CME Available for this Article at ACOEM.org

Development of an Environmental Relative Moldiness Index for US Homes

Stephen Vesper, PhD
Craig McKinstry, MS
Richard Haugland, PhD
Larry Wymer, MS
Karen Bradham, PhD
Peter Ashley, DrPH
David Cox, PhD
Gary Dewalt, PhD
Warren Friedman, PhD

Learning Objectives

- Point out the drawbacks and limitations of air sampling, the traditional means of measuring home mold concentrations.
- Describe the process of dust sampling and analysis that yields an Environmental Relative Moldiness Index (ERMI).
- Give examples of how the ERMI has been used to support the diagnosis of childhood respiratory disorders and monitor exposure after taking measures to eliminate mold.

Abstract

Objective: *The objective of this study was to establish a national relative moldiness index for homes in the United States.* ***Methods:*** *As part of the Housing and Urban Development's American Healthy Homes Survey, dust samples were collected by vacuuming 2 m^2 in the bedrooms plus 2 m^2 in the living rooms from a nationally representative 1096 homes in the United States using the Mitest sampler. Five milligrams of sieved (300 μm pore, nylon mesh) dust was analyzed by mold-specific quantitative polymerase chain reaction for the 36 indicator species in 1096 samples.* ***Results:*** *On the basis of this standardized national sampling and analysis, an "Environmental Relative Moldiness Index" was created with values ranging from about −10 to 20 or above (lowest to highest).* ***Conclusions:*** *The Environmental Relative Moldiness Index scale may be useful for home mold-burden estimates in epidemiological studies.* (J Occup Environ Med. *2007;49:829–833*)

From the United States Environmental Protection Agency (Drs Vesper and Haugland, and Mr Wymer), National Exposure Research Laboratory, Cincinnati, Ohio; Pacific Northwest National Laboratory (Dr McKinstry), Battelle, Richland, Washington; United States Environmental Protection Agency (Dr Bradham), National Exposure Research Laboratory, Research Triangle Park, North Carolina; Department of Housing and Urban Development (Drs Ashley and Friedman), District of Columbia, Washington; and QuanTech (Drs Cox and Dewalt), Arlington, Virginia.

Stephen Vesper and Richard Haugland receive a percentage of royalties paid to the EPA from companies that utilize Mold Technology: Aerotech Laboratories, Inc., Ambient Group, Inc., CENAS AG, EMSL Analytical, Inc., Forensic Analytical, Galson Laboratories, Genetic ID, Healthy Office Co. UK Limited, Microbac, Microbial Insights, Inc., Mycometrics, LLC, Pure Earth Environmental Laboratory, Inc., P & K Microbiology Services.

Address correspondence to: Stephen Vesper, PhD, US EPA, 26 West M. L. King Drive, Cincinnati, OH 45268; E-mail: vesper.stephen@epa.gov.

DOI: 10.1097/JOM.0b013e3181255e98

To understand the risk from mold exposure, the medical community needs a reliable process for determining the extent of the mold burden in a home based on standardized sampling and analytical methods. Air samples have primarily been used to measure mold concentrations. For practical reasons, air samples have been limited to very short durations, often 5 minutes or less with the resulting limitations in understanding long-term mold exposures.[1,2]

As an alternative to air samples, mold specific quantitative polymerase chain reaction (MSQPCR) analysis of dust has been proposed to measure the mold burden in a home.[3–6] Dust is a repository of the history of the mold condition of a home and of the environmental spore load and supports the view that settled dust represents more than temporary microbial conditions.[7] Nevertheless, it is impractical to measure all the molds in a home.

Therefore, indicator mold groups for water-damaged and non–water-damaged homes were selected from a total of 82 species.[6,8] Of the 82 species analyzed in samples of water-damaged and control homes, only 36 species were widely distributed. These 36 species were divided into 26 group 1 species associated with water damage and 10 group 2 species that are not associated with water damage.[6] By subtracting the sum of the log-transformed concentrations of group 2 species from the sum of the log-transformed concentrations of group 1 species, a "Relative Moldiness Index" (RMI) was

created.[6] This RMI was useful in predicting health outcomes in studies in Ohio homes.[6,8] We now sought to take this approach to a national survey of homes.

As part of the 2006 Housing and Urban Development's American Healthy Home Survey (AHHS), dust samples from a statistically representative set of homes across the United States were analyzed. This national spectrum of homes allowed us to extend the RMI from just Ohio and develop the "Environmental Relative Moldiness Index" (ERMI) for the United States.

