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The Environmental Relative Moldiness Index reveals changes in mold contamination in United States homes over time

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Abstract

The Environmental Relative Moldiness Index (ERMI) is a scale created to compare mold contamination levels in U.S. homes. The ERMI was developed as a result of the Department of Housing and Urban Development's (HUD) first American Healthy Homes Survey (AHHS I), which sampled 1,096 homes selected to be representative of the U.S. housing stock. In AHHS I, a dust sample from each home was analyzed using quantitative PCR assays (qPCR) for 36 common indoor molds: 26 Group 1 molds, which were associated with water damage in homes and 10 Group 2 molds, which primarily enter the home from the outside environment. In 2019, HUD completed AHHS II by sampling 695 homes. Because lead was banned from paint in 1978, a larger proportion of homes selected for AHHS II had been built before 1978 compared to AHHS I. The 36 ERMI molds were analyzed in AHHS II exactly as in AHHS I. For the 36-ERMI molds, the rates of detection, average concentrations, and geometric means were in significant concordance ($p < 0.001$) between AHHS I and II, indicating that the ERMI methodology was stable over time. However, the average ERMI value in AHHS II homes was greater than in AHHS I. The reason for the difference was investigated by examining the Group 1 and 2 mold populations. The average summed logs of Group 1 molds were significantly greater in homes built before 1978 than the average for homes built later. Conversely, the average summed logs of Group 2 mold populations were the same in homes built before 1978 and homes built later. Since the summed logs of Group 2 mold is subtracted from the summed logs of Group 1 molds in the ERMI calculation, the average ERMI value was higher in AHHS II homes than AHHS I. In conclusion, by using the ERMI metric, we were able to demonstrate that water damage and mold growth were more likely to occur as homes get older.

Keywords

American Healthy Homes Survey; ERMI; asthma; home; mold

Introduction

Moisture damage and mold contamination in homes and other buildings increase respiratory symptoms, asthma, and the risk of development of new cases of asthma (Reponen et al.

2011; Kanchongkittiphon et al. 2015; Pekkanen and Lampi 2015; Osborne et al. 2015; Sharpe et al. 2015; Oluwole et al. 2017; Thacher et al. 2017; Knibbs et al. 2018; Mendell et al. 2018). To provide an objective measure of mold contamination, the Environmental Relative Moldiness Index (ERMI) was created as a result of the qPCR analysis of molds in dust samples obtained during the first American Healthy Homes Survey (AHHS I) conducted by the Department of Housing and Urban Development (HUD) started in 2004 (Vesper et al. 2007).

In AHHS I, a standard floor-dust sample was obtained from a nationally representative set of homes (n = 1,096) and analyzed for mold using quantitative PCR (qPCR) assays (Haugland and Vesper 2002; Haugland et al. 2004). The ERMI resulted from the quantification of 36 common indoor molds, which were divided into two groups. The Group 1 molds (n = 26) are associated with water damage in homes, and the Group 2 molds (n = 10) primarily enter the home from the outside environment (Vesper et al. 2007). To calculate the ERMI value for a home, the summed common logarithms of the concentrations of Group 2 molds is subtracted from those of the Group 1 molds. This subtraction normalizes mold contamination differences in homes across the U.S., independent of water damage, due to other factors like cleaning habits, window use or air-conditioning, the outdoor ecosystem that surrounds the home, and others (Vesper 2011).

In 2019, HUD completed a second American Healthy Homes Survey (AHHS II) to track changes in the condition of the U.S. housing stock. One of our goals was to examine the stability of the ERMI scale in U.S. housing over time. Another goal was to document changes in mold contamination in homes built before 1978, the year lead was banned from paint in the U.S. (U.S. Consumer Product Safety Commission 1977).

Methods

Home selection process

The selection and recruitment of homes in AHHS II was the same process used in AHHS I but based on the 2010 census data, not the 2000 census data used in AHHS I (Vesper et al. 2007). However, due to budget constraints, only 695 homes were sampled for mold analysis in AHHS II compared to 1,096 in AHHS I. The homes in AHHS II were selected from the same U.S. States sampled in AHHS I, except no samples were obtained from Colorado in AHHS II. The density of samples obtained from each State was proportionally lowered in AHHS II to maintain the same representation as in AHHS I.

