CORRELATIONS OF PARTICLES, ALLERGENS, FUNGI AND BACTERIA IN AIR AND CHAIR DUST IN A HOSPITAL SETTING

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ABSTRACT
An environmental survey was conducted in two hospital buildings, one of which had a history of water incursion on the top floors and a cluster of work-related asthma. Sampling focused on air measurements for ultrafine particles (<1 μm), culturable fungi, culturable bacteria, fungal spores, endotoxin and latex allergen. Chair dust was analyzed for culturable fungi, culturable bacteria, endotoxin, mouse urinary protein, and latex, cat, dust mite, and cockroach allergens. In general, air concentrations were higher and chair concentrations were lower in the health complaint building. The air measurements for total fungal spores, total culturable fungi, ultrafine particles, and airborne endotoxin were significantly correlated with each other. Endotoxin concentration in chair dust was significantly correlated with total culturable fungi. No significant correlations were found between air and chair measurements. Our data suggest that exposure ranges both within and between buildings require consideration when investigating associations between health effects and environmental measures.

INDEX TERMS
Culturable fungi, Chair dust, Air endotoxin, Spore count, Ultrafine particle counts

INTRODUCTION
There is evidence that increased morbidity can arise from the indoor environments of water-damaged buildings (Bornehag, *et al*., 2001). Biological particles are thought to be the causative agents (Dales, *et al*., 1991). An epidemiological and environmental investigation was conducted in two hospital buildings of a healthcare campus in the western United States. It was undertaken in response to a reported cluster of workers with respiratory symptoms on the top floor of one building (Hospital A), an 8-story facility that houses most inpatient services. Nineteen percent (6/32) of the nurses and unit secretaries on the top floor had developed work-related asthma since 1998. The building had been undergoing renovations for the past 6 years and had a history of water incursions into the building envelope. We chose a comparison building, Hospital B, in which no work-related asthma cases had been reported since 1998. This is a 5-story facility, approximately 1 mile from the Hospital A. This paper investigates the distribution of biological contaminants between and within the two hospitals, and examines the correlations among the environmental measures and media (i.e., air and chair dust).

METHODS
We selected 18 sampling sites in Hospital A, and 9 sites in Hospital B, and 2 outdoor sites (one at each building). Selection of the sites for air and chair sampling took into consideration the number of people potentially exposed, the amount of time of potential exposure, the level of activity in the area and accessibility of the sampling site.

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For air sampling, 19 nurses' stations and 8 support staff locations were selected. Replicate integrated long-term air sampling for endotoxin was conducted at 18 sampling sites in Hospital A, 8 sites in Hospital B, and 1 outdoor location with 2-micrometer (μm) pore size, 37-millimeter (mm) diameter, polytetrafluroethylene (PTFE), open-faced cassettes operated at 3 liters/minute (L/min). Sampling time was during the hours of 7 A.M. to 7 P.M. for 6 days. The total sampling time for each cassette averaged 3049 minutes. Total mass was assessed. One set of filters was analyzed for latex allergen using a CAP inhibition assay according to Baur *et al*. (Baur, *et al*., 1995). The other set of filters was analyzed for endotoxin using a limulus amebocyte lysate (LAL) assay (Kinetic-QCL, Biowhittaker Inc., Walkerville, MD) according to the kit manufacturer's recommended procedures.

Short-term culturable air samples for fungi and bacteria were collected using N-6 Anderson multiple-hole impactors (SKC, Eighty Four, PA, USA). Malt extract agar (MEA) and R2A plates were used for fungi and bacteria counts and speciation, respectively. Samples were taken for 4 minutes at 28.3 L/min. Concurrent spore trap samples were taken using Air-O-Cell sampling cassettes (SKC, Eighty Four, PA, USA). Spore trap samples were taken for 4 minutes at 15 L/min. In addition, ultrafine particle counts were measured with a Model 8525 P-Trak (TSI, St. Paul, MN, USA) at each of the sample sites. The P-Trak is a condensation particle counter that measures the number of particles per unit volume in the size range of 0.02 to greater than 1 μm at 0.10 L/min. A 10-second averaging period was used. One morning and one afternoon sample were taken for two days and one sample was taken during floor vacuuming for a total of 5 sampling events for each site.

