, upgrades to the air handling system made, and windows caulked. In our study, the levels of culturable fungi in dust sampled in 2002, seven months after the 2001 questionnaire survey were low in comparison with those in other studies of office buildings (Chao et al. 2001), school buildings (Ebbehoj et al. 2005), and residential buildings (Hicks et al. 2005) with no apparent water damage. The historical levels of fungi before our study were higher. For example, three

consultant reports on 20 surface dusts from 2000 to 2001 showed a range of 12,000–7,800,000 cfu/g as compared to our current study range of 276–1,200,000 cfu/g in floor dust. Furthermore, fungal contamination was found in the walls. We surmise that the relative differences in occupants' exposure and fungal profile in the dust at individual workstations might have remained even though the remediation action changed the absolute levels of microbial contaminants. This would explain the association between the fungal exposure and health effects even at the low absolute levels of fungi in the dust.

The fungal profile in our dust samples was predominantly hydrophilic and mesophilic fungi. At the time of our study, the carpet and chairs generally showed low water activity (0.18-0.8 with a mean of 0.5). Historical reports indicated that the building had extensive water damage in the past, and our fungal profile analyses support that there must have been wet conditions. Hydrophilic fungi are not likely to become predominant unless wet conditions persist for an extended time (Flannigan and Miller 2001). In a study of houses without water damage, water indicator fungi (*Chaetomium* spp, *Ulocladium* spp., and *Stachybotrys* spp.) were largely absent from air and dust samples (Horner 2004). Furthermore, since the spores of the four most dominant fungi we found in both floor and chair dusts (yeasts, *Aureobasidium pullulans*, *Alternaria alternata*, and *Epicoccum nigrum*) have long survival times (Flannigan and Miller 2001), they can remain in relatively dry conditions as indicators of past dampness.

A number of different fungal genera and species have been used as indicators of water-damaged indoor environments (Burge and Otten 1999; Flannigan and Miller 2001). We know of no source which lists the water activities for all fungi. Furthermore, minimum and optimum water activity for growth of individual fungal species can differ depending on environmental condition such as temperature and nutrient availability (Burge and Otten 1999). We categorized

mesophilic and hydrophilic fungi based on three publications (Burge and Otten 1999; Flannigan and Miller 2001; Grant et al. 1989), and thus we may have some misclassification. However, since most of the fungi with prevalence of more than 10% were classifiable based on those reports, the misclassification in water activity categorization is not likely to change our findings in the study.

In conclusion, we showed that among employees in a building with a long history of water damage, respiratory symptoms and post-occupancy asthma were strongly associated in a linear exposure-response manner with fungi, especially the levels of hydrophilic fungi (including yeasts) in dust. These findings extend the conclusions of the Institute of Medicine of insufficient evidence for the development of asthma in relation to the presence of mold or other agents in damp indoor environments (Institute of Medicine 2004). Since the markers (total culturable fungi, hydrophilic fungi, and ergosterol) of potential mold exposure were associated with health outcomes, we suggest that further research to understand respiratory health effects in water-damaged indoor environments include measurements of both ergosterol and speciated culturable fungi in dust.

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Table 1. Demographics of respiratory cases, epidemiologically defined asthma cases, current post-occupancy asthma cases, and comparison group.

Demographics	Total: Respiratory case plus Comparison (n=352)	Respiratory cases[a] (n=200)	Epidemiologic Asthma cases[a] (n=123)	Post-occupancy Dr.-diagnosed Asthma cases[a] (n=49)	Comparison group[a] (n=152)
Age (Mean ± SD)	46.4 ± 8.6	46.3 ± 8.4	45.8 ± 7.1	46.4 ± 7.4	46.5 ± 8.8
Race (%)					
White	68.8	65.1	69.8	57.1	73.7
Black	20.7	23.6	20.2	28.6	16.9
Other	10.5	11.3	10.1	14.3	9.5
Gender (% Female)	59.3	70.0	66.7	75.5	45.0
Building occupancy in years (Mean ± SD)	6.0 ± 1.8	6.1 ± 1.6	6.2 ± 1.5	6.1 ± 1.4	5.9 ± 1.9
Smoking status (%)					
Current	13.1	15.5	17.1	6.1	9.9
Former	25.9	29.0	30.1	38.8	21.7
Never	61.1	55.5	52.9	55.1	68.4

a: See definitions of the three case groups and the comparison group in the methods section.

Table 2. Distributions of concentration and load of microbial agents in floor and chair dust.