Materials and Methods

Home Selection Process

AHHS targeted a nationally representative sample of permanently occupied homes or housing units. A housing unit is defined as a house, apartment, mobile home, a group of rooms, or a single room that is occupied as separate living quarters. Separate living quarters are those in which the occupants live and eat separately from any other persons in the building and which have direct access from the outside or through a common hall.

The first stage of the selection process was to choose 100 clusters called "primary sampling units" (PSUs), which covered the entire United States. The PSUs were selected with probability proportional to population in the 2000 census. The second stage of the selection process was to choose five "segments" in each PSU with probability proportional to the total number of housing units in the segment. A segment consists of a census block or a group of geographically contiguous blocks, and in most cases is similar to a city block. The third stage in selection of a sample of housing units required the use of a "sampling frame." A sampling frame is a list of all units in the segments from which the sample of housing units were drawn. A frame of housing units was created by a variation of a process called "listing." Listing is the process of identifying and recording the addresses of households (or their descriptions and locations, if they do not have addresses) on listing sheets. In this way, all of the places where people live, or might live, within the boundaries of the segment are eligible for inclusion in the study.

For this survey, lists of households in the sampled segments were acquired from commercially available sources. A sample of four residential addresses, plus two backup addresses, was randomly selected from the list in a typical segment to determine which households were eligible to be included in the sample. These lists were validated by a modified listing process in which interviewers visited the sampled segments with the acquired lists to compare them with the housing units actually present to validate a list for each designated segment within each PSU. This comparison resulted in some housing units being added to the lists and others being deleted from the lists. The result was the ultimate selection of 1144 homes for sampling. (For more detailed information about the selection process, go to www.hud.gov.)

Dust Sample Collection and Analysis

Dust samples were collected from the 1144 homes by vacuuming 2 m^2 in the living room and 2 m^2 in a bedroom for 5 minutes each with a Mitest sampler-fitted vacuum, directly adjacent to the sofa or bed, respectively. The dust was sieved through a 300-μm pore size nylon mesh (Gilson Company, Lewis Center, OH). Sieved dust samples from 1096 (96%) homes were analyzed. (Insufficient dust was collected in 48 homes.)

The analysis of the 1096 dust samples was completed by Environmental Protection Agency (EPA)-licensed commercial laboratories, and the analysis of 435 randomly selected samples were repeated at our EPA laboratory, as previously described.[9–11] All primer and probe sequences, as well as known species comprising the assay cluster, and the list of EPA-licensed commercial laboratory were published at the EPA web site: www.epa.gov/microbes/moldtech.htm. Primers and probes were synthesized commercially (Applied Biosystems, Foster City, CA; Integrated DNA Technologies, Coralville IA; Sigma Genosys, Woodlands, TX).

Statistical Methodology

Statistical analyses and graphics were performed using SAS (SAS Institute, Cary, NC) and the R Software environment for statistical computing and graphics (www.r-project.org). Mold concentration values observed below the minimum detection limit of 1 cell per milligram dust were treated as left-censored data, and all summary statistics referenced were estimated using a modified Kaplan–Meier survival model adapted for left-censored data.[12] The Kaplan–Meier model is widely used in the analysis of right-censored data as typically found in survival studies.[13] The modification involves transforming the data from left-censored to right censored, estimating summary statistics, and transforming back (as detailed).[12]

The three-stage sampling scheme provided a nationally representative sample of occupied housing units, and summary statistics for that population accounted for the probability weighting factors used in drawing the sample. The ERMI scale computation required that each home in the survey be given equal weight. Correlation between the commercial laboratory results and the results for the same sample analyzed at our EPA laboratory was determined by calculating the Pearson correlation coefficient.

Results

Table 1 shows the rate of occurrence, the average concentration and standard deviation, the geometric mean, and the highest concen-

TABLE 1
Mold Species Occurrence Percentages in Dust Collected From 1096 AHHS Homes, Averages (AVGs) Cell Equivalents (CEs) per Milligram of Dust With Standard Deviations (SDs), Highest Concentrations (CEs per Milligram of Dust), and Their Geometric Means (GMs) as CE per Milligram Dust