The samples were obtained from occupied 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.

One of HUD’s main goals for AHHS II was to determine if the distribution of lead-based paint hazards in U.S. homes had changed in the 15 years since AHHS I. To facilitate this goal, the AHHS II survey design included a longitudinal component in which homes

sampled in AHHS I, that had been built before 1978 (n = 504), were also targeted for recruitment in AHHS II (lead-based paint having been banned for use in homes in 1978) (U.S. Consumer Product Safety Commission 1977). Although an attempt was made to recruit all 504 homes for AHHS II, only 211 agreed to take part (resident turnover contributed to this low rate). In addition, dust samples could not be obtained from five of these homes for various reasons, including lack of electricity, and refusal. Therefore, only 206 of AHHS I homes built before 1978 were resampled in AHHS II. In AHHS II, one ERMI value, 42.95, in a home built before 1978 was determined to be an outlier, using the Grubbs test, leaving 205 resampled homes.

Dust sample collection, quantitative PCR (qPCR) analysis, and ERMI calculation

In AHHS II, dust samples were collected from the homes by vacuuming a 2 m^2 area in the living room and a 2 m^2 area in a bedroom, directly adjacent to the sofa or bed, for 5 min each with a Mitest sampler-fitted vacuum, exactly as performed in AHHS I. The dust was sieved through a 300-micron pore size nylon mesh (Gilson Company, Inc. Lewis Center, OH) and 5 mg from each of the sieved-dust samples were analyzed by a commercial laboratory that performs the ERMI analysis (Mycometrics LLC, Monmouth Junction, NJ). In order to ensure that the assays performed as they did in AHHS I, each assay in AHHS II used the same primers and probe as in AHHS I. In addition, each qPCR detector instrument used to AHHS II was calibrated using standard curves (Haugland and Vesper 2002; Haugland et al. 2004). The standard curves were generated using spore stocks of the Type Strain of each of the 36-ERMI molds (Haugland and Vesper 2002; Haugland et al. 2004). The amplification efficiency for each assay in AHHS II was matched to the amplification efficiency of each assay in AHHS I.

After the concentrations of each of the Group 1 and Group 2 molds were determined, the ERMI values were calculated, as shown in Equation 1. The summed logs of the concentrations of the Group 2 molds (s_2) was subtracted from the summed logs of the concentrations of Group 1 molds (s_1) to produce the ERMI value (Vesper et al. 2007).

$$\text{ERMI} = \sum_{i=1}^{26} \log_{10}(s_{1i}) - \sum_{j=1}^{10} \log_{10}(s_{2j}) \quad (1)$$

Statistical analysis

The distribution of ERMI values in AHHS I and AHHS II were shown by plotting their respective kernel density estimates. To compare the rate of detection (positive number samples per total number of samples), the average concentration and the geometric means for the 36 ERMI molds in AHHS I (n = 1,096) and AHHS II (n = 694), Kendall Tau concordance (correlation) analysis was used. Tau concordance expresses the probability, on a scale of −1 to +1, of agreement between AHHS I and II for each type of comparison (occurrence, concentration or geometric mean) for any arbitrary pair of mold species selected.

The average ERMI values in AHHS I and AHHS II were compared using a z-test. The average ERMI value in AHHS I or AHHS II homes built before 1978 was compared to the average ERMI value in AHHS I or AHHS II homes built after 1977 using the z-test. Also, using the z-test, the average summed logs of Group 1 and Group 2 molds in AHHS I or II homes built before 1978 were compared to these corresponding values for AHHS I or II homes built after 1977. Statistical analyses and graphics were performed using SAS (SAS Institute Inc., Cary, NC).