Chair dust was collected onto a 142-mm diameter glass fiber filter (Gelman Type A/E) with a crevice tool, a specialized filter holder and a backpack vacuum (100 CFM, 1.5 HP, L'il Hummer™). The filter holder was manufactured from polyvinyl chloride (PVC) and had a 1000-μm pore size prefilter. Four chairs per sampling site were vacuumed for 1.5 minutes each. The dusts collected from the seats of the four chairs were pooled for analyses. The pooled dust was partitioned and analyzed for culturable fungi and bacteria (on MEA and R2A, respectively), latex (CAP inhibition assay according to Baur *et al*. (Baur, *et al*., 1995), endotoxin (limulus amebocyte lysate assay as described above), and mouse urinary protein and cockroach (*Bla g2*), dust mite (*Der p1, Der f1*), and cat allergens (*Fel d1*) (enzyme-linked immunosorbent assay according to Chew *et al*. (Chew, *et al*., 1998)). Concentrations were expressed per chair.

Because environmental data are usually lognormally distributed (Eudey, *et al*., 1995), we expressed exposure concentrations in terms of geometric means or natural log transformations. Samples below the limit of detection (LOD) of the analytical method were assigned a value of 2/3(LOD). Statistical analyses were conducted using JMP, version 4 software (SAS Institute Inc., 2001)).

RESULTS

Although Hospital A tended to have higher air concentrations of endotoxin, culturable fungi, total spore count, and culturable bacteria than Hospital B (Table 1), these differences were not statistically significant. The geometric mean of endotoxin levels in air across all sampling locations for Hospital A was 1.48 Endotoxin Units per cubic meter (EU/m^3) and 1.15 EU/m^3 for Hospital B ($p = 0.16$). None of Hospital B's sampling locations (0/8) and 24% of Hospital A's sampling locations (4/17) had endotoxin levels greater than the outdoors. Indoor endotoxin levels in air ranged from 0.74 to 4.72 EU/m^3. The outdoor level was 2.09 EU/m^3.

Table 1. Air concentrations of bioaerosols and particles (<1 μm) in Hospitals A and B

Bldg/ Floor	Department	Culturable Fungi[a] (CFU[b]/m^3)	Fungal Spores[c] (Spores/m^3)	Culturable Bacteria[a] (CFU/m^3)	Endotoxin[d] (EU/m^3)	Total Ultrafine Particles[c] (particles/cm^3)
A1	Outside	107 (81.5)	926 (2.4)	578 (2.3)	2.09	4002 (1.2)
A1	Administration	16 (22.4)	110 (3)	152 (1.6)	1.11	4588 (1.6)
A1	Medical Records A	50 (72.4)	50 (11.1)	138 (1.6)	0.86	6390 (1.9)
A1	Office Hallway	16 (17.3)	11 (10.9)	80 (1.7)	2.39	2561 (1.8)
A1	Medical Records B	18 (23.8)	67 (3.3)	114 (1.6)	NS[e]	3522 (2.3)
A2	Surgical Care	30 (12)	164 (2.6)	193 (1.7)	1.35	3274 (2)
A2	Radiology	4 (4.9)	35 (9.4)	53 (2.8)	1.15	775 (2.3)
A2	Emergency Room	13 (12.1)	28 (7.6)	139 (2.3)	1.08	754 (2.3)
A2	Laboratory	4 (4.9)	26 (6.8)	57 (1.8)	1.07	2254 (2.6)
A2	Surgery	0 (0)	9 (14.2)	59 (2)	1.07	1233 (1.3)
A3	Orthopaedics/Neuro	20 (23.2)	103 (2.5)	167 (2.5)	1.39	3142 (1.7)
A3	Coronary Care	9 (15.6)	140 (2.1)	269 (1.5)	1.28	1486 (1.1)
A4	Pediatrics	23 (18.7)	201 (1.9)	187 (2.6)	2.59	2664 (1.4)
A4	Progressive Care	27 (28.5)	48 (9.9)	195 (2.4)	1.71	1636 (1.5)
A5	Obstetrics	20 (15.5)	22 (11.8)	188 (2.1)	1.68	2128 (1.4)
A5	Labor/Delivery	4 (4.9)	36 (8.4)	133 (1.3)	1.24	1361 (2)
A5	Neonatal ICU	3 (5.2)	8 (6.4)	69 (1.4)	0.9	3245 (1.8)
A6	Oncology	96 (112.4)	252 (2)	232 (1.4)	2.61	4380 (1.6)
A7	Medical Care	136 (146.9)	1099 (5.7)	277 (1.3)	4.72	7164 (1.4)
BG	Outside	76 (23.6)	326 (2.3)	216 (1.7)	NS	NS
BG	Restorative Services	16 (14.8)	20 (15.9)	207 (1.7)	1.03	1705 (1.3)
BG	Medical Transcrip.	40 (16.3)	44 (2.8)	92 (1.6)	0.92	1436 (1.6)
B1	Convenience Care	29 (17.3)	54 (2.5)	129 (1.5)	1.19	1106 (1.2)
B1	Radiation Therapy	13 (19.7)	7 (6.1)	99 (1.3)	0.74	985 (1.4)
B2	Rehabilitation	23 (18.3)	22 (6.7)	191 (1.9)	1.8	1445 (1.3)
B2	Speech Pathology	29 (17)	82 (1.5)	171 (1.9)	NS	1683 (1.6)
B3	Behavioral Health	36 (30.4)	28 (7.8)	190 (1.3)	1.14	2075 (1.6)
B4	Short Stay Unit	14 (9)	5 (6.4)	146 (1.4)	1.28	1768 (1.8)
B4	Transitional Care	31 (26.7)	32 (5.3)	143 (2.1)	1.36	1358 (1.5)

