Longitudinal Study of Dust and Airborne Endotoxin in the Home

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To characterize the seasonal variability of endotoxin levels, we measured endotoxin in dust from the bed, bedroom floor, and kitchen floor in 20 homes, and in air from the bedroom in 15 of the homes. All homes were located in the greater Boston, Massachusetts, area and were sampled each month from April 1995 to June 1996. Outdoor air was collected at two locations. We found greater within-home than between-home variance for bedroom floor, kitchen floor, and airborne endotoxin. However, the reverse was true for bed dust endotoxin. Thus, studies using single measurements of dust endotoxin are most likely to reliably distinguish between homes if bed dust is sampled. Dust endotoxin levels were not significantly associated with airborne endotoxin. Airborne endotoxin was significantly (p = 0.04) and positively associated with absolute humidity in a mixed-effect model adjusting for a random home effect and fixed effect of sampling month and home characteristics. This finding implies that indoor humidity may be an important factor controlling endotoxin exposure. We found a significant (p < 0.05) seasonal effect in kitchen floor dust (spring > fall) and bedroom airborne endotoxin (spring > winter), but not in the other indoor samples. We found significant seasonal pattern in outdoor airborne endotoxin (summer > winter). *Key words*: endotoxin, house dust, humidity, indoor air pollution, seasonal variability, temperature. *Environ Health Perspect* 108:1023–1028 (2000). [Online 5 October 2000]
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Endotoxin, a proinflammatory component of the outer membrane of gram-negative bacteria that produces airway inflammation when inhaled, is present in house dust (*1–5*), and thus exposure to endotoxin is common to everyone. Exposure to endotoxin, however, is not well characterized; for example, little is known about seasonal patterns, between- and within-home variability of endotoxin levels, correlation with home climatic factors, and sources of endotoxin.

Research on seasonal patterns of indoor and outdoor airborne endotoxin levels may be useful in understanding the seasonal pattern of respiratory disease (*6–8*) and in establishing a strategy for measurement of home endotoxin. Rizzo et al. (*3*) reported lower levels of house dust endotoxin during the winter on the basis of the repeated measurements in a group of homes over 1 year. In an occupational setting, DeLucca and Palmgren (*9*) reported seasonal variation of airborne endotoxin in respirable dust and in settled grain dusts sampled over 16 months at two grain terminals on the lower Mississippi River. To our knowledge however, there are no published reports about seasonal variation of airborne endotoxin in homes or in the outdoor, ambient air.

In occupational epidemiologic studies, information about within- and between-worker variability has been used (*10–14*) to establish optimal strategies for assessing exposure to workplace contaminants. Likewise, we may also be able to use information about variance components (between- and within-home variance) in endotoxin level to improve assessment of exposure to endotoxin at home. However, there are no previous reports of the within- and between-home variance components of endotoxin.

In this study we measured dust endotoxin in 20 homes and airborne endotoxin in 15 of those homes at monthly intervals for up to 13 months. With these repeated measurements, we analyzed within- and between-home variance components. We investigated the presence of seasonal and indoor climatic influences on endotoxin using the repeated within-home measurements. We also examined seasonal patterns of outdoor airborne endotoxin levels.

Methods

Study cohort. This study is a component of a longitudinal exposure measurement study designed to characterize seasonal variation in home allergen, fungus (*15*), and endotoxin levels. We recruited 20 subjects from the faculty, staff, and students at the Harvard School of Public Health who lived in the greater Boston, Massachusetts, area, who did not plan to move during the study period, and who agreed to help by collecting samples and measuring other environmental factors in their homes.