Sample type and microbial agents analyzed	< LOD[a] (n/total N)	Concentration GM (GSD)	IQR	Load[b] GM (GSD)	IQR
Floor dust:		(per g or mg)[c]		(per m^2 of floor)	
Total fungi (CFU)	3/328	7,700 (4.7)	14,300	2,000 (5.5)	4,700
Fungi with Aw ≥ 0.8	12/328	4,800 (5.6)	11,100	1,300 (6.6)	3,900
Hydrophilic fungi[d]	53/328	2,600 (7.0)	7,700	700 (7.9)	2,600
Yeasts only	78/328	1,700 (6.7)	5,600	500 (8.1)	1,600
Hydrophilic (no yeasts)	233/328	380 (4.2)	170	100 (4.5)	110
Ergosterol (ng)	0/334	0.5 (3.1)	0.7	126.2 (3.9)	231.8
Endotoxin (EU)	0/338	10.9 (4.1)	28.1	2,683.0 (4.8)	7,413.6
Cat allergen (μg)	3/314	2.5 (2.4)	2.3	0.7 (2.9)	0.9
Dog allergen (μg)	16/314	2.1 (2.8)	2.6	0.6 (3.2)	1.0
Chair dust:		(per g or mg)[c]		(per chair)	
Total fungi (CFU)	1/326	11,000 (5.4)	31,200	4,900 (5.7)	12,000
Fungi with Aw ≥ 0.8	8/326	7,000 (6.2)	15,700	3,100 (6.6)	7,400
Hydrophilic fungi[d]	56/326	2,600 (8.1)	7,000	1,200 (8.5)	3,800
Yeasts only	118/326	1,100 (6.5)	3,400	500 (7.0)	1,300
Hydrophilic (no yeasts)	181/326	610 (6.7)	600	270 (7.1)	330
Ergosterol (ng)	0/325	0.4 (2.5)	0.5	173.6 (2.9)	260.4
Endotoxin (EU)	0/327	2.1 (2.6)	2.7	932.8 (3.0)	1,268.8
Cat allergen (μg)	0/318	12.5 (3.6)	24.8	5.8 (4.1)	9.8
Dog allergen (μg)	4/318	5.7 (3.7)	12.2	2.6 (4.1)	6.5

GM=Geometric mean; GSD=Geometric standard deviation; IQR=Interquartile range (75^{th} percentile minus 25^{th} percentile); CFU=Colony forming unit; Aw=Water activity; EU= Endotoxin Units.

a. Limit of detections (LOD) are 0.002 EU/mg for Endotoxin, 350-400 CFU/g for total culturable fungi depending on the amount of samples analyzed, 0.5 μg/g for cat allergen, and 0.4 μg/g for dog allergen. For the samples below LOD, LOD/2 was assigned for total fungi. For the subgroups of total culturable fungi, 200 CFU/g was assigned for the samples below LOD.

b. Load of microbial agents was computed by multiplying the concentration (per g or mg) of the microbial agents by the total amount of dust in gram or milligram collected in each floor (or chair) sample and then dividing by 2 m^2 for floor samples.

c. The units of concentrations are ng/mg for ergosterol, EU/mg for endotoxin, CFU/g for fungi, and μg/g for allergens.

d. Hydrophilic fungi require 0.9 or higher water activity for growth, where water activity is defined as the amount of free or available water in substrates.

Table 3. The prevalence and levels of mesophilic and hydrophilic fungal species identified from the samples.[a]

	Floor dust samples			Chair dust samples		
	Total=328	Level (CFU/g dust)		Total=326	Level (CFU/g dust)	
Species found in samples	Prevalence: n (%)	Median	Maximum	Prevalence: n (%)	Median	Maximum
Yeasts[b]	250 (76.2)	2,400	$1.2x10^6$	208 (63.8)	2,340	$8.6x10^5$
Aureobasidium pullulans	175 (53.4)	770	$8.0x10^4$	244 (74.8)	1,500	$1.5x10^6$
Epicoccum nigrum	121 (36.9)	400	$1.4x10^4$	171 (52.4)	710	$3.3x10^4$
Alternaria alternata	70 (21.3)	710	$1.1x10^4$	74 (22.7)	740	$1.8x10^4$
Phoma herbarum[b]	54 (16.5)	2,850	$1.0x10^6$	71 (21.8)	3,600	$5.9x10^6$
Cladosporium sphaerospermum	48 (14.6)	1,100	$3.0x10^4$	51 (15.6)	1,200	$1.6x10^5$
Aspergillus niger	30 (9.1)	390	$6.1x10^4$	49 (15.0)	380	$3.6x10^4$
Chaetomium globosum[b]	27 (8.2)	770	$1.4x10^4$	50 (15.3)	390	$4.0x10^4$
Cladosporium cladosporioides	19 (5.8)	400	$2.9x10^4$	18 (5.5)	1,250	$7.7x10^3$
Fusarium solani	18 (5.5)	400	$8.0x10^3$	12 (3.7)	710	$4.0x10^3$
Cladosporium herbarum	16 (4.9)	1,800	$1.2x10^4$	13 (4)	710	$2.8x10^4$
Mucor plumbeus[b]	11 (3.4)	380	$3.7x10^4$	26 (8.0)	380	$4.0x10^4$
Rhizopus stolonifer[b]	9 (2.7)	370	$3.7x10^3$	23 (7.1)	370	$3.6x10^3$
Ulocladium chartarum	6 (1.8)	390	$3.6x10^3$	18 (5.5)	380	$7.1x10^3$
Stachybotrys chartarum[b]	4 (1.2)	2,200	$1.7x10^4$	5 (1.2)	380	$4.0x10^3$
Aspergillus flavus	2 (<1)	2,080	$3.8x10^3$	2 (<1)	2,550	$3.7x10^3$
Chrysonilia sitophila	2 (<1)	3,800	$3.8x10^3$	4 (1.2)	3,700	$3.6x10^4$
Penicillium expansum	2 (<1)	1,300	$1.5x10^3$	–	–	–

