Indoor Air 2005; 15 (Suppl 9): 89–97
www.blackwellpublishing.com/ina

INDOOR AIR

Use of surrogate markers of biological agents in air and settled dust samples to evaluate a water-damaged hospital

Abstract An environmental survey was conducted in two hospital buildings in Montana, one of which had historical water incursion on the top floors and higher prevalence of reported respiratory symptoms that improved when the occupants were away from work. We measured culturable fungi and bacteria, fungal spores, endotoxin, and sub-micron particles in air; and culturable fungi and bacteria, endotoxin, markers of fungi (extra-cellular polysaccharides specific for *Penicillium*/*Aspergillus*, ergosterol, and $\beta(1\rightarrow3)$ glucans) and cat allergen in chair and floor dusts. For the analytes measured in air, the correlation coefficients ranged from 0.43 to 0.78 ($P < 0.05$). In chair dust, $\beta(1\rightarrow3)$ glucan concentrations correlated with culturable fungi and ergosterol concentrations. We found that sub-micron particles and markers of microbiological agents, but not culturable microbiological agents, were significantly positively associated with the building that had both historical water damage and higher prevalence of reported respiratory symptoms. Chair dust measurements tended to be higher in the non-complaint building. These results suggest that air and floor dust measurements of marker compounds may be better indicators of current health risk in a water-damaged environment than chair dust measurements or measurements of culturable fungi or bacteria in air or settled dust.

C. Y. Rao[1], J. M. Cox-Ganser[1], G. L. Chew[2], G. Doekes[3], S. White[1]

[1]Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, WV, [2]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY, USA, [3]Institute for Risk Assessment Sciences (IRAS), University of Utrecht, Utrecht, the Netherlands

Key words: Fungi; Endotoxin; Glucans; Ergosterol; Bacteria; Hospitals; Particles; Cat allergen.

Carol Y. Rao
Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Suite 2800, Morgantown, WV 26505, USA
Tel.: 304 285 5987
Fax: 304 285 5820
e-mail: cnr3@cdc.gov

Practical Implications
Detection and quantification of nonculture-based microbiological markers and/or agents of disease may be useful methods to assess microbial contamination and to more accurately evaluate microbial exposures in the indoor environment for exposure-response studies.

Introduction

Microbiological agents such as fungi/fungal markers and bacteria/bacterial markers have been associated with respiratory disease and building-related symptoms (Burge, 1996; Chao et al., 2003; Douwes et al., 2000; Gyntelberg et al., 1994; Neas et al., 1996; Rylander, 1998). However, the nature of microbial exposures and the mechanisms of disease are not well-defined for the indoor environment. Although analytical techniques based on culturability are widely accepted and employed, there are no standardized methods to evaluate microbial contamination in an indoor environment (ACGIH, 1999; Rao et al., 1996). While species identification by culture is crucial to investigating building-related infections (e.g. aspergillosis), the relationship between species-specific culturable microbial concentrations and respiratory disease is unclear in epidemiological studies. Several studies have used visual assessment of molds and dampness as a surrogate indicator of the microbial contamination in the indoor environment (Brunekreef et al., 1989; Dales et al., 1997; Haverinen et al., 2002; Verhoeff et al., 1994; Zock et al., 2002). Detection and quantification of microbiological markers and/or agents of disease, such as ergosterol, endotoxins, $\beta(1\rightarrow3)$ glucans and extracellular polysaccharides (EPS), may be useful methods to assess microbial contamination and to more accurately evaluate microbial exposures in the indoor environment (Douwes et al., 2000; Larsson and Larsson, 2001; Miller and Young, 1997; Rylander, 1999; Verhoeff and Burge, 1997).

In April 2000, the National Institute for Occupational Safety and Health (NIOSH) received a health hazard evaluation request from the management of a large healthcare organization to investigate respiratory health and indoor air quality at a healthcare facility in Montana. The building had documented reports of water damage and employees with physician-diagnosed

work-related asthma. A comprehensive environmental investigation was conducted in August 2000 to evaluate the microbial contamination of the water-damaged hospital building as well as a comparison hospital building owned by the healthcare organization. Using a variety of analytical methods, we measured fungi, bacteria, allergens and particles in air, chair and floor dusts. We also assessed visual and olfactory indicators of moisture damage using a standardized checksheet. This study examines the relationships among the environmental measures to compare classical analytical methods (i.e. culture methods) with newer microbiological marker assays and investigates the differences in environmental measures between the two hospital buildings (problem building vs. non-problem building).

Methods

Study buildings

The majority of employees of the healthcare organization work primarily in two buildings we termed 'hospital A' (approximately 1300 employees) and 'hospital B' (approximately 400 employees). Hospital A is an eight-story facility that houses most inpatient services. It had a history of water damage (2–3 years prior) with water leaking around the windows and through the roof. The comparison building, hospital B, which was not known to have water incursion problems, is approximately 1.6 km from hospital A. Hospital B is a five-story facility that houses primarily outpatient services.

Nineteen percent (6/32) of the nurses and department secretaries on the eighth floor of hospital A had developed work-related physician-diagnosed asthma since 1998. Because of ongoing construction on the eighth floor during our site visit, all eighth floor employees had been relocated to the seventh floor. Concurrent with the environmental investigation, NIOSH administered a cross-sectional questionnaire survey to determine the prevalence of upper and lower respiratory building-related symptoms in the two hospitals. Briefly, hospital A participants had significantly more reported respiratory symptoms that improved when away from work than hospital B participants. The detailed results of the survey and population characteristics are presented elsewhere (Cox-Ganser and Rao, 2002).

Sampling strategy

We sampled air, chair and/or floor dusts in 27 departments in hospitals A and B, as well as two outdoor sites (Table 1). In each hospital, we sampled in approximately half of the departments. Nurses' stations and administrative departments were sampled, taking into consideration the number of people potentially exposed, duration of potential exposure, level of activity in the area and accessibility of the sampling site. We did not sample on the eighth floor of hospital A, which was closed for renovation during our investigation.