Environmental measurements. Each participant in the 20 homes collected three dust samples (bedroom bed, bedroom floor, and kitchen floor) on prescheduled days every month from April 1995 through July 1996. Participants in 15 homes also collected air samples, (4 ft above the floor and 1 ft from the nearest wall in the bedroom for 24 hr) every month before dust samples were taken. Vacuuming for collecting dust from the floors and beds followed the published protocol (*15*). Briefly, participants collected settled dust from all layers of bedding by vacuuming for 5 min with a modified Eureka Mighty-Mite II canister vacuum cleaner (Model 3621; The Eureka Co., Bloomington, IN) fitted with a 19 × 90 mm cellulose extraction thimble (Whatman International, Ltd., Maidstone, England). Using a separate thimble, a measured area of the bedroom floor (either 1 or 2 m^2) was vacuumed for 5 min; a third thimble was used to collect kitchen samples when the floor around all edges of cabinets, inside the cabinet under the sink, and around and behind the refrigerator was vacuumed for a total of 5 min. If there was a rug on the kitchen or bedroom floor, it was vacuumed for at least 1 min. The collected dust was weighed in the laboratory and sifted through a 425-µm mesh sieve, and the fine dust was reweighed and separated into aliquots for various analyses: allergens, culturable fungi, and endotoxin. Endotoxin was only assayed if there was sufficient fine dust after dust was used for all other assays.

Indoor air samples were collected from the bedroom of 15 homes. Air was sampled for 24 hr using a Gilian pump (model HFS 513A; Gilian Instrument Corp., West Caldwell, NJ) attached to a filter cassette assembled with a 0.4-µm preweighted polycarbonate filter. Outdoor air samples were collected weekly from two locations, one urban and one suburban, during spring,

summer, and fall, and at least every other week during the winter. Air was sampled for 2–3 days on each occasion. The urban site was located outside a first-floor apartment beside the Charles River in Cambridge, Massachusetts, 6 ft above the ground and 2 inches from the building. The suburban site was located outside of a single-family house, 6 ft above ground and 8 inches from the building, adjacent to a pasture and downwind of a commercial vegetable farm using large quantities of manure in Lexington, Massachusetts. Each air-sampling assembly was precalibrated at 2 L/min flow rate with a Gilian soap bubble flowmeter (P/N 800286; Gilian Instrument Corp.) before sampling, and was postcalibrated with the same instrument after sampling. After sampling, filters were weighed for total suspended particulate (TSP) analysis with an electrobalance (model Cahn 21; Cahn Instrument Inc., Cerritos, CA) at 65–75°F, 35–45% relative humidity, then assayed for endotoxin.

Participants completed a questionnaire about home characteristics each month. They also measured wet-bulb and dry-bulb temperature in the air 2–3 inches above the bedroom floor and on the surface of the bed and bedroom floor with a Microscanner D-501 (Exergen Coportion, Newton, MA). Relative humidity in air and on the surfaces [water activity: amount of water available for microorganisms on the sampling surface (16)] was calculated based on measured dry-bulb and wet-bulb temperature, and absolute humidity (grams per kilogram) was estimated using a psychrometric chart.

Endotoxin assay. Dust endotoxin and airborne endotoxin were assayed as previously described (2,17). Results were reported in endotoxin units with reference to the EC5 or EC6 reference standard endotoxin [U.S. Pharmacopoeia, Inc., Rockville, MD; 1 ng EC5 and EC6 = 10 endotoxin units (EU)]. In a previous report (2), we showed that lots of Limulus amebocyte lysate (LAL) differed in their sensitivity to environmental endotoxin. Therefore, we tested and compared the sensitivity of the LAL lots used in this study to house dust-associated endotoxin and adjusted the final estimates so that all data were on the same scale.

Blank filters for quality control of air sampling were subjected to all the procedures of calibration and storage (on average, four blank filters per month). If the blank filters indicated possible contamination during calibration, shipping, and storage, we excluded air samples collected between 1 day before the date of the contaminated blank and the date of next clean blank. Out of 334 air samples, 53 filters were excluded from data analysis because of contamination that occurred during calibration when soap solution in the

bubble generator became contaminated with gram-negative bacteria.

Data analysis. The normality of distribution for measured and log-transformed dust and airborne endotoxin was evaluated by the Shapiro-Wilk normality test (18). All log-transformed endotoxin measurements were approximately Gaussian except for those of log-transformed bedroom floor dust endotoxin, which were symmetrical. Therefore, all data analyses were performed with log-transformed data. For multiple comparisons, Scheffe's method (19) was used to correct *p*-values.

To analyze within- and between-home variance components, we applied the random effects models described by Rappaport and colleagues (11,12,20,21). Between- and within-home variance components for airborne endotoxin sampled in bedroom, and endotoxin in dust from bed, bedroom floor, and kitchen floor were analyzed in mixed-effect models with a random home effect and a fixed effect of season (spring: April–May; summer: June–August; fall: September–October; winter: November–March). The mixed-effect models were further adjusted for time-varying home characteristics (operating humidifier, windows open, and indoor climate parameters) to examine whether within-home variance could be explained by these factors.