Results

Table 1 presents the data for each ERMI mold's percent of detection, average cell concentration, and geometric mean in samples from AHHS I and AHHS II homes. Since the rate of detection, average concentration, and geometric mean in AHHS I homes were each in significant ($p < 0.001$) concordance with these same measurements in AHHS II homes, it appears that the ERMI's constituent molds performed consistently in each survey.

The density plots of ERMI values in AHHS I and AHHS II homes are shown in Figure 1. The ERMI values in AHHS II homes appeared to trend higher than the ERMI values in AHHS I homes, i.e., the AHHS II curve was shifted right. Therefore, this difference was investigated.

The average ERMI value (6.85) in AHHS II homes was significantly ($p < 0.001$) greater than average ERMI value (4.55) in AHHS I homes (Table 2, Comparison A), in agreement with the trend observed in Figure 1. However, the data for the year the homes were built was only available for 1,039 of the 1,096 of the AHHS I homes. Therefore, to confirm that the average ERMI value in these 1,039 homes was in the same relationship to the average ERMI value for the entire set of AHHS I homes, the average ERMI values in the subset ($n = 1,039$) and full set ($n = 1,096$) were compared, as shown below.

Table 2, Comparison B, shows that the average ERMI value (4.56) for the subset of AHHS I homes with built-year data ($n = 1,039$) was not significantly different from the average ERMI value (4.55) for the entire set of AHHS I homes ($n = 1,096$). Therefore, the ERMI values in the subset of AHHS I homes ($n = 1,039$) was representative of the entire AHHS I data set ($n = 1,096$). Using this subset, the comparison of average ERMI values for AHHS I and AHHS II homes was repeated. Again, the average ERMI value (6.85) for the AHHS II homes was significantly ($p < 0.001$) greater than the average ERMI value (4.56) for AHHS I homes (Table 2, Comparison C). Therefore, further comparisons were made, as described below, using this subset of AHHS I homes ($n = 1,039$).

Next, the homes in AHHS I and II built Pre-1978 and Post-1977 were compared. In AHHS I, the average age of the homes built Pre-1978 was 52.1 years old, with a range from 27–219 years old and in the Post-1977 homes, the average age of the homes was 14.0 years old, with a range from 0–26 years old. In AHHS II, the average age of the homes built Pre-1978 was 64.5 years old, with a range from 42–149 years old and in Post-1977 homes, the average age of the homes was 28.1 years old, with a range from 2–41 years old.

In Table 3, Comparison D, the homes in AHHS I (n = 1,039) were divided into those built before 1978 (n = 602) and those built after 1977 (n = 437). For the AHHS I homes built before 1978, the average ERMI value (4.93) was significantly ($p = 0.02$) greater than the average ERMI value (4.03) of AHHS I homes built after 1977. Similarly, the average ERMI value (7.61) in AHHS II homes built before 1978 (n = 468) was significantly ($p < 0.001$) greater than the average ERMI value (5.12) in AHHS II homes built after 1977 (n = 226) (Table 3, Comparison E).

Next, the average ERMI values in AHHS I and AHHS II homes built before 1978 were compared. The average ERMI value (7.61) in AHHS II homes built before 1978 was significantly ($p < 0.001$) greater than the average ERMI value (4.93) in the AHHS I homes built before 1978 (Table 3, Comparison F). Similarly, the average ERMI value (5.12) in AHHS II homes built after 1977 was significantly ($p = 0.03$) greater than the average ERMI value in AHHS I homes built after 1977 (Table 3, Comparison G). For the AHHS I homes built before 1978 that were resampled in AHHS II (n = 205), the average ERMI value (7.95) in AHHS II reselected homes was found to be significantly ($p < 0.001$) greater than the average ERMI value (5.19) for those same homes in AHHS I (Table 3, Comparison H). Therefore, the source of the higher average ERMI values in older homes was investigated by comparing the components of the ERMI metric, i.e., the summed logs of Group 1 and summed logs of Group 2 mold values.

