Interpreting Airborne Fungal Spore Samples

Part 2: *Databases of Sample Results*

by Joe Spurgeon, Ph.D., CIH

About the Author

Dr. Spurgeon, who is a Certified Industrial Hygienist, has extensive experience as an expert witness in mold litigation cases, and currently has a number of active cases in California and Texas. His experience includes both commercial and residential properties. His areas of expertise include microbial IAQ investigations, assessment strategies, sampling methods, and data analysis and interpretation. Dr. Spurgeon can be reached at cih@bi-air.com.

Introduction

The first article in this series briefly discussed referencing an airborne concentration of fungal spores to a statistical database. This article discusses the use of databases in more detail. In addition, summary data for two typical databases are included in the article as examples.

When a single airborne concentration is measured, it is just one small part of a distribution of possible concentrations that could have been measured. An important difference is that the measured concentration can change significantly between samples, but the distribution from which the sample was drawn can be rather stable. If the distribution is stable, it provides a reference for evaluating site-specific sample results. For example, the site-specific concentration can be characterized as low, moderate, high, or extreme relative to the expected distribution of concentrations.

Note: The 50th percentile concentration means that 50 percent of all sample results are expected to be less than that concentration — a typical concentration. The 95th percentile concentration means that only 5 percent of all sample results are expected to exceed that concentration — an unusually high concentration.

A second advantage of characterizing the distribution of concentrations is that extreme concentrations can be compared as well as average concentrations (although with less confidence). This is important because adverse health effects are typically associated with exposures to extreme concentrations, not average concentrations. The 95th percentile concentration of *Aspergillus versicolor* is more likely to cause an adverse health reaction than the 20th percentile concentration. For example, what is the significance of an airborne concentration of 240 colony forming units per cubic meter (cfu/m^3) of *Aspergillus versicolor*? It's difficult to even guess, because there isn't any point of reference. But, what if I can convince the reader that this is the 95th percentile concentration? Does that make it easier to assess that particular sample result?

Airborne Fungal Spores

The data presented in this section describe the lognormal distributions for *Cladosporium*, *Aspergillus/Penicillium* (*Asp/Pen*) type spores, and Total spores measured indoors. The data in Table 1 are for 51 individual airborne samples collected using the Air-O-Cell cassette.

Cladosporium was detected in 47 samples and *Asp/Pen* type spores in 40 of the 51 samples. The percentile concentrations, the average concentration, the geometric mean (GM) concentration, and the geometric standard deviation (GSD) are included for each distribution.

The data, as an example, can be interpreted as follows. Airborne concentrations that are equal to or less than the 75th percentile concentration might be considered in the normal to moderate range. Concentrations between the 75th percentile and the 90th percentile might be considered to be elevated, and those higher than the 90th percentile might be considered to be significantly elevated.

> Half (50 percent) of the indoor samples in Table 1 are expected to have a *Cladosporium* concentration of 607 spores/m^3 or less; and almost all of the

Table 1: Airborne Fungal Spores Collected Indoors: Air-O-Cell Cassette (spores/m_)

Cumulative Percent	*Cladosporium*	*Asp/Pen*	*Total Spores*
16 %	229	133	620
50 %	607	365	1,469
Average	962	598	2,109
75 %	1,172	723	2,629
84 %	1,609	1,005	3,479
95 %	3,019	1,932	6,067
99 %	5,888	3,866	10,953
GM	607	365	1,469
GSD	2.65	2.75	2.37

Table 2: Airborne Fungi Collected Indoors: N6 Sampler with MEA Plates (cfu/m_)

Cumulative Percent	*Cladosporium*	*Asp. spp.*	*A. ver.*	*Pen. spp.*	*Total Spores*
16 %	168	23	10	67	494
50 %	391	98	33	216	1,005
Average	555	258	65	420	1,285
75 %	693	258	75	478	1,622
84 %	912	411	111	700	2,043
95 %	1,574	1,040	241	1,494	3,229
99 %	2,811	2,787	550	3,343	5,250
GM	391	98	33	216	1,005
GSD	2.33	4.21	3.33	3.24	2.03

samples are expected to have a *Cladosporium* concentration of less than 5,888 spores/m^3.

The *Asp/Pen* data from Table 1 were discussed in the previous article. The maximum measured concentration was listed as 2,640 spores/m^3, which was true. The concentrations in Table 1 are calculated values representing *the expected percentile concentrations*, and may exceed the highest observed concentrations.

Airborne Culturable Fungi

The data in Table 2 are for 60 individual airborne samples collected indoors using the N6 Impaction Sampler with malt extract agar (MEA) culture plates. *Cladosporium* was detected in all 60 samples, *Aspergillus* species (*Asp*. spp.) in 41 samples, *Penicillium* species (*Pen*. Spp.) in 51 samples, and *Aspergillus versicolor* (*A. ver*.) in 19 samples.

One investigation concerned a single family house with three children in bed with cold and flu-like symptoms. In addition, all three children had bloody rhinitis, suggesting the possibility that cytotoxic fungi were present in the indoor air. The airborne samples averaged 900 cfu/m^3 of Aspergillus versicolor and 400 cfu/m^3 of *Stachybotrys chartarum* - both considered cytotoxic fungi. In addition, the airborne concentrations of both fungi were well above their 99th percentile concentrations.