Based on the estimated within- and between-home variance components, the

geometric standard deviation (GSD) of home endotoxin was calculated by taking the antilog of the square root of each variance. We also computed the ratio of within-home to between-home variance and the within-home correlation coefficient [the ratio of between-home to sum of within- and between-home variance, a measure of the reproducibility of repeated measurements (22)].

To examine the correlations between the average home climatic parameters and airborne and dust endotoxin (bed, bedroom floor, and kitchen floor) levels, we averaged all measured values over 13 months from each sampling location within homes. Because the number of homes in this study was relatively small (15 homes with airborne endotoxin and 20 homes with dust endotoxin), we calculated Spearman correlation coefficients.

We used regression models controlling for a random home effect and for the fixed effects of sampling month and room to examine the association of time-varying home climatic parameters with dust endotoxin levels. A regression model controlling for a random home effect and the fixed effect of sampling month was also fit to examine the association of time-varying climatic parameters with airborne endotoxin. We identified home characteristics significantly associated with endotoxin and adjusted for these factors in our final multivariate models to ensure that the association was not confounded by these home characteristics.

Table 1. Distribution of endotoxin level and TSP.

Parameter	Dust (EU/mg)			Air (EU/m^3)	TSP (µg/m^3)
	BB	BF	KF		
GM	43.5	76.8	105.4	0.64	52.9
Median	43.7	80.2	104.7	0.64	57
GSD	2.9	1.9	2.6	2.6	1.7
Minimum	2.8	5.2	4.2	0.02	9.3
Maximum	1057.2	459.5	844.4	19.82	173.8
IQR	53.8	53.4	147.0	0.75	41.2
Number of samples	118	200	128	142	195

Abbreviations: BB, bedroom bed BF, bedroom floor; KF, kitchen floor; GM, geometric mean; IQR, interquartile range.
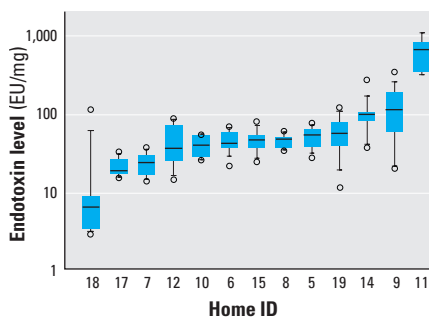

Figure 1. Distribution of bed dust endotoxin level by home. The lower and upper boundaries of each box indicate the 25th and 75th percentiles, respectively. The line within the box indicates the median, and whiskers above and below the box indicate the 90th and 10th percentiles, respectively. Circles are outliers.

Figure 2. Distribution of kitchen floor dust endotoxin level by home. The lower and upper boundaries of each box indicate the 25th and 75th percentiles, respectively. The line within the box indicates the median, and whiskers above and below the box indicate the 90th and 10th percentiles, respectively. Circles are outliers.
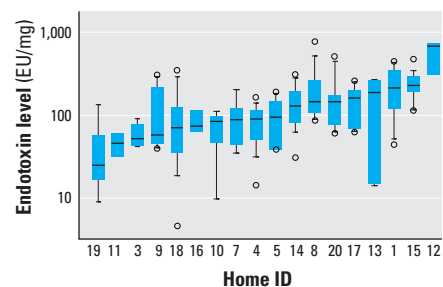
We analyzed seasonal variation in the dust and airborne endotoxin measurements using mixed linear regression models with a random home effect and a fixed season effect (19). In these mixed models, we categorized sampling month into four seasons based on the categorization of season used by Chew et al. (15) in their analysis of antigen levels in these homes. We also adjusted for time-varying home characteristics to determine whether they could explain the seasonal effect on endotoxin levels. To graphically examine time trends in relation to endotoxin levels, we applied the smoothing cubic spline technique with 8 degrees of freedom (23).