When the populations of Group 1 molds in homes built before 1978 were compared to homes built after 1977, the average of summed logs of Group 1 molds was significantly greater in the older homes in both AHHS I and II ($p = 0.04$ and $p < 0.001$, respectively) (Table 4). By contrast, the populations of Group 2 molds in the older and newer homes were not significantly different (Table 4). Therefore, the higher average ERMI value in AHHS I and II homes built before 1978 was the result of an increase in average Group 1 mold populations in these older homes and not with any change in the average Group 2 mold populations.

Discussion

Our first goal was to examine the stability of the ERMI scale in U.S. housing over time. This goal was addressed using the entire AHHS I and II cohorts of samples. The rates of detection, average concentrations and geometric means of the 36 ERMI molds measured in AHHS I and AHHS II were in significant concordance ($p < 0.001$) (Table 1), indicating that the ERMI methodology was stable between AHHS I and II. The higher average ERMI values in AHHS II homes (Figure 1) was the result of two major differences in home selection in AHHS II; fewer homes were surveyed in AHHS II and a greater proportion of the homes were built before 1978.

In AHHS II, 37% fewer homes were sampled than in AHHS I due to budget limitations. Therefore, although the same States were sampled in AHHS II (except for Colorado) the density of sampling was lower than in AHHS I but with the same proportional representation by State. Also, of the homes selected in AHHS II, a higher proportion were built before 1978 compared to AHHS I, 67.4% and 57.9%, respectively. This is relevant because, in both

AHHS I and AHHS II homes built before 1978, the average ERMI values were significantly higher compared to those built after 1977 (Table 3). Therefore, the repeat samples (n = 205) were used to address our second goal, to document changes in mold contamination in homes built before 1978.

The repeat samples (n = 205) addressed the increased likelihood that older homes were more likely to suffer water damage and the resulting mold growth. It appears that the home's infrastructure, e.g., roof and pipes, is increasing likely to deteriorate and fail, allowing for water infiltration and mold growth, as the home ages. To document this phenomenon, we compared the components of the ERMI, i.e., the Group 1 and 2 mold populations, in AHHS I or II homes built before 1978 to those built after 1977.

The analysis of the components of the ERMI metric showed that the increase in the average ERMI value in AHHS I or II homes built before 1978 was associated with an increase in the population of Group 1 molds compared to the population in homes built after 1977 (Table 4). There was no significant difference in average Group 2 mold populations in the homes built before 1978 compared to those built after 1977. Therefore, the higher average ERMI value observed in older AHHS I or II homes was the result of higher populations of Group 1 molds.

The Group 1 molds are those selected to reveal excess moisture and the resulting mold growth (Vesper et al. 2007; Vesper 2011). By quantifying the Group 1 mold populations in AHHS II, we were able to confirm that older homes, built before 1978, were more likely to suffer water damage and mold growth than newer homes. Conversely, the population of Group 2 molds in the AHHS II homes built before 1978 did not change significantly. Since the Group 2 molds primarily enter the home from the outside environment, the Group 2 mold populations in homes do not change because of water damage. Rather, Group 2 mold populations change, if cleaning habits in the home changed, if the frequency of opening or closing windows changed, if air-conditioning was added to the home, or if the outside ecosystem changed significantly. Our results showed that, in the intervening 15 years between surveys, these kinds of changes were not a common occurrence and, therefore, the average Group 2 mold populations stayed about the same. These results demonstrate the value of the objective quantification of the Group 1 and 2 mold populations to understanding mold contamination and its relationship/response to water damage.

Because of the negative health effects resulting from mold exposure, especially for asthma, it is important to be able to objectively quantify mold contamination. The ERMI metric has been shown to provide an objective, predictive measure of the relationship between mold contamination and asthma in epidemiological studies (Vesper and Wymer 2016). Other methods of mold contamination assessment, e.g., short air samples that are cultured or counted or visual/olfactory inspections, do not provide objective and quantitative mold contamination assessments.

Short-term air samples are still the most common methods used by the indoor-air community for mold quantification (Reboux et al. 2019). However, culture-based results are limited by the media selected to culture the molds from the samples and by the differential

rates of mold growth, as well as mold overcrowding on the culture-plates for longer air samples. Also, identifying colonies on a culture plate requires significant mycological expertise and is limited to the count of only viable mold spores.

