

Survey of the Asp f 1 Allergen in Office Environments

Timothy J. Ryan,¹ Lawrence W. Whitehead,² Thomas H. Connor,²
and Keith D. Burau²

¹School of Health Sciences, College of Health and Human Services, Ohio University, Athens, Ohio;

²University of Texas-Houston, School of Public Health, Houston, Texas

Sick Building Syndrome remains a prevalent problem with patient complaints similar to typical allergy symptoms. Unlike household allergens typically found in domestic reservoirs, the allergen from a common fungus like *Aspergillus fumigatus* (i.e., Asp f 1) is conceivably widespread in the work environment. This project surveyed airborne levels of the Asp f 1 allergen in office and non-industrial occupational environments, as well as the dust reservoirs of *A. fumigatus* believed to be responsible for those levels. Airborne and bulk dust samples were collected, extracted, and assayed for Asp f 1. Concurrently, bulk dusts collected from the same locations were selectively cultured for *A. fumigatus*, and mesophilic fungi and bacteria. Samples were collected during both wet and dry climatological conditions from paired wet and dry building locations to examine the possibility of Asp f 1 increases due to fungal growth blooms. Very low levels of Asp f 1 were detected but only in the airborne samples (2/120 positive samples, with 3.6 ng/m³ and 1.8 ng/m³; LOD < 1.2 ng/m³). No dust samples showed even detectable traces of the allergen (LOD = 5 ng/g dust). Although *A. fumigatus* counts from dusts fluctuated significantly with exterior moisture events, analysis of wet versus dry period samples showed no differences in Asp f 1 levels. These results indicate that even in the presence of measurable fungal concentrations, background levels of Asp f 1 are low. Non-industrial office buildings devoid of indoor air quality issues were not observed to have significant levels of the Asp f 1 allergen in the geographical region studied.

Keywords Allergens, Asp f1, *Aspergillus fumigatus*, Dew Point, Dust, IAQ, Microbial Blooms, Relative Humidity, Sick Building Syndrome

ACRONYMS

- IAQ: Indoor air quality
- IMA: Inhibitory mold agar
- kDa: Kilo Daltons

- PBS-BSA-T: Phosphate buffered saline-bovine serum albumin-Tween 20
- RH: Relative humidity
- RM: Room moisture
- SBS: Sick building syndrome
- TSA: Trypticase soy agar

A recent report by Meyer et al.⁽¹⁾ of Sick Building Syndrome (SBS) symptoms associated with serum Ig-E specific to *Penicillium chrysogenum* has provided a compelling link between a case of SBS and environmental exposure to this year-round mold.⁽²⁾ That result comes at a time when others have found a significant correlation between increased *Penicillium* prevalence and SBS in school buildings involved in indoor air quality (IAQ) complaints.⁽³⁾ The definitive association of a widespread fungus with a documented instance of SBS signals an important opportunity for the application of standard immunological tools to the assessment of office air issues.

Although half of all SBS cases are due to improper facility ventilation systems, in approximately 13 percent of cases no adequate cause can be determined.⁽⁴⁾ The problematic role of gross fungal growth generating office or other non-industrial IAQ issues has been widely reported.⁽⁵⁻¹²⁾ The *P. chrysogenum* finding justifies increased attention to the role of aeroallergens in certain IAQ investigations. Such studies may help elucidate otherwise unrecognized causes of SBS and help assess the impact of particular aeroallergens in the workplace. In addition, a number of sensitization and asthma studies has lead Platts-Mills⁽¹³⁾ and others to indicate that allergens of all types are a primary cause of the inflammation underlying asthma. Because asthma is one of the most common chronic diseases, with an estimated 9.9 million persons directly affected⁽¹⁴⁾ the workplace levels of an ostensibly common allergen from a well known indoor fungal contaminant is of growing significance given the increasing incidence of asthma.