Results

Distribution and variance components of endotoxin levels. The distributions of endotoxin in bedroom bed and kitchen floor dust, and distributions of airborne endotoxin and TSP were approximately lognormal (Shapiro-Wilk normality test: $p = 0.23, 0.27, 0.89,$

and 0.15, respectively). Endotoxin levels (Table 1) in kitchen floor and bedroom floor dust were significantly higher ($p < 0.05$) than in dust from beds. Endotoxin levels in kitchen floor dust were also significantly higher ($p < 0.05$) than in bedroom floor dust.

The home-specific medians of repeated endotoxin measurements for bed and kitchen floor dust were within two orders of magnitude across all homes studied (Figures 1 and 2). The range of median bedroom floor dust endotoxin levels covered one order of magnitude (Figure 3). The crude GSD for endotoxin in bed dust was larger than the GSDs for bedroom floor and kitchen floor dust (Table 1). However, when the total variance was partitioned into within-home and between-home variance, the between-home GSD was greater than within-home GSD for bed dust endotoxin; on the other hand, within-home GSDs were greater for endotoxin in floor dust from bedrooms and kitchens (Table 2). When all sources of house dust

endotoxin were analyzed together (pooled data) in a mixed-effect model with a random home effect controlling for the fixed effects of season and room, the within-home GSD was greater than the between-home GSD.

The overall crude GSD of airborne endotoxin was larger than that of TSP (Table 1). The large GSD for airborne endotoxin seemed to be driven by a large within-home variance component (Table 2 and Figure 4). The ratio of within-home to between-home variance of airborne endotoxin was larger than the ratio for any of the dust endotoxin levels.

Correlation between endotoxin measurements. Mean bed endotoxin computed for each home was not significantly correlated with mean bedroom or kitchen floor endotoxin at $\alpha = 0.05$. Mean bedroom and kitchen floor endotoxin were significantly correlated ($p < 0.05$). Mean TSP and airborne endotoxin were not significantly correlated (Table 3).

None of the mean dust endotoxin measurements (bed, bedroom, and kitchen floor) was significantly correlated with mean airborne endotoxin (Table 3). The relationship of dust and airborne endotoxin levels was further examined with a mixed-effect linear regression model, controlling for a random home effect and a fixed sampling month effect. However, we did not observe a significant association between dust and airborne endotoxin levels.

Endotoxin levels and home characteristics and climate parameters. In the crude analysis of correlations between average climate factors and average airborne endotoxin levels within homes, airborne endotoxin levels tended toward weak positive correlations with humidity (except for absolute humidity) and weak negative correlations with temperature (except for wet-bulb temperature); none of these correlations was significant. Correlations of dust endotoxin with climatic factors showed similar patterns, as did airborne endotoxin (weakly positive with humidity and weakly negative with temperature). However, kitchen floor dust endotoxin was significantly correlated with bed and bedroom floor surface temperature at $\alpha = 0.05$ (Table 4). A total-home mean dust endotoxin level, calculated by averaging all types of dust endotoxin measurements within a home (bed, bedroom floor, and kitchen floor) was significantly and negatively associated with wet-bulb temperature.

The association of endotoxin levels with home characteristics and indoor climate parameters was further examined with mixed-effect regression models that included a random home effect and a fixed sampling month effect. We found that certain home characteristics (wool bedding on bed, type of
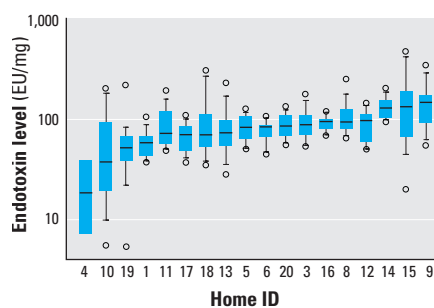

Figure 3. Distribution of bedroom floor dust endotoxin level by home. The lower and upper boundaries of each box indicate the 25th and 75th percentiles, respectively. The line within the box indicates the median, and whiskers above and below the box indicate the 90th and 10th percentiles, respectively. Circles are outliers.