a. Limit of detection ranged between 350 and 400 colony forming units/g dust. Samples below limit of detection for individual fungi are not included in statistics of the table.

b. Hydrophilic fungi requiring 0.9 or higher minimum water activity which is defined as the amount of free or available water in substrates; all other species in the table are mesophilic fungi requiring a water activity between 0.8 and 0.9.

Table 4: Associations of microbial agents in floor and chair dust with respiratory, epidemiologic asthma, or current post-occupancy asthma cases in single environmental variable models adjusted for age, gender, race, smoking status, and building occupancy time.

	Odd Ratios (95% CI)[a] for different outcome models					
	Respiratory cases[b]		Epi-asthma cases[b]		Post-occupancy Dr.-diagnosed asthma cases[b]	
Environmental variable	Floor dust[c]	Chair dust[c]	Floor dust[c]	Chair dust[c]	Floor dust[c]	Chair dust[c]
Total culturable fungi	1.66**	1.37**	1.72**	1.58**	1.56*	1.67**
	(1.19–2.33)	(1.02–1.85)	(1.21–2.46)	(1.13–2.20)	(0.96–2.53)	(1.07–2.60)
$A_w \geq 0.8$ fungi[d]	1.66**	1.31**	1.69**	1.46**	1.72**	1.56**
	(1.17–2.38)	(1.00–1.72)	(1.15–2.47)	(1.09–1.97)	(1.03–2.88)	(1.05–2.30)
Fungi not classified as	1.11	1.09	1.21	1.11	0.80	1.20
$A_w \geq 0.8$ fungi	(0.80–1.54)	(0.76–1.56)	(0.84–1.72)	(0.75–1.64)	(0.47–1.37)	(0.67–2.18)
Hydrophilic fungi[d]	1.73**	1.45**	1.80**	1.63**	2.19**	1.85**
	(1.20–2.51)	(1.07–1.97)	(1.20–2.69)	(1.16–2.28)	(1.23–3.89)	(1.19–2.89)
Fungi not classified as	1.08	1.25*	1.12	1.31*	0.71	1.31
hydrophilic	(0.79–1.50)	(0.96–1.62)	(0.79–1.59)	(0.99–1.74)	(0.42–1.19)	(0.88–1.96)
Yeasts only	1.37*	1.46**	1.46*	1.42**	1.77**	1.55*
	(0.97–1.93)	(1.06–2.01)	(1.00–2.13)	(1.01–1.99)	(1.05–3.01)	(1.00–2.41)
Hydrophilic fungi	1.50**	1.21*	1.47**	1.36**	1.43*	1.44**
without yeasts	(1.18–1.91)	(0.97–1.51)	(1.13–1.91)	(1.06–1.74)	(0.99–2.05)	(1.05–1.98)
Ergosterol	1.56**	1.38*	1.60**	1.54**	1.37	1.63*
	(1.13–2.16)	(0.98–1.93)	(1.13–2.28)	(1.05–2.26)	(0.87–2.17)	(0.95–2.81)
Endotoxin	1.60**	1.10	1.54**	1.09	1.40	1.15
	(1.09–2.37)	(0.82–1.48)	(1.01–2.34)	(0.79–1.52)	(0.79–2.50)	(0.71–1.87)
Cat allergen (Fel d 1)	1.33*	1.21	1.35*	1.37*	1.16	1.55*
	(0.96–1.83)	(0.91–1.63)	(0.95–1.92)	(1.00–1.88)	(0.72–1.88)	(1.00–2.39)
Dog allergen (Can f 1)	1.18	1.26	1.09	1.20	1.01	1.10
	(0.85–1.65)	(0.86–1.83)	(0.76–1.57)	(0.78–1.83)	(0.62–1.65)	(0.61–2.00)