Air sampling We collected long-term integrated air samples using open-faced sampling techniques on 2-μm pore size, 37-mm polytetrafluoroethylene filters housed in three-piece cassettes (Millipore, Billerica, MA, USA) at a rate of 3 l/min. Sampling time was during the hours of 7 AM to 7 PM over 4 days. The total sampling time for each sample averaged 3049 min (approximately 51 h total). NIOSH analyzed the filters for endotoxin using a *Limulus* amoebocyte lysate (LAL) assay (Kinetic-QCL, Biowhittaker Inc., Walkerville, MD, USA) (Chun et al., 2002).

Short-term culturable air samples for fungi and bacteria were collected using N-6 multiple-hole impactors for 4 min at 28.3 l/min (SKC, Eighty Four, PA, USA). We used malt extract agar (MEA) and Reasoner's 2A (R2A) media for fungi and bacteria, respectively, for colony counts and speciation. We obtained concurrent spore trap samples for 4 min at 15 l/min using Air-O-Cell® sampling cassettes (Zefon International Inc., Ocala, FL, USA). The spore trace was mounted in lacto-phenol and microscopically counted under 20× and 40×. Spores were identified to genera level. We sampled in the morning and afternoon on 2 days and once during floor vacuum sampling, for a total of five sampling events for each site. The MEA and R2A impaction plates for airborne culturable fungi and bacteria were incubated at 25°C for 4 days. Positive-hole correction factor was applied to the number of colonies recovered on an impaction plate (Macher, 1989).

In addition, we used P-TRAKs^{TM} (Model 8525, TSI, St Paul, MN, USA) to measure particle counts, averaged over 10 s, at each of the sampling sites. The P-TRAK^{TM} is a condensation particle counter that measures the number of particles per unit volume in the size range of 0.02–1 μm at 0.1 l/min. We sampled in the morning and afternoon on 2 days and once during floor vacuum sampling, for a total of five sampling events for each site.

Dust sampling We collected floor and chair dust onto a pre-weighed 142-mm diameter glass fiber filter (Gelman Type A/E) with a crevice tool, a specialized filter holder and a commercial backpack vacuum (100 CFM, 1.5 HP) (L'il Hummer; Miracle Marketing Corp., Salt Lake City, UT, USA). The filter holder was manufactured from polyvinyl chloride and had a 1000-μm prefilter. The crevice tools and filter holders were cleaned with isopropyl alcohol before each sampling. We vacuumed the seats of four chairs per sampling site for 1.5 min each. The dust collected from the four chairs was pooled for analyses. For settled floor dust

Table 1 Sampling type, sample number and measurement

Sample or observation type	Hospital A	Hospital B	Outdoor	Measurement
Long-term air filter cassette (~3000 min at 3 l/min for 12 h/day for 4 days)	$n = 17$	$n = 9$	$n = 1$	Total dust ($\mu g/m^3$) Endotoxin
Short-term air N-6 impactor (4 min at 28.3 l/min)	$n = 18$ 5×/site	$n = 9$ 5×/site	$n = 2$ 4×/site	Culturable fungi Culturable bacteria
Short-term Air-O-Cell® spore trap (4 min at 15 l/min)	$n = 18$ 5×/site	$n = 9$ 5×/site	$n = 2$ 4×/site	Fungal spores
Short-term air P-TRAK™ counts (10 s at 0.1 l/min)	$n = 18$ 5×/site	$n = 9$ 5×/site	$n = 2$ 4×/site	Particle counts
Air temperature and air relative humidity	$n = 18$ 4×/site	$n = 9$ 4×/site	$n = 2$ 4×/site	°C and % RH
Vacuumed floor dust	$n = 15$	$n = 8$	–	Total dust (g/m^2 or g/chair)
Vacuumed chair dust	$n = 17$	$n = 8$	–	Culturable fungi Culturable bacteria Ergosterol EPS-*Pen/Asp* $\beta(1\rightarrow3)$ glucans Endotoxin Dustmite allergen Cockroach allergen Cat allergen Mouse urinary protein
Surface wet/dry bulb temperature	$n = 15$	$n = 8$	–	Floor surface water activity
Signs of water damage and mold odor	$n = 30$	$n = 19$	–	Subjective score index

Three sampling sites in hospital A and one site in hospital B were vinyl-tiled floors. In hospital A, one site was not sampled for air or floor dust because the carpet had been replaced the prior week. In hospital A, two sites were not sampled for either chair or floor dust because it was too disruptive for the patients (i.e. Surgery and the Neonatal Intensive Care Unit). In hospital B, one chair sample and one floor sample were lost because of technical error. One chair dust analysis for EPS-*Pen/Asp* in hospital B was lost because of technical error.

sampling, a 0.836 m^2 floor area was vacuumed for 5 min.

The filter was post-weighed and the collected dust was partitioned for analysis of culturable fungi (on MEA, cellulose and DG-18 media incubated at 25°C) and bacteria (on R2A media incubated at 25°C), endotoxin by LAL (Chun et al., 2002), EPS specific for *Penicillium* and *Aspergillus* fungal species (EPS-*Pen/Asp*) and $\beta(1\rightarrow3)$ glucans by enzyme immuoassay (Chew et al., 2001; Douwes et al., 1996, 1999), ergosterol by gas chromatography/mass spectrophotometry (Larsson and Saraf, 1997), mouse urinary protein (Chew et al., 2003a) and cockroach (Bla g 2) (Pollart et al., 1991), dustmite (Der p 1, Der f 1) (Luczynska et al., 1989), and cat allergens (Fel d 1) (Chapman et al., 1988) by enzyme-linked immunosorbent assay (Indoor Biotechnologies, Charlottesville, VA, USA). For some sampling sites, there was insufficient dust collected to conduct all of the analyses. The priority for analysis was: culturable fungi, culturable bacteria, allergens and mouse urinary protein, ergosterol, endotoxin, EPS-*Pen/Asp*, and β1–3 glucans. Concentrations of the environmental measures were expressed per gram of collected dust.