[a] Arithmetic means (standard deviations)
[b] CFU = Colony Forming Units
[c] Geometric means (geometric standard deviations)
[d] EU = Endotoxin Units
[e] NS = No sample taken

Total culturable fungal concentrations in air were averaged for each sampling site (Table 1) The fungal species found outdoors were primarily *Cladosporium herbarum*, *Epicoccum*, and basidiomycetes. The indoor fungal types were similar to the outdoors except for the 6th and 7th floors in Hospital A. *Penicillium chrysogenum* was detected in 55% of the samples indoors (6 out of 11 samples taken). The average concentration of *P. chrysogenum* was 77 CFU/m^3 on the 6th and 7th floors that accounted for 55% of the fungal species detected. Fourteen percent of outdoor samples (1 out of 7 samples) were positive for *P. chrysogenum*.

The average concentration of *P. chrysogenum* was 1 CFU/m^3 that accounted for 1.2% of the total fungal species detected outdoors.

The geometric mean of total fungal spore counts in air ranged from 5 to 1099 spores/m^3 (Table 1). The pattern of concentration and species distribution between Hospitals A and B and the floors was similar to the culturable fungi results. *Penicillium* and *Aspergillus* spore counts were higher on the 6th and 7th floors of Hospital A than outdoors or in other areas of either building. In some samples on the 7th floor, *Pen/Asp* accounted for more than 80% of the fungal species detected.

The geometric mean of total culturable bacteria in air ranged from 52 to 277 CFU/m^3 indoors (Table 1). 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.

Total dust mass in air was significantly higher in Hospital A (range 6.2-68.9 μg/m3, geometric mean = 19.8) than in Hospital B (range 3.9-17.3 μg/m3, geometric mean = 8.6). Overall ultrafine particle counts on Hospital A (geometric mean = 2427) were significantly higher than Hospital B (geometric mean = 1485) (Table 1). None of Hospital B's sampling locations (0/8) and 24% of Hospital A's sampling locations (4/17) had ultrafine particle counts greater than the outdoors (4002 particles/m^3).

Total fungal spores, total culturable fungi, ultrafine particles, total dust mass and airborne endotoxin were significantly correlated with each other (Table 2).

Table 2. Correlation coefficients between airborne environmental measurements

	Mass (μg/m^3)	Endotoxin (EU/m^3)	Fungi CFU/m^3	Particles/cm^3
Spores/m^3	0.57	0.71	0.78	0.64
Fungi (CFU/m^3)	0.49	0.64		0.62
Particles/cm^3	0.48	0.41	0.62	

In chair dust, dust mite allergen (*Der p1, Der f1*), cockroach allergen (*Bla g2*) and mouse urinary protein were below the limit of detection of the analytical method. There were no significant differences in total culturable bacteria, total culturable fungi, cat allergen (*Fel d1*) concentrations in chair dust between the buildings (Figure 1). *Bacillus* spp., *Coryneform* bacteria, *Micrococcus luteus*, and *Rhodococcus* were the predominant bacteria recovered from the chairs. *Alternaria alternata, Aureobasidium pullulans, Cladosporium herbarum, Epicoccum nigrum, Penicillium chrysogenum* and yeasts (other than *Rhodotorula*) were the predominant fungal species recovered from chairs. The overall geometric means across chair sampling sites were 22.9 μg Fel d1/chair, 3.8 x 10^4 CFU bacteria/chair, and 9.5 x 10^4 CFU fungi/chair in the chair samples. Endotoxin levels were significantly higher in chairs in Hospital B than Hospital A (Figure 1). The geometric mean across all chair sampling sites was 7.74 EU/chair. Endotoxin concentration in chair dust was significantly correlated with total culturable fungi (r = 0.44) and cat allergen (r = 0.64). No air measurements were significantly correlated with any chair dust analyte measurement.