Counting mold spores captured on sticky surfaces is limited by the difficulty in identifying molds by spore appearance alone and the differential efficiency in spore capturing devices, as well as overcrowding on the sticky surface in only but the shortest of air samples (Vesper 2011). Also, short-term air samples, usually 5–10 min, provide only a glimpse of the total mold burden in homes.

Another method used to estimate mold contamination is visual inspection and/or olfactory detection (Shiue 2015; Moses et al. 2019). Although these are useful techniques for assessments by the same inspector or investigator, they cannot be applied consistently because of the lack of objective criteria that are independent of the individual inspector or investigator.

Microbiome analyses, e.g., next-generation sequencing, pyrosequencing, or high-throughput sequencing, have been used in some studies to estimate mold contamination (Fu et al. 2020; Hanson et al. 2016). Such approaches can differentiate fungal/mold communities in water-damaged homes (Karvonen et al. 2014; Sylvain et al. 2019). The results from such methods might suggest additional molds for inclusion in an expanded mold index someday.

There are potential limitations to our study. The AHHS II was not as comprehensive as AHHS I. Fewer homes were sampled in AHHS II and a higher proportion were built before 1978. Despite these differences, we were able to achieve our goal of documenting changes in mold contamination in homes built before 1978.

Since many molds in these homes were not quantified, this could be a potential limitation. However, the 36-ERMI molds quantified were adequate to document the differences in mold contamination in the AHHS I and II homes over time, and the reasons for the differences. Also, the names of some of the 36 molds have changed since the ERMI was developed because of changes in taxonomy. Some might consider this to be a limitation. However, the mold names for each of the 36 molds were based on the Type Strain, the official name given when an isolate was deposited in a recognized culture collection (Haugland and Vesper 2002). The qPCR assays for each of the 36 molds were based on the DNA sequences for the Type Strain of each named mold. In addition, since the ERMI value itself is not based on these names but rather the DNA sequences measured by each assay, name changes are not a limitation to the application of the ERMI.

Some have suggested that a few of the assays for the 36 ERMI molds are not optimized. Although these assays have all been well documented (Haugland and Vesper 2002; Haugland et al. 2004), it is not the specific assay performance but the fact that each assay provides consistent results from sample to sample that is critical to the application of the ERMI metric.

Another potential limitation of the comparison of results from AHHS I and II is the fact that the ERMI methodology has not been the subject of a multi-laboratory validation study. Such

a validation study is necessary if the ERMI metric is to become widely applicable. Therefore, without the method validation study, the interpretation of the results presented in this manuscript may be questioned. Despite the inevitable improvements in qPCR instruments and changes in personnel, our best efforts have been made to utilize the same critical elements of the methodology, primers and probes and standard curves, in AHHS II that were used in AHHS I.

Conclusions

By using the ERMI metric, we were able to demonstrate that water damage and mold growth were more likely to occur as homes get older.