If fungal allergens are an important cause of IAQ issues, then background as well as problematic concentrations must be determined. Safe levels of agents acceptable to nearly all workers⁽¹⁵⁾ in industrial settings will be less than protective to

more susceptible office populations, where the “healthy worker” effect is minimized. Although no universally accepted thresholds have been established for all recognized allergens, mite allergen levels in excess of 2 $\mu\text{g}/\text{gm}$ dust of group I allergen are considered of importance to atopic individuals.⁽¹⁶⁾ In the case of the highly asthmogenic natural latex rubber allergen, an airborne concentration of only 0.6 ng/m^3 can be at issue.⁽¹⁷⁾ Despite the existence of such recommendations, in the reported association of *P. chrysogenum* with SBS symptoms no environmental exposure data to the allergenic dusts were presented. Previous studies conducted without specific immunological assay have mentioned a doubling of weakly asthmatic or allergic symptoms resulting from fungal exposures,⁽¹⁸⁾ again without the benefit of concomitant dust allergen measurements.

With the relatively recent amino acid sequencing and identification of the *Aspergillus* Asp f 1 allergen⁽¹⁹⁾ (formerly Ag3⁽²⁰⁾), the opportunity exists to accurately study the prevalence of this particular allergen in the non-industrial occupational setting. Asp f 1 is a major 18-kDa *A. fumigatus* allergen and is a member of the mitogillin family of cytotoxins believed act as a virulence factor for *A. fumigatus*.⁽²¹⁾ The only known study⁽²²⁾ of indoor and outdoor exposures to Asp f 1 found it detectable in 5 percent of house dust extracts (15/296) at a mean concentration of 38 ng/g dust. One key relationship shown in the domestic study was an increase in the Asp f 1 leaf extract concentration following heavy rainfall episodes (9 ng Asp f 1/g in dry periods, 44 ng/g post-rainfall). That finding, considered in conjunction with the known association between mold-generated IAQ issues and moisture or water intrusion events,^(7,9,23) supports the hypothesis of Asp f 1 allergen increases resulting from SBS episodes involving increased water availability contributing to mold growth.

This study determined the levels of Asp f 1 in non-domestic settings. Since all facilities were free of SBS complaints, detected concentrations could be considered background and representative of acceptable indoor environmental quality with respect to this allergen. This study also sought to examine what role, if any, interior moisture increases had on Asp f 1 concentrations.

METHODS AND MATERIALS

Study Design

To obtain statistical power, 30 university buildings were selected for study. Occupancies included office spaces ($n = 19$), research settings ($n = 7$), classrooms (empty, $n = 2$), and support functions ($n = 2$). Buildings with known mold risk factors (i.e., water intrusion history and/or basement rooms) were included to maximize the possibility of detecting allergen generation from fungal growth.

Within each building, both a potentially moist location (e.g., basement level) and a typically dry location (e.g., second story or higher) were randomly identified. To determine

the effect moisture and temperature had on microbial growth paired location samples were collected from all facilities, during climatologically defined dry periods and again during wet periods. Dry periods were defined as all days of 0.1 inches or less of precipitation subsequent to at least a consecutive three-day (72 hour) period of 0.1 inches or less of precipitation (i.e., dry periods began three days after the most recent rainfall of 0.1 inches or less). Sampling days with no/minimal rainfall were termed “Hi” climatological periods (in reference to the typically high barometric pressure associated with such weather patterns) to avoid confusion with sampling location dry areas, which were termed “Dry.”

Asp f 1 production is detectable 12 hours after spore germination,⁽²¹⁾ and so wet periods were defined as any 60-hour period commencing 12 hours following at least 0.5 inches of precipitation, inclusive (i.e., 12–72 hours after the beginning of rainfall exceeding 0.5 inches in a 24-hour period). Wet climatological periods were termed “Lows” (in reference to the typically low barometric pressure associated with such weather patterns) to avoid confusion with wet sampling locations, which were termed “Wet.” The rainfall definitions were selected based on Harris County, Texas, flood control plan action measures used to qualify rainfall events as heavy. Hi and Low period categorization was based on official National Weather Service climatological data for an airport six miles away from the sampling site, as reported by the local National Oceanic and Atmospheric Administration office. Actual on-site rainfall was verified with instruments atop one of the study buildings.