a. Odds ratio (OR) and 95% confidence interval were computed based on change of the inter-quartile range in the environmental variable. b. The number of samples for each model varies from 286 to 303 for respiratory cases, 225 to 241 for epi-asthma cases, and, 170 to 183 for post-occupancy asthma cases. c. The units of the environmental variables are colony forming units (cfu)/m^2 for fungi in floor dust and cfu/chair for fungi in chair dust, endotoxin unit (EU)/m^2 for endotoxin in floor dust and EU/chair for endotoxin in chair

dust, ng/m^2 for ergosterol in floor dust and ng/chair for ergosterol in chair dust, and mg/m^2 for allergen in floor dust and mg/chair for allergen in chair dust. d. Aw≥0.8 fungi are mesophilic and hydrophilic fungi (Table 3). Hydrophilic fungi include Yeasts, *Phoma herbarum*, *Chaetomium globosum*, *Mucor plumbeus*, *Rhizopus stolonifer*, and *Stachybotrys chartarum*. ** Odds ratios are statistically significant at α=0.05. * Odds ratios are statistically significant at α=0.1.

Table 5: Associations of microbial agents measured in floor and chair dust with respiratory, epidemiologic asthma, or current post-occupancy asthma cases in multiple environmental variable models adjusted for age, gender, race, smoking status, and building occupancy time.

Multiple environmental variable models	Odd Ratios (95% CI)[a] for different outcome models					
	Respiratory cases[b]		Epi-asthma cases[b]		Post-occupancy Dr.-diagnosed asthma cases[b]	
	Floor dust[c]	Chair dust[c]	Floor dust[c]	Chair dust[c]	Floor dust[c]	Chair dust[c]
Total fungi models:						
Total culturable fungi	1.46** (1.02–2.10)	1.36* (0.99–1.87)	1.55** (1.05–2.27)	1.57** (1.09–2.25)	1.46 (0.88–2.44)	1.60* (0.99–2.58)
Ergosterol	1.40* (0.97–2.04)	1.33 (0.93–1.91)	1.54** (1.02–2.34)	1.46* (0.96–2.23)	1.22 (0.71–2.11)	1.48 (0.82–2.67)
Endotoxin	1.20 (0.75–1.90)	0.91 (0.65-1.27)	1.07 (0.64-1.80)	0.83 (0.57–1.21)	1.05 (0.53–2.08)	0.87 (0.51–1.48)
Hydrophilic fungi models:						
Hydrophilic fungi[d]	1.54** (1.05–2.27)	1.42** (1.03–1.95)	1.62** (1.06–2.48)	1.57** (1.11–2.23)	2.09** (1.15–3.79)	1.79** (1.12–2.85)
Ergosterol	1.41* (0.97–2.05)	1.32 (0.92–1.89)	1.55** (1.02–2.36)	1.46* (0.96–2.22)	1.19 (0.68–2.07)	1.47 (0.81–2.63)
Endotoxin	1.21 (0.76–1.92)	0.93 (0.67–1.28)	1.10 (0.66–1.84)	0.88 (0.61–1.26)	1.02 (0.51–2.05)	0.87 (0.51–1.47)

a. Odds ratio and 95% confidence interval were computed based on change of the inter-quartile range in the environmental variable for each model. b. The number of samples for each model is 294 or 295 for respiratory cases, 233 or 234 for epi-asthma cases, and 178 or 179 for post-occupancy asthma cases, depending on the total amount of dust available for the analyses of the microbial agents which were collected from the participants defined as cases and comparison. Priority of the analysis was endotoxin, culturable fungi, ergosterol, and then allergens. c. The units of the environmental variables are colony forming units (cfu)/m^2 for fungi in floor dust and cfu/chair for fungi in chair dust, endotoxin unit (EU)/m^2 for endotoxin in floor dust and EU/chair for endotoxin in chair dust, ng/m^2 for ergosterol in floor dust and ng/chair for ergosterol in chair dust, and mg/m^2 for allergen in floor dust and mg/chair for allergen in chair dust. d. Hydrophilic fungi include Yeasts, *Phoma herbarum*, *Chaetomium globosum*, *Mucor plumbeus*, *Rhizopus stolonifer*, and *Stachybotrys chartarum*. ** Odds ratios are statistically significant at α=0.05. * Odds ratios are statistically significant at α=0.1.