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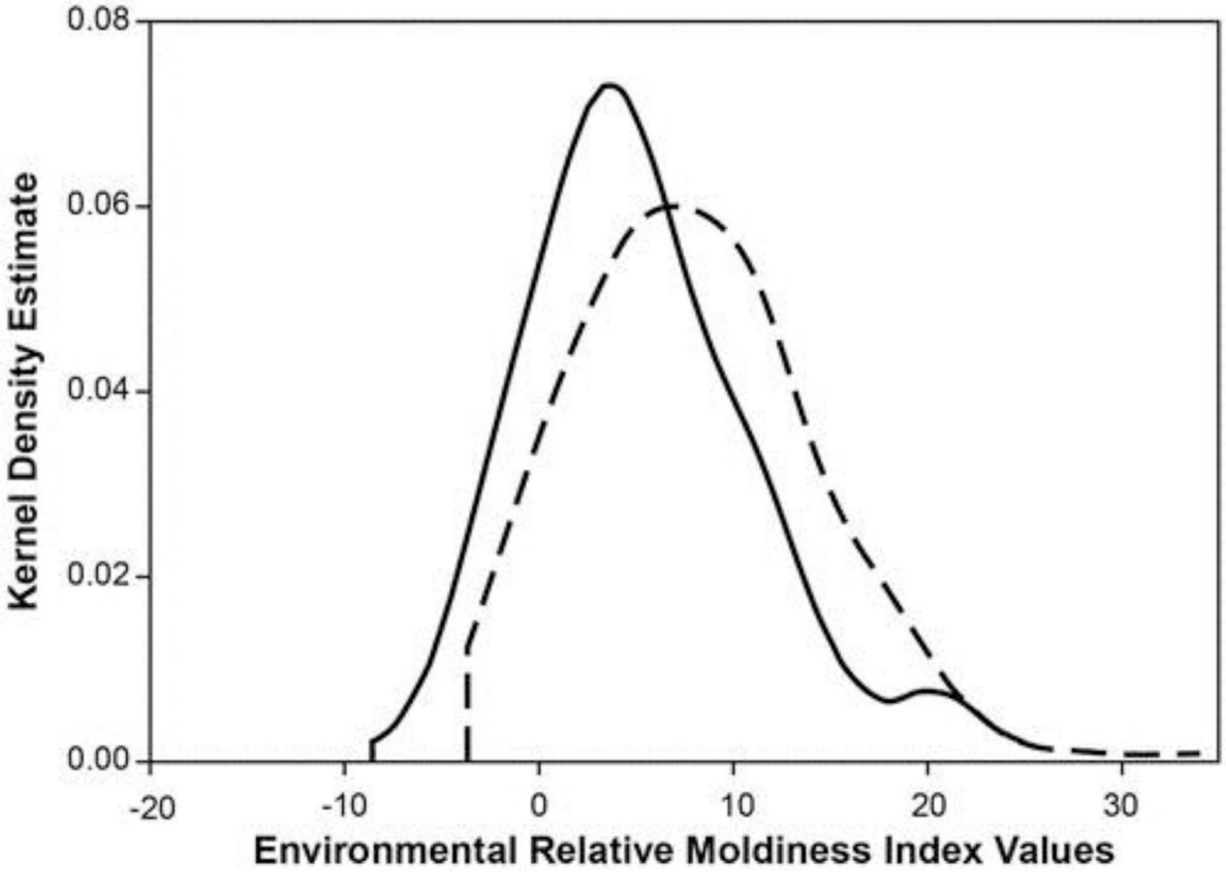

Figure 1

. Kernel density estimate plots of the Environmental Relative Moldiness Index values for the homes sampled in AHHS I (n = 1,096) (solid curved line) and AHHS II (n = 694) (dashed curved line).

Table 1.

Kendall Tau concordance results for comparisons of the percent occurrence in all samples (% Occur), average (AVG) cell-equivalent concentration per mg dust (CE/mg) and geometric mean (GM) in samples from the first American Healthy Homes Survey (AHHS I) (n = 1,096) and AHHS II (n = 694).

Mold species in Groups	% Occur AHHS I	% Occur AHHS II	AVG CE/mg AHHS I	AVG CE/mg AHHS II	GM CE/mg AHHS I	GM CE/mg AHHS II
Group 1						
Aspergillus flavus	36	47	18	14	2	1
Aspergillus fumigatus	62	70	19	5	3	2
Aspergillus niger	69	97	99	824	4	18
Aspergillus ochraceus	27	74	34	27	2	3
Aspergillus penicillioides	90	99	8609	5041	91	140
Aspergillus restrictus	12	76	51	141	2	6
Aspergillus sclerotiorum	26	54	6	12	2	2
Aspergillus sydowii	29	6	60	159	3	6
Aspergillus unguis	20	36	16	9	2	1
Aspergillus versicolor	30	70	28	236	2	14
Aureobasidium pullulans	94	100	1719	876	263	335
Chaetomium globosum	51	72	45	13	2	3
Cladosporium sphaerospermum	82	98	1497	286	13	47
Eurotium amstelodami	98	100	3758	2002	155	71
Paecilomyces variotii	46	64	208	398	2	2
Penicillium brevicompactum	52	89	98	42	5	6
Penicillium corylophilum	17	68	16	41	2	4
Penicillium group 2	8	63	19	51	1	6
Penicillium purpurogenum	15	25	2	8	1	1
Penicillium spinulosum	20	5	5	11	1	1
Penicillium variabile	50	87	18	19	3	6
Scopulariopsis brevicaulis	53	64	18	26	2	2
Scopulariopsis chartarum	38	75	5	15	2	3
Stachybotrys chartarum	35	38	23	2	2	1
Trichoderma viride	27	78	3	10	2	3
Wallemia sebi	75	100	962	4251	18	155
Group 2						
Acremonium strictum	57	82	16	35	4	7
Alternaria alternata	88	100	169	236	35	75
Aspergillus ustus	40	60	6	12	2	2
Cladosporium cladosporioides 1	99	100	1497	1866	331	892