Airborne allergen samples were collected for the four sampling conditions (Hi/Dry, Hi/Wet, Low/Dry, and Low/Wet). In addition, for Hi/Dry and Low/Wet (i.e., the worst-case conditions), bulk dust serial dilution pour plates were prepared on two varieties of solid agar media.

Environmental Measurements

Environmental factors including room and floor relative humidity (RH), room and floor temperature, moisture content of floor areas, and moisture content of room materials (i.e., walls, ceiling, carpet, etc.) were recorded for all areas. Temperature and relative humidity of each sampling area during each sampling run were assessed with a Testoterm model 6100 direct reading temperature/relative humidity sensor (Davis Instruments, Baltimore, MD). Floor temperature was assessed with a Raytek Raynger model ST2 infrared thermometer (Raytek, Santa Cruz, CA). Moisture content in concrete floors was assessed with a Tramex Concrete Encounter meter (Tramex Ltd., Co. Dublin, Ireland), which is capable of non-invasively measuring between 2–6 percent moisture content of set concrete. Wall, ceiling or carpet moisture content was determined with a Delmhorst moisture meter (Delmhorst Instrument Co., Towaco, NJ) capable of measuring between 6–40 percent moisture when using invasive 1–4” pins. Based on temperature and RH measurements, dewpoints for floors and rooms were calculated using standard formulae.⁽²⁴⁾

Allergen Sampling and Quantification

Airborne and dust allergen samples were collected and prepared following Custovic's methods utilized for similarly sized aerosol or dust collections onto filters.^(25,26) Briefly, air sampling was conducted using a high-volume Eberline Model RAP-1 sampling pump (Eberline, Inc., Santa Fe, NM), calibrated with a dry gas meter to a flow rate of 50 liters per minute. Airborne samples were collected on 42.5 mm Whatman GFA micro-glass fiber filters (Maidstone, U.K.). According to the manufacturer, these filters provide fine particle retention at high flow rates with high efficiency (>99.97% retention of particles 1.6 μm and larger).

Aggressive sampling was performed by vigorously sweeping the area with a hand broom for a 10-minute sample period (sampled air volume 0.5 m^3 ; airborne limit of detection [LOD] = 1.2 ng/m^3). Neither the total nor viable particulate concentration generated by this technique was determined. Filters were transported to the laboratory in sealed plastic bags where they were folded with tweezers and placed into 7 ml vials containing 3 ml of 1 percent Phosphate Buffered Saline-Bovine Serum Albumin with 0.05 percent Tween (PBS-BSA-T) added (Sigma Chemical Co., St. Louis, MO). Vials were rotated at 180 rpm at 4°C overnight. An aliquot of extraction fluid was then transferred into a 3 ml conical screw cap tube and centrifuged at 2500 RPM for 20 minutes at 4°C. Supernatant liquid was removed with a Pasteur pipette and placed in a second screw cap tube, coded, and stored at -8°C until shipment for analysis.

Collection of bulk dust samples was performed using a Dayton model 4Z906 hand-held vacuum cleaner (Dayton Electric Manufacturing Co., Niles, IL), utilizing collector "socks" (ALK Indoor Allergen Analysis Laboratory, Spring Mills, PA) for the purposes of fine dust retention. The volumetric flow of the vacuum cleaner and the collection efficiency of the vacuum/sock combination were unknown. It was necessary to sample a total of 2 m^2 of floor area in Dry locations in order to obtain a desired minimum of 100 mg fine dust. Dust socks were transported to the laboratory in sealed plastic bags, where the contents were weighed and then sieved through a 355- μm -diameter NBS traceable No. 45 mesh screen (VWR Scientific, West Chester, VA) to remove large particles and fibers. Approximately 100 mg of this fine dust was placed in a 3 ml conical test tube with 1.0 ml of 1 percent PBS-BSA-T, resuspended via a vortex mixer, and rotated at 180 rpm overnight at 4°C. Samples were then centrifuged for 20 minutes at 2500 RPM at 4°C, the supernatant removed and placed in a second screw cap tube, coded, and stored at -8°C until shipment for analysis.