Mold Species and Groups	% Occurrence	AVG CE/mg	SD AVG	Highest Concentration CE/mg	GM CE/mg
Group 1					
Aspergillus flavus[a]	36	18	159	4,768	2
A. fumigatus[b]	62	19	205	5,800	3
A. niger[c]	69	99	1,918	6,200	4
A. ochraceus[d]	27	34	432	12,000	2
A. penicillioides	90	8,609	18,1759	6,000,000	91
A. restrictus[e]	12	51	5,864	25,000	2
A. sclerotiorum	26	6	787	890	2
A. sydowii	29	60	41	14,666	3
A. unguis	20	16	579	5,588	2
A. versicolor	30	28	195	3,574	2
Aureobasidium pullulans	94	1,719	178	130,000	263
Chaetomium globosum	51	45	709	21,000	2
Cladosporium sphaerospermum	82	1,497	888	23,000	13
Eurotium amstelodami[f]	98	3,758	43,103	1,100,000	155
Paecilomyces variotii	46	208	450	204,539	2
Penicillium brevicompactum	52	98	113	6,200	5
P. corylophilum	17	16	281	2,600	2
Penicillium (Group 2)[g]	8	19	9	8,275	1
P. purpurogenum	15	2	60	260	1
P. spinulosum[h]	20	5	65	1,901	1
P. variabile	50	18	6,177	1,023	3
Scopulariopsis brevicaulis	53	18	175	5,200	2
S. chartarum	38	5	45	1,429	2
Stachybotrys chartarum	35	23	123	2,000	2
Trichoderma viride[i]	27	3	11	236	2
Wallemia sebi	75	962	12798	400000	18
Group 2					
Acremonium strictum	57	16	517	970	4
Alternaria alternata	88	169	49	10,993	35
Aspergillus ustus	40	6	33	738	2
Cladosporium cladosporioides (Type 1)	99	1,497	6,355	140,000	331
C. cladosporioides (Type 2)	70	32	182	4,100	4
C. herbarum	84	432	2,004	52,000	31
Epicoccum nigrum	93	2,394	12,291	250,000	117
Mucor racemosus[j]	92	146	953	22,000	15
Penicillium chrysogenum (Type 2)[k]	66	129	1,533	38,000	5
Rhizopus stolonifer	29	3	21	530	1

[a]Includes *A. flavus* and *A. oryzae.*
[b]Includes *A. fumigatus* and *Neosartorya fischeri.*
[c]Includes *A. niger*, *A. foetidus*, and *A. pheonicis.*
[d]Includes *A. ochraceus* and *A. ostianus.*
[e]Includes *A. restrictus*, *A. caesillus*, and *A. conicus.*
[f]Includes *E. amstelodami*, *E. chevalieri*, *E. herbariorum*, *E. rubrum*, and *E. repens.*
[g]Includes *P. crustosum*, *P. camembertii*, *P. commune*, *P. echinulatum*, and *P. solitum.*
[h]Includes *P. spinulosum*, *P. glabrum*, *P. lividum*, *P. pupurescens*, and *P. thomii.*
[i]Includes *T. viride*, *T. atroviride*, and *T. koningii.*
[j]Includes *M. amphibiorum*, *M. circinelloides*, *M. hiemalis*, *M. indicus*, *M. mucedo*, *M. racemosus*, *M. ramosissimus*, *R. azygosporus*, *R. homothallicus*, *R. microsporus*, *R. oligosporus*, and *R. oryzae.*
[k]This is the dominant subgroup of species.

tration of each of the 36 species found in the dust of the 1096 AHHS homes. The Pearson correlation coefficient between the 435 homes analyzed by our EPA laboratory and these same samples analyzed by the licensed commercial laboratories was 0.734.

For each home, the mold burden was computed by taking the sum of log-transformed group 1 mold species concentrations minus the sum of log-transformed group 2 mold species concentrations.[6,8] Assembling these mold burden values from low-

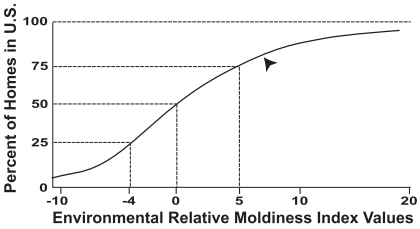

Fig. 1. Environmental relative moldiness index (ERMI) scale. The black curved line (at arrow) represents the ERMI values of the 1096 homes assembled from lowest to highest. The scale is divided into quartiles to facilitate interpretation. Because the result of each of the 36 MSQPCR assays has a standard deviation of ±half a log, each ERMI value has a standard deviation of 3.

est to highest resulted in a scale designated the ERMI (Fig. 1). (Because the standard deviation of each of the 36 MSQPCR assays is ±half of a log,[11] the sum of the standard deviations produces a standard deviation of 3 for any ERMI value.) The ERMI values from the survey homes were divided into quartiles based on the percentage of homes falling within those particular ERMI value ranges.