Mold species in Groups	% Occur AHHS I	% Occur AHHS II	AVG CE/mg AHHS I	AVG CE/mg AHHS II	GM CE/mg AHHS I	GM CE/mg AHHS II
Cladosporium cladosporioides 2	70	95	32	59	4	13
Cladosporium herbarum	84	99	432	973	31	180
Epicoccum nigrum	93	98	2394	271	117	59
Mucor racemosus	92	97	146	161	15	17
Penicillium chrysogenum 2	66	95	129	386	5	24
Rhizopus stolonifer	29	52	3	12	1	2
Kendall Tau Concordance	0.662		0.694		0.737	
p-value	<0.001		<0.001		<0.001	

Table 2.

Comparisons made in the mean ERMI values in the first American Healthy Homes Survey (AHHS I) and AHHS II homes. (Significant differences are bolded).

	Comparisons	ERMI Value, Mean	P
A	All homes		
	AHHS I (n = 1,096)	**4.55**	**<0.001**
	AHHS II (n = 694)	**6.85**	
B	AHHS I		
	With year built (n = 1,039)	4.56	0.89
	All AHHS I (n = 1,096)	4.55	
C	AHHS I & II		
	AHHS I (n = 1,039)	**4.56**	**<0.001**
	All AHHS II (n = 694)	**6.85**	

Table 3.

Comparisons made in the mean ERMI values in the first American Healthy Homes Survey (AHHS I) and AHHS II in homes built before 1978 (Pre-1978) and homes built after 1977 (Post-1977).

	Comparisons	ERMI Value, Mean	p
D	AHHS I		
	Pre-1978 (n = 602)	4.93	0.02
	Post-1977 (n = 437)	4.03	
E	AHHS II		
	Pre-1978 (n = 468)	7.61	<0.001
	Post-1977 (n = 226)	5.12	
F	AHHS I & II Pre-1978		
	AHHS I Pre-1978 (n = 602)	4.93	<0.001
	AHHS II Pre-1978 (n = 468)	7.61	
G	AHHS I & II Post-1977		
	AHHS I Post-1977 (n = 602)	4.03	0.03
	AHHS II Post-1977 (n = 226)	5.12	
H	AHHS I & II Pre-1978		
	AHHS I Repeats (n = 205)	5.19	<0.001
	AHHS II Repeats (n = 205)	7.95	

Table 4.

Comparison of the mean summed logs of the Group 1 mold populations (Group 1), summed logs of Group 2 mold populations (Group 2) and ERMI values in the American Healthy Homes Surveys I and II (AHHS I and II) homes built before 1978 (Pre-1978) and homes built after 1977 (Post-1977).

	Comparison	Group 1	Group 2	ERMI
AHHS I (n = 602)	Pre-1978	19.09	14.16	4.93
AHHS I (n = 437)	Post-1978	18.24	14.21	4.03
p-value		0.04	0.63	0.02
AHHS II (n = 468)	Pre-1978	21.69	14.07	7.61
AHHS II (n = 226)	Post-1978	19.02	13.9	5.12
p-value		<0.001	0.49	<0.001