Both the filter extracts and sieved dust preparations were shipped in insulated cold-packed containers via overnight delivery to the allergen analysis laboratory, where a two-site ELISA method was used to quantify the Asp f 1 allergen.⁽²⁷⁾ Briefly, microtiter plates were coated with an anti-Asp f 1 mAb, 4A6, and incubated for 1 hour with *Aspergillus* extracts. Bound Asp f 1 was detected by the addition of a rabbit anti-*A. fumigatus* antiserum (1:1,000) followed by incubation with peroxidase-conjugated goat anti-rabbit IgG. Plates were developed with

0.01 M 2,2'-azino-bis(3-ethylbenz)-thiazoline-6-sulfonic acid in 0.07 M citrate-phosphate buffer, pH 4.2, containing 0.03% H_2O_2 , and A_{405} was determined by an ELISA plate reader. Values were calculated from a control curve (concentration range, 0.5 to 150 ng Asp f 1/ml dusts, 0.2 to 250 ng/Asp f 1/ml airborne).

Microbial Sampling and Quantification

A. fumigatus in dust was determined by suspending approximately 10 mg of the fine dust in 1 ml sterile distilled water and making three serial dilutions using the method described by Benson.⁽²⁸⁾ A general purpose, non-selective media (Trypticase Soy Agar, TSA; Becton Dickinson, Cockeysville, MD) was utilized for mesophilic fungi and total bacteria assessment, while a media selective for fungi (BBL Inhibitory Mold Agar, IMA; Becton Dickinson) was used for selective fungi enumeration. TSA dilution plates were grown out under fluorescent light at room temperature while the IMA plates were incubated in the dark at 37°C. Plates were initially read after 48 hours, with final plate counts determined 48–120 hours post-sampling. All colonies were visually counted under a 10 \times plate counter. Only *A. fumigatus* identification was performed; no other genera or species identification was conducted. Identification of *A. fumigatus* colonies was accomplished from comparison of lactophenol cotton blue (Becton Dickinson Microbiology Systems, Sparks, MD) stained wet mounts to keys and morphology supplied by Larone.⁽²⁹⁾ Results are reported in colony forming units per gram dust (CFU/g).

Data Analysis

Descriptive point estimates and measures of variability for collected dusts results were computed on the data as observed and following log transformation. The Shapiro-Wilk "W" statistic⁽³⁰⁾ was calculated to test for normality of the log transformed data. Comparisons across climatological condition and sampling location groups were computed using two-sample t-tests. Environmental measures of relative humidity, dewpoint, and relative moisture were compared across climatological condition and building sampling location dichotomies using two-sample t-tests. All statistics were calculated using Minitab Statistical Software, version 8.21 (Minitab, Inc., State College, PA).

RESULTS

Airborne sampling detected very low levels of Asp f 1 in only two samples (3.6 ng/m^3 and 1.8 ng/m^3) from typically Dry areas during Hi climatological conditions (2/120, 1.6%); all other airborne Asp f 1 samples were below the airborne limit of detection (1.2 ng/m^3).⁽³¹⁾ The "worst-case" bulk dust samples (i.e., Hi/Dry and Low/Wet) were analyzed for Asp f 1. Dust extracts from these areas (n = 60) were all negative for Asp f 1 (dust sample LOD = 5 ng/g dust).⁽³²⁾

Environmental data collected simultaneously with Asp f 1 sampling showed distinct differences between room mid-level

TABLE I
Environmental measures by climatological condition

Climate condition/ sampling location	Room % RH ^A ± SD ^B	Room dew point ^C ± SD	Room Relative Moisture ± SD
Hi/Dry ^D (n = 30)	42.0 ± 6.5	48.1 ± 4.0	8.4 ± 1.3
Hi/Wet (n = 30)	44.2 ± 8.8	49.9 ± 6.4	8.7 ± 1.5
Low/Dry ^D (n = 30)	51.3 ± 5.9	52.8 ± 4.2	8.7 ± 1.4
Low/Wet (n = 30)	51.4 ± 7.4	54.5 ± 5.3	10.3 ± 3.0 ^E

^ARelative Humidity.