Temperature, relative humidity and water activity measurements Temperature and relative humidity in air were measured with VWR Hygrometer/Thermometer Pens (VWR, Westchester, PA, USA). We took morning and afternoon measurements for 2 days and averaged the temperature and relative humidity measurements for each site.

Wet and dry bulb temperatures were measured at the surface of the floor with an infrared thermometer (D-series, Exergen Corp., Watertown, MA, USA). Floor water activity (the relative availability of water in a substance) was calculated from the wet and dry bulb temperatures (ASTM, 1996).

Subjective assessment scoring Both hospital buildings were assessed for signs of water incursions and moisture damage. Floor plans were used prior to the investigation to select areas, based on 55 departments, within these two buildings. Working in pairs, we examined each area and used a standardized assessment checksheet to rate each area for dampness and potential microbial contamination based on five parameters: current moisture, stains, rust, visible mold growth, and odor. Areas within each department were individually scored from 0–3 (none to profuse) for moisture, stains, and rust; and from 0–2 (none to profuse) for mold, and 0–2 (none to strong) for mold odor.

Data analysis

Statistical analyses were conducted using JMP (version 5.01) and SAS (version 8.02) software packages (SAS

Institute, Cary, NC, USA). We expressed exposure concentrations in terms of geometric mean (GM) or natural log transformations as the data were approximately log-normally distributed. Samples below the limit of detection (LOD) of the analytical method were assigned a value of LOD/$\sqrt{2}$ (Hornung and Reed, 1990).

Restricted maximum likelihood variance components were assessed for the log-transformed replicate air samples for particles, culturable fungi, culturable bacteria and fungal spores to examine the intra-site and inter-site variability. The additional random effect of the variable site (building) was assessed, while the residual errors were used to estimate the inter-site component of variation. Air samples taken during vacuuming were excluded from the variance component analysis. If significantly different, the factors of hospital building, morning/afternoon sampling and interaction of building and morning/afternoon sampling were included as a fixed effect in the mixed-effects model.

A subjective assessment index for each department was created by summing the subjective assessment scores for visible mold, mold odor, water or damp area, water stains, and rust. The scores from several rooms or areas in a department were summed to determine the score for the entire department.

The GM were calculated for the five air sampling events for culturable fungi, fungal spores, culturable bacteria and particles for each sampling site. Pearson correlation coefficients were calculated for comparing environmental measurements within and between each sampling type (air, chair or floor). One-way analysis of variance (ANOVA) was performed to compare environmental measurements in hospitals A and B.

Because many of the samples were below the LOD for culturable *Penicillium* and *Aspergillus*/*Eurotium* fungal species, total culturable *Penicillium*/*Aspergillus*/*Eurotium* concentrations in chair and floor dusts were also dichotomized as present/absent and at the 75th percentile (5000 CFU/g for both chair and floor dusts). A chi-square test was then used to evaluate the association between EPS-*Pen*/*Asp* and culturable *Penicillium*, *Aspergillus* and *Eurotium* species.

Results

In general, indoor concentrations of culturable fungi, fungal spores, culturable bacteria and particles in air were lower than outdoors (Table 2). For the indoor air measurements, 29% of the samples (38/131) for culturable fungi and 17% of the spore traps (22/131) were below the LOD. Air samples (i.e. culturable fungi, culturable bacteria, fungal spore count and particle count) taken during activity (i.e. floor vacuuming) were not significantly different than the quiescent samples. Particle counts were significantly higher in the morning (GM = 2591 particles/cm^3) than in the afternoon (GM = 1640 particles/cm^3) ($P < 0.05$).

The intra-site temporal variability contributed more to overall variability than inter-site variability for airborne fungi and bacteria, while for airborne particles, the inter-site variability was the larger component. For particles in air, the significant fixed effects in the model were hospital building, morning/afternoon sampling period and the interaction of hospital building and morning/afternoon sampling period. For particles in air, about 40% of the variability was due to differences within the sampling sites (intra-site) and about 60% was due to differences between the sampling sites (inter-site). For culturable bacteria, culturable fungi and fungal spore counts in air, the significant fixed effect in the model was hospital building. The intra-site variability accounted for 80% (culturable bacteria), 73% (culturable fungi), and 70% (fungal spores) of the variability.

In the chair and floor dust samples, dustmite allergen (Der p 1, Der f 1: LOD = 0.08 μg/g), cockroach allergen (Bla g 2: LOD = 0.08 U/g = 3.2 ng/g) and mouse urinary protein (LOD = 0.25 μg/g) were below the LOD of the analytical method. Twenty-four percent of the chair and floor dust samples (15/62 samples) were below the LOD for EPS-*Pen*/*Asp* (LOD = 2344 U/g). All other environmental measures were detectable in the samples.

Comparisons within air, chair and floor dust samples

Total culturable fungi, total culturable bacteria, total fungal spores, endotoxin, and particle counts in air were correlated with each other (correlation coefficients ranged from 0.43 to 0.78, $P < 0.05$) (Table 3). $\beta(1\rightarrow3)$ glucan concentrations were correlated with total culturable fungi and ergosterol levels in chair dusts ($r =$ 0.48 and 0.61, respectively, $P < 0.05$). $\beta(1\rightarrow3)$ glucan concentrations were inversely correlated with total culturable fungi levels in floor dusts ($r = -0.74$, $P < 0.05$). EPS-*Pen*/*Asp* was not correlated with total culturable *Penicillium*/*Aspergillus*/*Eurotium* in chair or floor dust (assessed using either the continuous data or the dichotomized data) or with any other fungal measurement.