This was one of the rare times that I have recommended that occupants be removed from their environment. I'm not familiar with any published data indicating what are considered hazardous concentrations for these fungi. Therefore, The database was crucial in supporting my recommendation.

First, without reference to my database, I would not have had any way to support this recommendation; or to determine that these concentrations were extreme. Second, once I was able to establish that the concentrations were extreme, it was easier for an attorney to associate the adverse health effects with the extreme concentrations that were detected.

Exposure Assessments

The book *Bioaerosols: Management and Control* (J. Macher, Ed., ACGIH) is often referred to as "the" primary reference for microbial investigations. It is that, and it contains a wealth of information. However, as an industrial hygienist, I am generally asked for two products — an exposure assessment and/or a scope of remediation.

When asked to perform an exposure assessment, I often refer to the American Industrial Hygiene Association (AIHA) Exposure Assessment Strategy, part of which

continued on page 59

agricultural products for mycotoxin contamination,[13] it should be clarified that these are tests that actually measure the mycotoxins directly, rather than indirectly measuring *antibodies* against mycotoxins in environmental samples. Any attempt to describe the approval of such environmental ELISA tests as evidence of the general acceptance of the method as a biomarker of exposure to mycotoxins in humans would be a mischaracterization. Given that there are many different types of ELISA methods and applications in which the technique is utilized in medicine and in other applications, the emergence of mycotoxin antibody testing in the legal setting is likely to present challenges to attorneys, experts, and factfinders in cases involving mold, mycotoxins, and personal injuries.

References

[1] Centers for Disease Control and Prevention. Second National Report on Human Exposure to Environmental Chemicals. Division of Laboratory Sciences, National Center for Environmental Health. 2003. http://www.cdc.gov/exposurereport/ (accessed January 24, 2004)

[2] Sudakin DL. Dietary aflatoxin exposure and chemoprevention of cancer: a clinical review. J Toxicol Clin Toxicol. 2003; 41:195-204

[3] Monaci L, Palmisano F. Determination of ochratoxin A in foods: state-of-the-art and analytical challenges. Anal Bioanal Chem. 2004; 378:96-103

[4] Skaug MA. Levels of ochratoxin A and IgG against conidia of Penicillium verrucosum in blood samples from healthy farm workers. Ann Agric Environ Med. 2003; 10:73-77

[5] Meky FA, Turner PC, Ashcroft AE et al. Development of a urinary biomarker of human exposure to deoxynivalenol. Food Chem Toxicol. 2003; 41:265-273

[6] Food and Agriculture Organization, World Health Organization. Joint FAO/WHO Expert Committee on Food Additives. Pages 1-33. 2-15-2001. http://www.who.int/pcs/jecfa/Summary56.pdf (accessed January 24, 2004)

[7] Pestka JJ. Deoxynivalenol-induced IgA production and IgA nephropathy-aberrant mucosal immune response with systemic repercussions. Toxicol Lett. 2003; 140-141:287-295

[8] Trout D, Bernstein J, Martinez K et al. Bioaerosol lung damage in a worker with repeated exposure to fungi in a water-damaged building. Environ Health Perspect. 2001; 109:641-644

[9] Vojdani A, Kashanian A, Vojdani E et al. Saliva secretory IgA antibodies against molds and mycotoxins in patients exposed to toxigenic fungi. Immunopharmacol Immunotoxicol. 2003; 25:595-614

[10] College of American Pathologists, Commission on Laboratory Accreditation. Laboratory General Checklist. 2003. http://www.cap.org/apps/docs/laboratory_accreditation/checklists/laboratory_general_july2003.pdf (accessed January 24, 2004)

[11] Nielsen KF, Smedsgaard J. Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology. J Chromatogr A. 2003; 1002:111-136

[12] Crowther JR. The ELISA Guidebook. Volume 149 vol. Totowa, NJ: Human Press, 2001

[13] United States Department of Agriculture. Grain Fungal Diseases and Mycotoxin Reference. GIPSA, Technical Services Division. 1999. Kansas City, MO, USDA. http://www.usda.gov/gipsa/pubs/mycobook.pdf (accessed January 24, 2004)

Interpreting Airborne
continued from page 5

was described in this article [*A Strategy for Assessing and Managing Occupational Exposures, 2nd Ed.; J. Mulhausen, J. Damiano; 1998 (aiha.org)*]. This is a detailed, systematic method for assessing exposures, and works quite well for most environmental contaminants, including fungi.

Summary

It should be obvious that I am the only consultant in possession of the specific data presented in this article. Therefore, the distributions, although "real", should be viewed as examples. In order to be useful in a practical sense, consultants may have to establish their own databases. The distributions of concentrations would then be specific to the seasons, sampling methods, laboratory, types of properties, and geographical areas they actually sample.

I believe it would be beneficial for microbial consultants to establish these databases, and even share them through publication. If for no other reason, analyzing the data gives the consultant, attorney, and property manager an intuitive feel for airborne concentrations. It then becomes easier to judge whether a particular airborne concentration is high, moderate, or low.

Remember, "to assess" means "to determine the significance of". In the absence of consensus guidelines, the AIHA Exposure Assessment Strategy, part of which was described in this article, is the only practical method that I am aware of for assessing airborne fungal concentrations.