^BStandard Deviation.

^CDegrees Fahrenheit.

^DDifferences between Hi/ and Low/categorical data were highly significant ($p < 0.001$) for both room % RH and room dew point (two-tailed two-sample t-test).

^EThe difference between Low/Wet data and all three other conditions was highly significant ($p < 0.002$) for both room % RH and room dew point (two-tailed two-sample t-test).

(i.e., 1.5 meters above floor level) relative humidity and dewpoint according to climatological condition (Table I). Differences in Hi/Dry and Low/Wet RH and dewpoint measurements taken directly at floor level were likewise significantly different (data not presented). There was a strong correlation (0.967) between mid-level and floor-level RH values and therefore, as would be expected, between mid-level dewpoint and floor-level dewpoint (0.854) temperatures. Somewhat surprisingly, room relative moisture values did not correlate well with dewpoints (0.133 Hi/Dry; 0.116 Low/Wet). Room relative moisture values were essentially the same under all climate conditions at all locations with the exception of damp locations sampled under climatologically wet circumstances. The difference between Low/Wet data and all three other conditions was highly significant ($p < 0.002$) for both Room % RH and Room Dew point.

Colony recoveries of mesophilic fungi and bacteria from dusts, selective fungi, and *A. fumigatus* colonies/g dust are shown in Table II. Bulk dusts were cultured from Hi/Dry and Low/Wet conditions (n = 60) for total mesophilic fungi and bacteria on TSA, selectively for fungi only on IMA, and specifically for *A. fumigatus* on IMA at 37°C. Analysis by climate condition/sampling location showed no significant differences

between mesophilic fungi and bacteria or selective fungi CFU/g dust. However, log transformed *A. fumigatus* concentrations between the two categories were significantly different ($p = 0.042$). *A. fumigatus* appeared with essentially the same frequency (50% of Hi/Dry samples, 47% of Low/Wet samples) in both sets of samples.

DISCUSSION

Results demonstrate that non-industrial office building occupancies in this geographical region do not have significant levels of the Asp f 1 allergen. Even in the presence of culturable *A. fumigatus*, levels of Asp f 1 are very low and generally not detectable by the methods employed in this study. At the airborne levels tested for in this study (i.e., $> 1.2 \text{ ng/m}^3$) Asp f 1 did not increase as the result of exterior climatological moisture events, despite demonstrated increases in both interior moisture and recoverable *A. fumigatus* from dusts collected during those events.

A review was made of the two buildings where Asp f 1 was detected, but nothing remarkable was seen in those environments. The first facility was an office building where 3.6 ng/m^3 Asp f 1

TABLE II
Colony recoveries from dusts by climatological condition

Climate condition/ sampling location	Mesophilic Fungi and Bacteria		Selective Fungi		<i>A. fumigatus</i>	
	Mean CFU/g	GM ^A CFU/g	Mean CFU/g	GM CFU/g	Mean CFU/g	GM CFU/g
Hi/Dry (n = 30)	4.2×10^6	9.5×10^5	2040	1426	120	148 ^B (n = 15) ^C
Low/Wet (n = 30)	3.3×10^6	7.9×10^5	4424	1747	287	330 ^B (n = 14) ^C

^AGeometric mean.

^BSignificant difference ($p = 0.042$); based on a comparison of the nine data pairs from both Hi/Dry and Low/Wet datasets which had no zero values.

^CNumber of non-zero values in sample.

was found in a carpeted fourth floor office, while the second building was a religion center with faculty offices. The second floor atrium in this facility demonstrated 1.8 ng/m^3 of Asp f 1. Environmental measures were not unusual for either building in either sampling location.