The subjective assessment index ranged from 0 to 20 with a median of 4. Subjective assessment indices were significantly correlated with endotoxin levels in air and $\beta(1\rightarrow3)$ glucan concentrations in floor dusts ($r = 0.46$ and 0.58 respectively with $P < 0.05$). Subjective assessment indices were not significantly correlated with any chair measurement (Table 3).

The median water activity (a_w) measured at the floor surface was 0.45 (range 0.35–0.75). Water activity at the floor surface was weakly correlated with $\beta(1\rightarrow3)$ glucan concentrations in floor dust ($r = 0.44$, $P < 0.1$). Air temperature was inversely correlated with relative humidity in air ($r = -0.50$, $P < 0.05$).

Table 2 Geometric mean and one-way ANOVA results comparing environmental measures in air (a), chair (b) and floor (c) dusts between hospitals

	Hospital A		Hospital B			
Environmental measure	*n*	GM (GSD)	*n*	GM (GSD)	Outdoor GM (GSD)	Overall range
(a) Air measurements						
Fungal spore counts (spores/m^3)	18	83 (2.6)	9	40** (1.7)	510 (2.6)	17–1099[a]
Culturable fungi (CFU/m^3)	18	15 (2.1)	9	20 (1.5)	78 (1.7)	6–75[a]
Culturable bacteria (CFU/m^3)	18	133 (1.7)	9	147 (1.3)	330 (2.2)	53–277[a]
Endotoxin (EU/m^3)	17	1.5 (1.6)	8	1.1 (1.3)	2.1 (one sample)	0.7–4.7
Particle counts (particles/cm^3)	18	2.4×10^3 (1.9)	9	1.5×10^3** (1.3)	4.0×10^3 (1.2)	7.5×10^2–7.2×10^3[a]
Dust loading ($\mu g/m^3$)	18	35.3 (2.2)	9	15.0 (1.8)	193.8 (1 sample)	6.5–193.8
Temperature (°C)	18	24.7 (1.0)	9	23.7** (1.0)	30.8 (1.2)	22.6–26.4
Relative humidity (% RH)	18	27.9 (1.1)	9	34.3** (1.1)	24.6 (1.4)	25.0–42.2
(b) Chair measurements[b]						
Dust loading (g/chair)	17	1.4 (1.5)	8	1.4 (1.4)		0.8–2.7
Culturable fungi (CFU/g)	17	6.6×10^4 (1.4)	8	7.3×10^4 (1.5)		3.0×10^4–1.6×10^5
$\beta(1\rightarrow3)$ glucans ($\mu g/g$)	17	1.1×10^3 (1.5)	8	1.4×10^3 (1.4)		612–2.1×10^3
EPS-*Pen/Asp* (U/g)	17	6.8×10^3 (1.8)	7	8.7×10^3 (1.2)		1.7×10^3–1.7×10^4
Ergosterol (pg/g)	17	1.0×10^5 (1.5)	8	1.1×10^5 (1.3)		5.1×10^4–1.7×10^5
Culturable bacteria (CFU/g)	17	2.4×10^4 (7.0)	8	3.9×10^4 (6.0)		1.7×10^3–1.1×10^6
Endotoxin (EU/mg)	17	4.6 (2.1)	8	8.0* (1.3)		0.4–10.7
Cat allergen (Fel d 1) ($\mu g/g$)	17	12.8 (2.7)	8	25.5* (1.8)		2.0–79.7
(c) Floor measurements[b]						
Dust loading (g/m^2)	15	0.9 (2.6)	8	1.0 (1.7)		0.2–8.9
Culturable fungi (CFU/g)	15	3.9×10^4 (2.3)	8	4.3×10^4 (2.8)		5.0×10^3–1.6×10^5
$\beta(1\rightarrow3)$ glucans ($\mu g/g$)	10	1.6×10^3 (1.5)	6	0.6×10^3** (2.0)		220–3.3×10^3
EPS-*Pen/Asp* (U/g)	10	6.6×10^3 (2.3)	6	5.0×10^3 (2.6)		1.7×10^3–3.4×10^4
Ergosterol (pg/g)	14	14.2×10^4 (2.0)	7	7.7×10^4 (2.8)		1.5×10^4–3.0×10^5
Culturable bacteria (CFU/g)	14	4.2×10^5 (9.5)	8	1.2×10^5 (8.6)		4.9×10^3–8.3×10^6
Endotoxin (EU/mg)	13	20.1 (8.1)	6	5.8 (1.6)		2.8–3945
Cat allergen (Fel d 1) ($\mu g/g$)	14	1.0 (3.1)	8	0.6 (5.3)		0.02–5.6

CFU, colony-forming unit; EU, endotoxin unit; GSD, geometric standard deviation.
[a]Range of the GM values of the five air sampling events.
[b]Other allergens (i.e. mouse urinary protein, cockroach allergen and dustmite allergens) were below the limit of detection for all samples.
*$P < 0.10$; **$P < 0.05$.

Table 3 Correlations between environmental measures in air, chair dust and floor dust

	Air	Chair dust	Floor dust
Air	Culturable fungi, fungal spores, culturable bacteria, endotoxin, particle counts** Temperature and relative humidity** (inversely) Endotoxin and subjective assessment index**		
Chair dust	Total culturable bacteria*	$\beta(1\rightarrow3)$ glucans and culturable fungi** $\beta(1\rightarrow3)$ glucans and ergosterol**	
Floor dust	None	None	$\beta(1\rightarrow3)$ glucans and culturable fungi** (inversely) $\beta(1\rightarrow3)$ glucans and water activity* $\beta(1\rightarrow3)$ glucans and subjective assessment index*

*$P < 0.10$; **$P < 0.05$.