Discussion

Past surveys of mold concentrations have relied on air samples. For example, Shelton et al[14] reported on the analysis of over 9000 indoor air samples and 2400 outdoor air samples from 1717 buildings. They found that *Cladosporium*, *Penicillium*, non-sporulating fungi, and *Aspergillus* were the most common molds. Nevertheless, these samples were not collected at random but came from buildings with employee health complaints, in the evaluation of visible mold growth or odors, or from a "proactive" indoor air quality program. So there was an inherent bias in the sampling locations. Also, the samples were short-term air samples, and these types of samples have many inherent problems.

For example, in one study using N6-Andersen samplers, in combination with DG-18 agar and a sampling time of 5 minutes, it was reported that "the number of CFU/m^3 in the indoor and outdoor air varied widely" and the "low predictive value . . . limits their use in epidemiological studies."[15] Spicer and Gangloff[16] showed that "the levels of fungi in the outdoor air varied significantly between morning and afternoon . . . with no pattern by species, time of day, or location." Also, the methods used to analyze air samples, either culturing or counting, have there own set of limitations.

For culture-based analysis of air samples, culture plates can be quickly overgrown. Also, different molds have different growth requirements and different growth rates. Identification of the colonies on a plate can require significant expertise in mycology. Therefore, analysis of samples necessarily favors the discovery and quantification of certain species.

For counting-based methods of air sample analysis, short duration sampling is again necessitated by the limited surface area of the various capture devices. These devices can be quickly overwhelmed and the mold cells not captured efficiently, hidden by debris, or distorted by drying. Also, because many mold spores appear alike, similar appearing spores tend to be combined into large groups like "Pen-Asp," which includes not only *Penicillium* and *Aspergillus* species but also any other species with small round spores. Therefore, the counts can be greatly affected by the expertise of the person doing the counting. In a recent comparison test, identical air samples were sent to various commercial laboratories for testing.[17] Significant variability was reported between laboratories and between the researcher's own laboratory and the commercial laboratories. For these reasons, we have selected dust as the most practical substrate for comparing the mold burdens in homes in the United States and a DNA-based method to identify and quantify the molds.

Dust has been used to estimate the mold burden in homes,[4–6,18] which we define by both the concentrations of the species as well as the diversity of species in the home. Nevertheless, it was not practical to measure all molds in homes, so our goal was to measure enough indicator species to quantify mold burdens in homes across the United States on a relative basis. The 36 indicator species were selected because they were widely found.

The result of this approach is a simple numeric scale, the ERMI (Fig. 1). Because the ERMI scale was developed using a nationally representative sampling of homes, one can now place any newly sampled home in the United States on this ERMI scale and assess its relative mold burden (lowest 25%, highest 25%, etc) within the standard deviation of the ERMI value, which is 3.[10] This relatively high standard deviation indicates that the ERMI is not capable of separating small differences in mold burdens, eg, a home with an ERMI value of 2 is not different from a 1 or a 4. Rather, the ERMI is designed to distinguish homes with major differences in ERMI values. Thus, for example, a home with an ERMI of 12 has a greater mold burden than a home with an ERMI of 2.

It is also important to note that the ERMI is a mold index not a health index. Each individual's genetic make-up and health status makes their particular response to mold exposures unique. Nevertheless, epidemiological studies can help us to understand group or population centered responses to mold. For example, two epidemiological studies in Ohio were conducted related to asthma and the RMI. In Cleveland, asthmatic children living in water-damaged homes were studied and the dust in these homes analyzed by MSQPCR. There was an 80% likelihood of finding an asthmatic child in a home with a RMI value of about 1 or greater.[6] Remediation of the moisture and mold in these homes significantly reduced the asthmatic child's need for either emergency room visits or hospital admissions during follow-up.[19] In a

prospective study of atopic infants in Cincinnati, the risk of developing wheeze/rhinitis was 70% at a RMI value of −2.5 or higher.[8]

The use of a highly standardized, objective approach to mold analysis centered on a DNA-based method of identification and quantification should provide more reliable measures of the mold burden in homes. The resulting ERMI provides a simple numeric estimate of this mold burden. The fact that the ERMI is based on accumulated dust means that it is not an instantaneous measure but a long-term measure of the mold burden. On-going epidemiological studies should help us determine if the ERMI improves our understanding of the role of molds in human health.

Acknowledgments

The US Environmental Protection Agency (EPA) through its Office of Research and Development and Housing and Urban Development (HUD), funded and collaborated in the research described here. It has been subjected to each Agency's peer review and has been approved as an EPA publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use. Since MSQPCR technology is patented by the US EPA, the Agency has a financial interest in its commercial use.