Table I indicates that exterior rainfall events can result in moisture loading indoors. This observation challenges the view that building interior air is rigidly maintained at precise air handler set-points and therefore controlled in isolation from exterior events. With indoor temperatures in buildings maintained at 70°F , any significant elevation of the indoor dew point could bring temperature and dew point values close enough to result in localized condensation at surfaces. Those events could potentially account for the CFU/g dust blooms presented in Table II, since *A. fumigatus* in carpet dust was previously seen to significantly increase in another study examining domestic allergens and mold spore levels after absolute humidity increases (20 CFU/g geometric mean prior versus 60 CFU/g post).⁽³³⁾ In this study culturable *A. fumigatus* colonies from interior dusts fluctuated significantly with exterior moisture events, although no visible surface condensation effect was observed.

Factors which could have accounted for the low levels of Asp f 1 reported might include poor environmental stability of Asp f 1, low antigenicity of the *A. fumigatus* strains present, poor recovery of the allergen from the ALK sampling socks, lack of actively growing *A. fumigatus* in the study region, and average concentrations below our 1.2 ng/m^3 airborne LOD. The environmental half-life of an Asp f 1 aerosol is unknown and it is therefore not possible to elaborate on the importance of the aerosol age sampled in this study. Antigenicity of *A. fumigatus* has been noted to be highly variable⁽³⁴⁾ and it is possible that the prevalent strains in the study area were only low Asp f 1 producers.

When removing dust samples from an ALK sock, it was usual that visible portions of fine dust remained trapped in the fabric of the device. For this reason it is possible that allergen content in the dust was underestimated if unbound Asp f 1 was predominantly associated with that fraction of the dust. Only actively growing *A. fumigatus* will produce the characteristic Asp f 1 allergen. Since none of the study buildings were associated with SBS at the time of the study, and little airborne Asp f 1 was detected, it is probable that the dust results are based on growth of the fungus from otherwise quiescent spores and not from collected hyphae actively growing in the environment. Concentrations of mold species are known to vary widely by geographic region and there are at least two specific reports of substantially different *A. fumigatus* exposures in disparate locales.^(35,36)

Although this study demonstrated culturable *A. fumigatus* in half of all floor dusts, its prevalence is quite variable, it is seldom a predominant genera, and much higher counts of *Cladosporium*, *Alternaria*, and *Penicillium* are typically seen.^(2,5,10,35) Poor collection efficiency of allergen on the airborne filters used is an unlikely cause for poor detection in that allergens are typically conveyed aboard larger particles⁽³⁷⁾ which would have been col-

lected by the filters used. Therefore, the most likely cause for the low allergen recoveries detected in this study is a lack of production of the allergens under the indoor environmental conditions studied.

CONCLUSION

The Asp f 1 allergen is essentially absent from non-industrial occupational environments. Based on the biology of production of the allergen it is rational to believe levels could be higher in facilities experiencing water-related SBS issues. Even though microbial blooms from dusts were temporally associated with interior moisture increases, Asp f 1 levels showed no increases above the lower limits of detection in this study. However, a finding of minimal allergen content under these conditions does not rule out Asp f 1 or some other allergenic component involvement under atypical building environment conditions.

RECOMMENDATIONS

A future study might examine Asp f 1 levels in facilities actively involved in SBS issues where water intrusion is a predominant issue, or in industries where *A. fumigatus* and high moisture routinely occur simultaneously (e.g., composting, timber processing, farming).

Dust recovery techniques employed in this study are representative of many research allergists' practices. Yet it appears from this experience that a more efficient process could be developed for obtaining the finer particulate fraction from bulk dusts. The allergen recovery coefficient of the dust socks should be determined and dust sock extraction methods should be refined and standardized.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Dr. Martin Chapman, Asthma and Allergic Diseases Center, Department of Medicine, University of Virginia, for providing the Asp f 1 allergen assays.