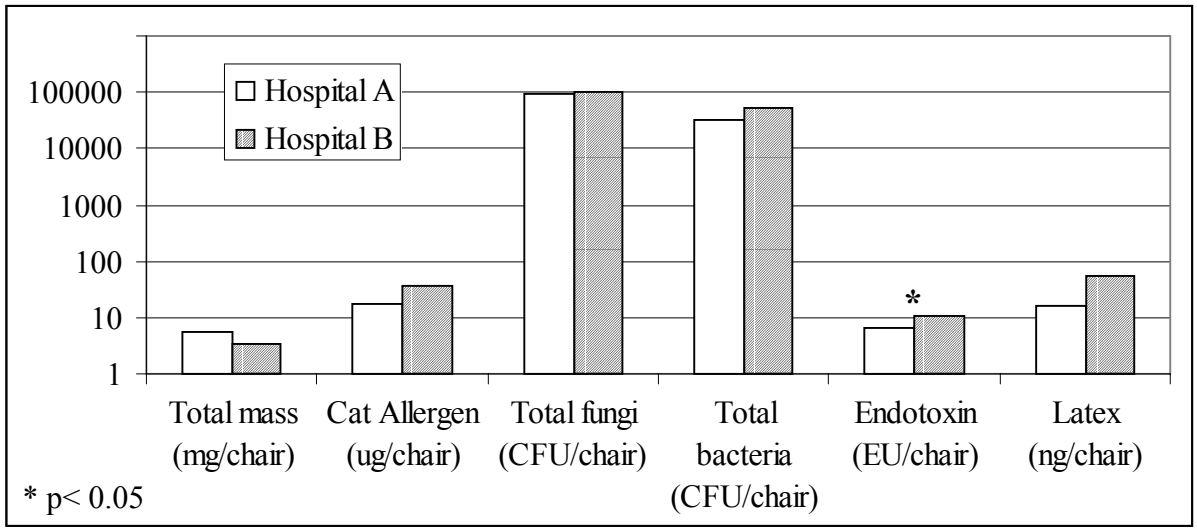

Figure 1. Geometric means of analytes in chair dust (concentration units are on the x-axis).

DISCUSSION

Although there were elevated airborne levels of *P. chrysogenum* on the top two floors of Hospital A, we did not find statistically significant differences for airborne biological contaminants (i.e., endotoxin, culturable bacteria and fungi and spore counts between Hospital A (with the initial asthma cluster) and Hospital B. In general, air concentrations were higher and chair concentrations were lower in Hospital A than in Hospital B. However, upon examining sampling sites results overall, certain departments in Hospital A had as high or higher concentrations in chair dust than Hospital B. In addition, Hospital A had statistically significantly higher ultrafine particle concentrations than Hospital B.

For the air measurements, fungal spores, total culturable fungi, ultrafine particles and endotoxin were significantly and strongly correlated. From the questionnaire data, the univariate models on air contaminants showed positive associations between overall and work-related lower respiratory symptoms and endotoxin, ultrafine particles, total culturable fungi, and total fungal spore count. Given the correlations between the four variables, it is difficult to know which were acting as etiologic agents or surrogate markers. Ultrafine particles are likely a surrogate marker of physical disturbance in the indoor environment rather than actually tracking culturable fungi and fungal spores concentrations because most fungal spores are greater than 1 μm in size. Ultrafine particles may have more severe respiratory effects because the small size allows deposition deep in the lungs. There is very little literature linking ultrafine particle exposures and respiratory health effects in the indoor environment. Wichmann and Peters found that outdoor particles less than 2.5 μm in diameter were related to decreases in lung function (Wichmann and Peters, 2000). No air measurements were significantly correlated with any chair dust analyte measurement. This is consistent with chair dust sampling being a possible historical marker for past exposures rather than current exposures.

Concurrent with the environmental sampling, NIOSH undertook a respiratory symptoms questionnaire survey of all employees in the two buildings. We found that the medical outcomes correlated with personal exposure indices based on the environmental measurements taken at all sampling sites. Therefore, even though we found no marked differences between average contaminant levels between buildings, use of intra-building

exposure data led to detection of exposure-response effects. Hence, based on these results, we advocate employment of data reflecting both inter- and intra-building exposure variation. Failure to do so, such as in allocating the same exposure to all building occupants, could lead to exposure misclassification, and resulting loss of power, and lack of ability to detect relationships between exposure and health outcomes.

CONCLUSIONS AND IMPLICATIONS

Our data suggest that the ranges of exposure within buildings should be considered when investigating associations between work-related health effects and environmental measures. Ultrafine particle exposures in the indoor environment should be further studied in relation to health effects.

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