This research was supported by funding from the EPA Asthma Initiative. The technical assistance of Melissa Rogers, John Kavanaugh, and Chris Hartmann was much appreciated. The contributions of Alexa Fraser, Susan Marie Viet, David Marker, and coworkers at Westat, Inc., who designed the AHHS, were greatly appreciated.

References

1. O'Connor GT, Walter M, Mitchell H, et al. Airborne fungi in the homes of children with asthma in low-income urban communities: the inner-city asthma study. *J Allergy Clin Immunol*. 2004;114: 599–606.
2. Meklin T, Reponen T, McKinstry C, et al. Comparison of mold concentrations in indoor and outdoor air sampled simultaneously and quantified by MSQPCR. *Sci Total Environ.* 2007;382;130–134. Epub April 30, 2007.
3. Vesper SJ, Varma M, Wymer LJ, Dearborn DG, Sobolewski J, Haugland RA. Quantitative PCR analysis of fungi in dust from homes of infants who developed idiopathic pulmonary hemorrhaging. *J Occup Environ Med*. 2004;46: 596–601.
4. Meklin T, Haugland RA, Reponen T, et al. Quantitative PCR analysis of house dust can reveal abnormal mold conditions. *J Environ Monitor*. 2004;6:615–620.
5. Haugland RA, Meklin T, Varma M, et al. Method to classify environmental samples based on mold analysis by QPCR. In: Johanning E, ed. *Bioaerosols, Fungi, Bacteria, Mycotoxions and Human Health: Pathophysiology, Clinical Effects, Exposure Assessment, Prevention and Control in Indoor Environments and Work.* Albany: Fungal Research Group; 2005:327–334.
6. Vesper SJ, McKinstry C, Yang C, et al. Specific molds associated with asthma. *J Occup Environ Med*. 2006;48:852–858.
7. Chao GL, Milton DK, Schwartz J, Burge HA. Dustborne fungi in large office buildings. *Mycopathologia*. 2002;154: 93–106.
8. Vesper SJ, McKinstry C, Haugland RA, et al. Relative moldiness index as predictor of childhood respiratory illness. *J Expo Sci Environ Epidemiol*. 2007; 17:88–94.
9. Brinkman NE, Haugland RA, Wymer LJ, Byappanahalli M, Whitman RL, Vesper SJ. Evaluation of a rapid, quantitative real-time PCR method for cellular enumeration of pathogenic *Candida* species in water. *Appl Environ Microbiol.* 2003; 69:1775–1782.
10. Haugland RA, Brinkman NE, Vesper SJ. Evaluation of rapid DNA extraction methods for the quantitative detection of fungal cells using real time PCR analysis. *J Microbiol Methods.* 2002; 50:319–323.
11. Haugland RA, Varma M, Wymer LJ, Vesper SJ. Quantitative PCR of selected *Aspergillus, Penicillium* and *Paecilomyces* species. *Syst Appl Microbiol.* 2004; 27:198–210.
12. Helsel DR. *Nondetects and Data Analysis, Statistics for Censored Environmental Data.* Hoboken, NJ: Wiley; 2005.
13. Collett D. *Modeling Survival Data in Medical Research*. 2nd ed. London: Chapman and Hall; 2003.
14. Shelton BG, Kirkland KH, Flanders WD, Morris GK. Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl Environ Microbiol*. 2002;68:1743–1753.
15. Verhoeff AP, van Wijnen JH, Brunekreef B, Fischer P, van Reenen-Hoekstra ES, Samson RA. Presence of viable mould propagules in indoor air in relation to house damp and outdoor air. *Allergy*. 1992;47:83–91.
16. Spicer R, Gangloff H. Establishing site specific reference levels for fungi in outdoor air for building evaluation. *J Occup Environ Hyg*. 2005;2:257–266.
17. Godish T, Godish D. Inter-laboratory variability in total mold particle counts based on co-located, concurrently collected total airborne samples. In: *Indoor Environmental Quality: Problems, Research and Solutions.* Research Triangle Park, NC: USEPA; 2006, July 17–19. USEPA/AWMA ISBN 10 0-9232-0475-Y, ISBN 13 978-0-9232-0475-4.
18. Chew GL, Rogers C, Burge HA, Muilenberg ML, Gold DR. Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics. *Allergy*. 2003;58:13–20.
19. Kercsmar CM, Dearborn DG, Schluchter MD, et al. Reduction in asthma morbidity in children as a result of home remediation aimed at moisture sources. *Environ Health Perspect*. 1006;114:1574–1580.