REFERENCES

1. Meyer, H.W.; Larsen, F.O.; Jacobi, H.H.; et al.: Sick Building Syndrome: Association of Symptoms with Serum IgE Specific to Fungi. *Inflamm Res* 47(suppl. 1):S9-S10 (1998).
2. Gravesen, S.: Identification and Prevalence of Culturable Mesophilic Microfungi in House Dust from 100 Danish Homes. *Allergy* 33:268-272 (1978).
3. Cooley, J.D.; Wong, W.C.; Jumper, C.A.; et al.: Correlation Between the Prevalence of Certain Fungi and Sick Building Syndrome. *Occup Environ Med* 55:579-584 (1998).
4. Seitz, T.A.: NIOSH Indoor Air Quality Investigations: 1971 Through 1988. In: *The Practitioner's Approach to Indoor Air Quality Investigations*, 1990, Weeks, D.M.; Gammage, R.B. Eds., pp. 163-171. Proceedings of the Indoor Air Quality International

- Symposium. American Industrial Hygiene Association, Fairfax, VA (1990).
5. Burge, H.A.: Bioaerosols: Prevalence and Health Effects in the Indoor Environment. *J Allergy Clin Immunol* 86(5):687–701 (1990).
 6. Crawford, G.N.; Filip, G.: Hidden Mold and Concealed Moisture Sources. [abstract]. In: Abstracts. American Industrial Hygiene Conference and Exposition, 1998, p. 41. American Industrial Hygiene Association, Fairfax, VA (1998).
 7. Croft, W.A.; Jarvis, B.B.; Yatawara, C.S.: Airborne Outbreak of Trichothecene Toxicosis. *Atmos Environ* 20(3):549–552 (1986).
 8. Harrison, J.; Pickering, C.A.C.; Faragher, E.B.; et al.: An Investigation of the Relationship Between Microbial and Particulate Indoor Air Pollution and the Sick Building Syndrome. *Resp Med* 86:225–235 (1992).
 9. Hodgson, M.J.; Morey, P.; Leung, W.-Y.; et al.: Building-Associated Pulmonary Disease from Exposure to *Stachybotrys chartarum* and *Aspergillus versicolor*. *J Occup Environ Med* 40(3):241–249 (1998).
 10. Flannigan, B.; Miller, J.D.: Health Implications of Fungi in Indoor Environments — An Overview. In: Health Implications of Fungi in Indoor Environments, 1994, Samson, R.A.; Flannigan, B. Eds., pp. 3–28, Air Quality Monographs. Vol. 2. Elsevier, Amsterdam (1994).
 11. Ryan, T.J.; Eason, T.; Stenvall, H.: Unusual Microbial Contamination of a University Rare Book Collection. [abstract]. In: Abstracts. American Industrial Hygiene Conference and Exposition, 1998, p. 22. American Industrial Hygiene Association, Fairfax, VA (1998).
 12. Samimi, B.: Prevalence of *Aspergillus versicolor* in Residential and Commercial Buildings with a History of Water Intrusion. [abstract]. In: Abstracts. American Industrial Hygiene Conference and Exposition, 1998, pp. 25–26. American Industrial Hygiene Association, Fairfax, VA (1998).
 13. Platts-Mills, T.A.E.: How Environment Affects Patients with Allergic Disease: Indoor Allergens and Asthma. *Ann Allergy* 72:381–384 (1994).
 14. Weiss, K.B.; Wagener, D.K.: Changing Patterns of Asthma Mortality. *JAMA* 264:1683–1687 (1990).
 15. ACGIH Worldwide. 2000 TLVs and BEIs Threshold Limit Values for Chemical and Physical Agents, p. 3. American Conference of Governmental Industrial Hygienists, Cincinnati, OH (1999).
 16. Platts-Mills, T.A.E.; Thomas, W.R.; Aalberse, R.C.; et al.: Dust Mite Allergens and Asthma: Report of a Second International Workshop. *J Allergy Clin Immunol* 89(5):1046–1060 (1992).
 17. Evans, III, R.; Mullally, D.I.; Wilson, R.W.: National Trends in the Morbidity and Mortality of Asthma in the U.S.—Prevalence, Hospitalization and Death from Asthma over Two Decades: 1965–1984. *Chest* 91(suppl.):65S–74S (1987).