Comparisons between air, chair and floor dust samples

Total culturable bacteria in chair dust was weakly correlated with total culturable bacteria in air (correlation coefficient = 0.36, $P < 0.1$). Endotoxin in air was not correlated with endotoxin concentrations in chair or floor dusts. Dust loading in air (μg of dust/m^3) was not correlated with dust loadings in chair (g/chair) or floor (g/m^2). Total airborne culturable fungi and spore counts, taken either during vacuuming or pooled across five sampling events, were not significantly correlated with EPS-*Pen/Asp*, $\beta(1\rightarrow3)$ glucan, or culturable fungi concentrations in chair or floor dusts.

Comparison of hospital buildings

Particle counts, total fungal spores (counted microscopically), and temperature were significantly higher in hospital A than hospital B (Table 2). None of hospital B's sampling locations (0/8) and 24% of hospital A's sampling locations (4/17) had particle

counts greater than the outdoors (4002 particles/cm^3). *Penicillium* and *Aspergillus* (*Pen*/*Asp*) spore counts were higher on the sixth and seventh floors (average = 152 and 2484 *Pen*/*Asp* spores/m^3 respectively) of hospital A than in other areas of either building. *Pen*/*Asp* spores were detected in 82% of the samples (9/11) taken on the sixth and seventh floors while *Pen*/*Asp* spores were detected in only 18% of the samples taken in the other areas of either building (maximum concentration = 283 *Pen*/*Asp* spores/m^3). In some samples on the seventh floor, *Penicillium* and *Aspergillus* accounted for more than 80% of the fungal genera detected. When data from the sixth and seventh floors were excluded from the analyses, the fungal spores (not significantly) and particles in air were still higher in hospital A than in hospital B (data not shown).

The patterns of airborne culturable fungi concentration and species distribution between hospitals A and B were similar to the microscopically counted fungal spore results. The dominant culturable outdoor fungal species were *Cladosporium herbarum*, *Epicoccum*, and Basidiomycetes. Indoor fungal types were similar to the outdoors except for the sixth and seventh floors in hospital A. *Penicillium chrysogenum* was detected in 55% (6/11) of the samples taken on the sixth and seventh floors of hospital A compared with 4% (5/120) of the samples taken in other areas of both hospitals. The average concentration of *P. chrysogenum* was 79 CFU/m^3 on the sixth and seventh floors and accounted for 55% of the total culturable fungi detected. Fourteen percent of outdoor samples (1/7) were positive for *P. chrysogenum*. The average concentration of *P. chrysogenum* was 6 CFU/m^3 and accounted for 1.2% of the total culturable fungi detected outdoors.

None of hospital B's sampling locations (0/8) and 24% of hospital A's sampling locations (4/17) had airborne endotoxin levels greater than the outdoors. The most commonly recovered bacteria were gram-positive bacteria, such as *Coryneform* bacteria, *Bacillus*, *Micrococcus* and *Rhodococcus*. The distribution of detected species of bacteria was similar indoors and outdoors.

In chair dusts, hospital B had higher concentrations of all measured microbiological agents than hospital A (Table 2). Endotoxin and cat allergen concentrations in chair dust were significantly higher in hospital B. In floor dusts, hospital A tended to have higher concentrations than hospital B (Table 2). $\beta(1\rightarrow3)$ glucan concentrations were significantly higher in hospital A floor dust samples. *Bacillus* spp., *Coryneform* bacteria, *Micrococcus luteus*, and *Rhodococcus* were the predominant bacteria species recovered from chair and floor dusts in both hospitals. *Alternaria alternata*, *Aureobasidium pullulans*, *C. herbarum*, *Epicoccum nigrum*, *P. chrysogenum* and yeasts (other than *Rhodotorula*) were the predominant fungal species recovered from chair and floor dusts in both hospitals.

For the subjective score assessment, hospital A (GM = 5.5) was higher than hospital B (GM = 3.4), although the difference was not significant ($P = 0.11$). Water activity (a_w) at the floor surface was higher in hospital A (a_w GM = 0.48) than in hospital B (a_w GM = 0.41) ($P < 0.1$).

Discussion

For short-term air sampling, analytes with less intra-site variability may be more suitable to detect differences between buildings. Several studies have found high inter- and intra-site variability of airborne fungi (Heinrich et al., 2003; Hunter et al., 1988; Verhoeff et al., 1990). We found higher variability within the sampling sites than between the sampling sites for fungi and bacteria in air. Particle concentrations in air within our sampling sites appeared to be less variable. Concentrations of smaller particles are less variable than larger particles (>5 μm) over time (Leese et al., 1997). In our initial walk-through investigation, we found that more than 90% of the particles at all sites (measured size range from 0.3 to 20 μm) were <1 μm in size. Sub-micron particles have a long aerosol half-life, the time required for the concentration of airborne particles to become halved. Using Stokes' law and a first-order decay process equation, we estimated that the aerosol half-life of 0.5 μm diameter particles is four times longer than 1 μm diameter particles and 100 times longer than 5 μm diameter particles. Sub-micron particle concentrations are more stable in the air, which could explain why we found less within-site variability than between-site variability for our airborne sub-micron particle count measurements. We detected significant differences between hospitals A and B for particle count and fungal spores but not culturable fungi and bacteria concentrations in air. Particle counts and fungal spores had less intra-site variability than culturable fungi and bacteria concentrations in air, which may account for our ability to detect inter-site differences.