 18. Molhave, L.: The Sick Building Syndrome (SBS) Caused by Exposures to Volatile Organic Compounds (VOCs). In: The Practitioner's Approach to Indoor Air Quality Investigations, 1990, Weeks, D.M.; Gammage, R.B. Eds., pp. 1–18. Proceedings of the Indoor Air Quality International Symposium. American Industrial Hygiene Association, Fairfax, VA (1990).
 19. Arruda, L.K.; Platts-Mills, T.A.E.; Fox, J.W.; et al.: *Aspergillus fumigatus* Allergen I, a Major IgE-Binding Protein, Is a Member of the Mitogillin Family of Cytotoxins. *J Exp Med* 172:1529–1532 (1990).
 20. Latge, J.P.: Tools and Trends in the Detection of *Aspergillus fumigatus*. In: Current Topics in Medical Mycology, 1995, Borgers, M.; Rodrick, H.; Rinaldi, M.G. Eds., pp. 245–281, Vol. 6. J.R. Prous Science, Philadelphia (1995).
 21. Arruda, L.K.; Mann, B.J.; Chapman, M.D.: Selective Expression of a Major Allergen and Cytotoxins, Asp f I, in *Aspergillus fumigatus*. *J Immunol* 149:3354–3359 (1992).
 22. Sporik, R.B.; Arruda, L.K.; Woodfolk, J.; et al.: Environmental Exposure to *Aspergillus fumigatus* Allergen (Asp f 1). *Clin Exp Allergy* 23:326–331 (1993).
 23. Reynolds, S.J.; Streifel, A.J.; McJilton, C.E.: Elevated Airborne Concentrations of Fungi in Residential and Office Environments. *Am Indus Hyg Assoc J* 51(11):601–604 (1990).
 24. National Weather Service—El Paso, Texas. Moisture Conversions: Dewpoint and Wet-bulb from Relative Humidity. Available from: <http://nwselp.epcc.edu/elp/wxcalc.html>
 25. Custovic, A.; Taggart, S.C.O.; Woodcock, A.: House Dust Mite and Cat Allergen in Different Indoor Environments. *Clin Exp Allergy* 24:1164–1168 (1994).
 26. Custovic, A.; Green, R.; Fletcher, A.; Smith, A.; Pickering, C.A.; Chapman, M.D.; Woodcock, A.: Aerodynamic Properties of the Major Dog Allergen Can f1: Distribution in Homes, Concentration, and Particle Size of Allergen in the Air. *Am J Respir Crit Care Med* 155(1):94–98 (1997).
 27. Chapman, M.D.: Analytical Methods: Immunoassays. In: Bioaerosols, Burge, H.A. Ed., pp. 235–248. CRC Press, Boca Raton, FL (1995).
 28. Benson, H.J.: Microbiological Applications. A Laboratory Manual in General Microbiology, 5th ed., pp. 87–90. Wm. C. Brown Publishers, Dubuque IA (1990).
 29. Larone, D.H.: Medically Important Fungi—A Guide to Identification, pp. 190–192. American Society for Microbiology, Washington, D.C. (1995).
 30. Shapiro, S.S.; Wilk, M.B.: An Analysis of Variance Test for Normality. *Biometrika* 52:591–611 (1965).
 31. Chapman, M.D.: Personal communication. July 14, 1998.
 32. Chapman, M.D.: Personal communication. April 27, 1998.
 33. Hirsch, T.; Hering, M.; Burkner, K.; et al.: House-Dust-Mite Allergen Concentrations (Der f1) and Mold Spores in Apartment Bedrooms Before and After Installation of Insulated Windows and Central Heating Systems. *Allergy* 55(1):79–83 (2000).
 34. Reed, C.: Variability of Antigenicity of *Aspergillus fumigatus*. *J Allergy Clin Immunol* 61(4):227–229 (1978).
 35. Burge, H.P.: Fungi in Libraries: An Aerometric Survey. *Mycopathologia* 64(2):67–72 (1978).
 36. Khan, Z.U.; Khan, M.A.; Chandy, R.; et al.: *Aspergillus* and Other Moulds in the Air of Kuwait. *Mycopathologia* 146(1):25–32 (1999).
 37. Chapman, M.D.; Heymann, P.W.; Sporik, R.B.; et al.: Monitoring Allergen Exposure in Asthma: New Treatment Strategies. *Allergy* 50(suppl. 25):29–33 (1995).