For approximately 50% of our air samples for culturable fungi, we detected very low absolute colony counts. The GM across all indoor sampling sites (Table 2) represents collection of about two colony counts on the impaction plate. The large measurement error resulting from the low absolute counts probably contributed to the high variability in the airborne culturable fungi and our inability to detect differences in airborne culturable fungi between hospitals. We did not see such low counts, however, in the concurrent samples for culturable bacteria or microscopic fungal spore counts. The high intra-site variability that we also found in airborne culturable bacteria levels indicates that short-term culturable methods may not be the optimal method to evaluate airborne microbial contamination. For example, we found significant

differences between hospitals for the non-culturable methods (i.e. fungal spore counts and particle counts) but not for the culturable methods (i.e. culturable fungi and bacteria).

Sub-micron particle counts were strongly correlated with fungal spores, total culturable fungi and total culturable bacteria. The sub-micron particle measurements are unlikely to be direct measurements of fungi and bacteria. Our bioaerosol sampling techniques capture bioaerosols that are mostly >1 μm in size (ACGIH, 1995). Sub-micron particles are likely a surrogate marker of physical disturbance in the indoor environment, such as human activity, ventilation, housekeeping and on-going renovations (Luoma and Batterman, 2001). Our findings are consistent with the idea that real-time data-logged particle counts would be useful in detecting potential peaks in total aerosols rather than as a surrogate measure of airborne microbiological agents (Parat et al., 1999).

We found that $\beta(1\rightarrow3)$ glucan concentrations were positively correlated with ergosterol and total culturable fungi levels in chair dusts. These nonculture-based measures of total fungi may be good markers for fungal load in an indoor environment. EPS-*Pen*/*Asp* concentrations were not correlated with other measures of fungi in chair dusts. $\beta(1\rightarrow3)$ glucans, ergosterol and total culturable fungi are measures of total fungi or fungal biomass while EPS-*Pen*/*Asp* are specific for *Penicillium*, *Aspergillus* and *Eurotium*. Culturable *Penicillium*, *Aspergillus* and *Eurotium* were not prevalent in the chair dusts samples (when present, prevalence was $<15\%$). In 28% of our chair dust samples, we did not detect any of these fungal genera by culture methods (data not shown). Other studies have found significant correlations between EPS-*Pen*/*Asp* and culturable fungi in settled dust. The studies reported higher prevalences of these fungal genera ($>50\%$) in their dust samples than we detected in our dust samples, which may contribute to our differing results (Chew et al., 2001; Douwes et al., 1999).

No air measurements were significantly correlated with any chair or floor dust environmental measurement. We may have not been able to detect correlations in comparing two disparate sampling methods (i.e. short-term air measurements vs. 'long-term' settled dust measurements). Air samples for culturable fungi and fungal spores taken during vacuuming, which would have been most indicative of aerosolized floor dust, were not associated with any fungal measurement in chair or floor dust. This is consistent with surface dust sampling being a possible historical marker for cumulative exposures rather than current exposures (Gyntelberg et al., 1994; Verhoeff and Burge, 1997; Wickman et al., 1992). Sources of chair and floor dust contaminants are not solely dependent upon settling from the air and vice versa (i.e. air contaminants are not solely dependent upon chair and floor contaminants). For example, cat allergen was detectable in all of the chair and floor dust samples, indicating that contaminants can be carried in by the building occupants in substantial amounts. Several studies have found that concentrations of contaminants in settled dust were not well associated with concentrations of airborne biological agents (Buttner and Stetzenbach, 1993; Hines et al., 2003). In residential environments, dust-borne and airborne biological agents may represent different ecological niches (Chew et al., 2003b; Rosas et al., 1997).

Outdoor infiltration of microbiological agents seem to be a source of indoor fungi and bacteria, except for the top floors of hospital A. Renovations under containment on the eighth floor of hospital A were on-going during the sampling period. The sixth and seventh floors of hospital A had higher levels of culturable fungi and fungal spores than the other areas of either hospital building. In our preliminary site visit, we had determined that there was *Penicillium*/*Aspergillus* contamination inside the walls of the seventh and eighth floors, based upon in-wall spore sampling (Cox-Ganser and Rao, 2002). The activities during construction probably contributed to the higher concentrations of fungi on the sixth and seventh floors. Fungal spores can penetrate into the occupied space during construction activities even with high-efficiency particulate air filtration (Cornet et al., 1999).

This study was a relatively small study of two buildings and the results may not be generalizable to other buildings especially in other geographical locations. Our data indicate that air and floor dust sampling may be better measures of current sources of microbial exposures than chair dust sampling, which may reflect other exposures. Hospital A, which had a higher prevalence of respiratory symptoms that improved when occupants were away from the building, had higher concentrations of air and floor dust contaminants than hospital B. In chair dusts, however, hospital A tended to have lower concentrations of contaminants than hospital B. As chairs are rarely cleaned, sampling of chair dust is perhaps less indicative of current building contamination than air or floor dust sampling as the primary sources of contaminants in chair dust are likely deposition from people sitting in the chairs rather than microbiological growth. Current microbial growth in the water-damaged building envelope and carpets would likely contribute more to concentrations found in air and floor dusts than chair dust. Moisture in the carpets (as measured by water activity) was higher in hospital A than hospital B. Our subjective visual/olfactory assessment index of mold contamination correlated with endotoxin levels in air and $\beta(1\rightarrow3)$ glucan concentrations in floor dust but did not correlate with any chair environmental measurement. In our study, using microbial measurements in chair dust to classify health risk by building would have led to exposure misclassification.

In summary, we found that measures of microbiological agents were correlated in air and chair dusts, but not in floor dusts. In addition, we found that non-culture based indicators of microbiological agents (i.e. fungal spores in air and $\beta(1\rightarrow3)$ glucan concentrations in floor dust) were significantly positively associated with the problem building (i.e. reported water damage and higher prevalence of reported current respiratory illness). These results indicate that air and floor dust measurements may be better indicators of the current health risk than chair dust measurements. Sub-micron particle levels were also positively associated with the problem building and should be further studied in relation to health effects. Detection and quantification of nonculture-based microbiological markers and/or agents of disease may be useful methods to assess microbial contamination and to more accurately evaluate microbial exposures in the indoor environment for exposure-response studies.

Acknowledgements

We would like to acknowledge Lennart Larsson at the University of Lund for performing the ergosterol analysis, Michael Whitmer and Daniel Lewis of the Health Effects Laboratory Division of NIOSH for performing the endotoxin analyses, Environmental Microbiology Laboratory (San Bruno, CA, USA) for performing the spore count and culturable biological analyses, William Miller for statistical assistance, Daniel Yereb for coordinating the field sampling and Michael Berakis, Randy Boylstein, Thomas Jefferson, Greg Kullman, Chris Piacitelli, Liesa Sabatino, and Michelle Vingle for assisting in the field sampling. We would also like to acknowledge the cooperation of the hospital management for the unlimited access to the buildings during our site visits. This project is supported by NIOSH intramural funds for the National Occupational Research Agenda.

References

ACGIH (1995) *Air Sampling Instruments for Evaluation of Atmospheric Contaminants*, Cincinnati OH, American Conference of Governmental Industrial Hygienists.

ACGIH (1999) *Bioaerosols: Assessment and Control*, Cincinnati OH, American Conference of Governmental Industrial Hygienists.

ASTM (1996) *Standard Test Method for Measuring Humidity with a Psychrometer (The Measurement of Wet- and Dry-Bulb Temperatures)*, West Conshohocken, PA, American Society for Testing and Materials (ASTM Standard E 377).

Brunekreef, B., Dockery, D.W., Speizer, F.E., Ware, J.H., Spengler, J.D. and Ferris, B.G. (1989) Home dampness and respiratory morbidity in children, *Am. Rev. Respir. Dis.*, **140**, 1363–1367.

Burge, H.A. (1996) Health effects of biological contaminants. In: Gammage, R.B. and Berven, B.A. (eds) *Indoor Air and Human Health*, Boca Raton, FL, CRC Press, 171–178.

Buttner, M.P. and Stetzenbach, L.D. (1993) Monitoring airborne fungal spores in an experimental indoor environment to evaluate sampling methods and the effects of human activity on air sampling, *Appl. Environ. Microbiol.*, **59**, 219–226.

Chao, H.J., Schwartz, J., Milton, D.K. and Burge, H.A. (2003) The work environment and workers' health in four large office buildings, *Environ. Health. Perspect.*, **111**, 1242–1248.

Chapman, M.D., Aalberse, R.C., Brown, M.J. and Platts-Mills, T.A.E. (1988) Monoclonal antibodies to the major feline allergen Fel d 1. Single step affinity purification of Fel d 1, N-terminal sequence analysis and development of a sensitive 2 site immunoassay to assess Fel d 1 exposure, *J. Immunol.*, **140**, 812–818.

Chew, G.L., Douwes, J., Doekes, G., Higgins, K.M., van Strien, R., Spithoven, J. and Brunekreef, B. (2001) Fungal extracellular polysaccharides, $(1\rightarrow3)$ glucans and culturable fungi in repeated sampling of house dust, *Indoor Air*, **11**, 171–178.

Chew, G.L., Perzanowski, M.S., Miller, R.L., Correa, J.C., Hoepner, L.A., Jusino, C.M., Becker, M.G. and Kinney, P.L. (2003a) Distribution and determinants of mouse allergen exposure in low-income New York City apartments, *Environ. Health. Perspect.*, **111**, 1348–1351.

Chew, G.L., Rogers, C., Burge, H.A., Muilenberg, M.L. and Gold, D.R. (2003b) Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics, *Allergy*, **58**, 13–20.

Chun, D.T., Chew, V., Bartlett, K., Gordon, T., Jacobs, R.R., Larsson, B.M., Lewis, D.M., Liesivuori, J., Michel, O., Rylander, R., Thorne, P.S., White, E.M., Gunn, V.C. and Wurtz, H. (2002) Second inter-laboratory study comparing endotoxin assay results from cotton dust, *Ann. Agric. Environ. Med.*, **9**, 49–53.

Cornet, M., Levy, V., Fleury, L., Lortholary, J., Barquins, S., Coureul, M.H., Deliere, E., Zittoun, R., Brucker, G. and Bouvet, A. (1999) Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against *Aspergillus* airborne contamination during hospital renovation, *Infect. Control Hosp. Epidemiol.*, **20**, 508–513.

Cox-Ganser, J. and Rao, C.Y. (2002) *Benefis Healthcare, Great Falls, Montana*, Cincinnati, OH, U.S. Department of Health and Human Services (HETA 2000–0255–2868). Available at: http://www.cdc.gov/niosh/hhe/reports/pdfs/2000-0255-2868.pdf.

Dales, R.E., Miller, D. and McMullen, E. (1997) Indoor air quality and health: validity and determinants of reported home dampness and moulds, *Int. J. Epidemiol.*, **26**, 120–125.

Douwes, J., Doekes, G., Montijn, R., Heederik, D. and Brunekreef, B. (1996) Measurement of $(1\rightarrow3)$ glucans in occupational and home environments with an inhibition enzyme immunoassay, *Appl. Environ. Microbiol.*, **62**, 3176–3182.

Douwes, J., van der Sluis, B., Doekes, G., van Leusden, F., Wijnands, L., van Strien, R., Verhoeff, A. and Brunekreef, B. (1999) Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms, *J. Allergy Clin. Immunol.*, **103**, 494–500.

Douwes, J., Zuidhof, A., Doekes, G., van der Zee, S., Wouters, I., Boezen, H.M. and Brunekreef, B. (2000) $(1\rightarrow3)$ β-D-glucan and endotoxin in house dust and peak flow variability in children, *Am. J. Respir. Crit. Care. Med.*, **162**, 1348–1354.

Gyntelberg, F., Suadicani, P., Nielsen, J.W., Skov, P., Valbjorn, O., Nielsen, P.A., Schneider, T., Jorgensen, O., Wolkoff, P., Wilkins, C.K., Gravesen, S. and Norn, S. (1994) Dust and the sick building syndrome, *Indoor Air*, **4**, 223–238.

Haverinen, U., Husman, T., Pekkanen, J., Vahteristo, M., Moschandreas, D. and Nevalainen, A. (2002) Characteristics of moisture damage in houses and their association with self-reported symptoms

of the occupants, *Indoor Built Environ*., **10**, 83–94.

Heinrich, J., Holscher, B., Douwes, J., Richter, K., Koch, A., Bischof, W., Fahlbusch, B., Kinne, R.W. and Wichmann, H.E. (2003) Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment, *J. Expo. Anal. Environ. Epidemiol*., **13**, 152–160.

Hines, C.J., Waters, M.A., Larsson, L., Petersen, M.R., Saraf, A. and Milton, D.K. (2003) Characterization of endotoxin and 3-hydroxy fatty acid levels in air and settled dust from commercial aircraft cabins, *Indoor Air*, **13**, 166–173.

Hornung, R.W. and Reed, L. (1990) Estimation of average concentration in the presence of nondetectable values, *Appl. Occup. Environ. Hyg*., **5**, 46–51.

Hunter, C.A., Grant, C., Flannigan, G. and Bravery, A.F. (1988) Moulds in buildings: the air spora of domestic dwellings, *Int. Biodeteriorat*., **24**, 81–101.

Larsson, L. and Larsson, P.F. (2001) Analysis of chemical markers as a means of characterizing airborne micro-organisms in indoor environments: a case study, *Indoor Built Environ*., **10**, 232–237.

Larsson, L. and Saraf, A. (1997) Use of gas chromatography-ion trap tandem mass spectrometry for the detection and characterization of microorganisms in complex samples, *Mol. Biotechnol*., **7**, 279–287.

Leese, K.E., Cole, E.C., Hall, R.M. and Berry, M.A. (1997) Measurement of airborne and floor dusts in a nonproblem building, *Am. Ind. Hyg. Assoc. J*., **58**, 432–438.

Luczynska, C.M., Arruda, L.K., Platts-Mills, T.A., Miller, J.D., Lopez, M. and Chapman, M.D. (1989) A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, *Der p* I and *Der f* I, *J. Immunol. Methods*, **118**, 227–235.

Luoma, M. and Batterman, S.A. (2001) Characterization of particulate emissions from occupant activities in offices, *Indoor Air*, **11**, 35–48.

Macher, J.M. (1989) Positive-hole correction of multiple-jet impactors for collecting viable microorganisms, *Am. Ind. Hyg. Assoc. J*., **50**, 561–568.

Miller, J.D. and Young, J.C. (1997) The use of ergosterol to measure exposure to fungal propagules in indoor air, *Am. Ind. Hyg. Assoc. J*., **58**, 39–43.

Neas, L.M., Dockery, D.W., Burge, H., Koutrakis, P. and Speizer, F.E. (1996) Fungus spores, air pollutants, and other determinants of peak expiratory flow rate in children, *Am. J. Epidemiol*., **143**, 797–807.

Parat, S., Perdrix, A., Mann, S. and Baconnier, P. (1999) Contribution of particle counting in assessment of exposure to airborne microorganisms, *Atmos. Environ*., **33**, 951–959.

Pollart, S.M., Smith, T.F., Morris, E.C., Gelber, L.E., Platts-Mills, T.A. and Chapman, M.D. (1991) Environmental exposure to cockroach allergens: analysis with monoclonal antibody-based enzyme immunoassays, *J. Allergy Clin. Immunol*., **87**, 505–510.

Rao, C.Y., Burge, H.A. and Chang, J.C. (1996) Review of quantitative standards and guidelines for fungi in indoor air, *J. Air. Waste Manag. Assoc*., **46**, 899–908.

Rosas, I., Salinas, E., Yela, A., Calva, E., Eslava, C. and Cravioto, A. (1997) *Escherichia coli* in settled-dust and air samples collected in residential environments in Mexico City, *Appl. Environ. Microbiol*., **63**, 4093–4095.

Rylander, R. (1998) Microbial cell wall constituents in indoor air and their relation to disease, *Indoor Air*, **Suppl. 4**, 59–65.

Rylander, R. (1999) Indoor air-related effects and airborne (1→3) β-D-glucan, *Environ. Health Perspect*., **107**, 501–503.

Verhoeff, A.P. and Burge, H.A. (1997) Health risk assessment of fungi in home environments, *Ann. Allergy Asthma Immunol*., **78**, 544–554.

Verhoeff, A.P., van Wijnen, J.H., Boleij, J.S., Brunekreef, B., Reenen-Hoekstra, E.S. and Samson, R.A. (1990) Enumeration and identification of airborne viable mould propagules in houses. A field comparison of selected techniques, *Allergy*, **45**, 275–284.

Verhoeff, A.P., van Wijnen, J.H., van Reenen-Hoekstra, E.S., Samson, R.A., van Strien, R.T. and Brunekreef, B. (1994) Fungal propagules in house dust. II. Relation with residential characteristics and respiratory symptoms, *Allergy*, **49**, 540–547.

Wickman, M., Gravesen, S., Nordvall, S.L., Pershagen, G. and Sundell, J. (1992) Indoor viable dust-bound microfungi in relation to residential characteristics, living habits, and symptoms in atopic and control children, *J. Allergy Clin. Immunol*., **89**, 752–759.

Zock, J.P., Jarvis, D., Luczynska, C., Sunyer, J. and Burney, P. (2002) Housing characteristics, reported mold exposure, and asthma in the European Community Respiratory Health Survey, *J. Allergy Clin. Immunol*., **110**, 285–292.