Figure 4. Distribution of indoor airborne endotoxin level by home. The lower and upper boundaries of each box indicate the 25th and 75th percentiles, respectively. The line within the box indicates the median, and whiskers above and below the box indicate the 90th and 10th percentiles, respectively. Circles are outliers.
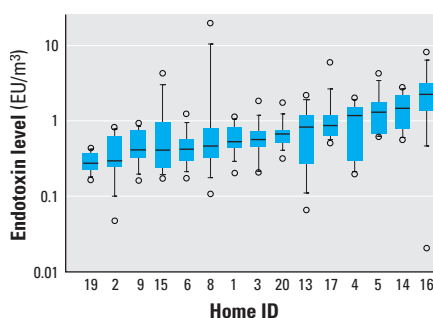
Table 2. Variance components of log-transformed home endotoxin level.

Endotoxin Model[a]	No. of homes	No. of samples[b]	GSD Within-home	GSD Between-home	Variance ratio (within/between)	Within-home correlation[c]
Bed dust						
A	20	118	1.82	2.76	0.35	0.74
B			1.80	2.89	0.31	0.76
Bedroom floor dust						
A	20	200	1.71	1.53	1.61	0.38
B			1.70	1.55	1.48	0.40
Kitchen floor dust						
A	20	128	2.15	1.75	1.88	0.35
B			2.15	1.81	1.65	0.38
Airborne endotoxin						
A	15	139	2.30	1.52	3.93	0.20
B			2.15	1.61	2.56	0.28

[a]A: Variance components were estimated using a mixed-effect model with a random home effect and fixed season effect; B: Variance components were estimated using a mixed-effect model with a random home effect and fixed season effect controlling for time-varying home characteristics within home (operating humidifier and windows open) for bed and bedroom floor dust endotoxin. For kitchen floor dust endotoxin and bedroom airborne endotoxin, models additionally included indoor climate parameters (wet- and dry-bulb temperature and absolute and relative humidity). [b]There was a total of 446 dust samples with dust available for the entoxin assay; 139 air samples were used in data analysis. [c]Ratio of between-home to sum of within-home and between-home variance represents reproducibility of repeated measurements within a home.

rug vacuumed in kitchen floor, foam pillow on bed, cotton bedding on bed, and operating humidifier) were significantly associated with airborne endotoxin. Of the climate parameters, only absolute humidity was positively [$\beta = 0.5$ (EU/m^3)/(10g H$_2$O/kg air)] and significantly ($p = 0.01$) associated with airborne endotoxin levels. This association remained significant ($p = 0.04$) in a multivariate model after adjusting for the significant home characteristics.

In the mixed regression model controlling for the random home effect and the fixed effect of sampling month and sample type, none of the indoor climate parameters was significantly associated with dust endotoxin levels. We found that mattress type on bed, type of rug vacuumed in kitchen floor, type of rug vacuumed in bedroom floor, and operating humidifier were significantly associated with dust endotoxin in the same mixed models. The lack of association between climactic parameters and dust endotoxin did not change after adjusting for the significant home characteristics.

Seasonal variation of indoor dust and airborne endotoxin levels. Our data did not suggest a consistent temporal pattern in endotoxin levels in settled dust. When we categorized sampling month by season, significant seasonal effects were observed in kitchen floor dust endotoxin levels; dust endotoxin level was highest in spring and lowest in fall. The seasonal effect on kitchen dust endotoxin remained significant ($p < 0.01$) in a multivariate model controlling for the time-varying home characteristics including home climate parameters (Table 5). However, we did not observe a seasonal influence on endotoxin levels in bed and bedroom floor dust.

Our data showed a significant seasonal effect on airborne endotoxin levels (Table 5). Airborne endotoxin was highest in the spring and lowest in the winter, both before and after adjusting for time-varying home characteristics (Table 5). The only significant contrast between seasons, after adjusting for multiple comparisons, was between spring and winter.

Outdoor airborne endotoxin. Overall (crude) mean indoor airborne endotoxin levels (Table 1) appeared to be higher (GM = 0.64) than those in outdoor air ($n = 70$, GM = 0.46, GSD = 2.6). However, the mixed-effect regression model [including fixed effects for sampling site (indoor/outdoor), season, and a sampling site by season interaction, controlling for the random home effect] indicated that the seasonal effect and the sampling site by season interaction effects were both significant ($p < 0.001$ and $p = 0.001$, respectively). The airborne endotoxin level was not consistently higher indoors than outdoors (Figure 5). From September through April, indoor airborne endotoxin levels were generally higher than those outdoors;

Table 3. Spearman correlation coefficient between average[a] values of airborne endotoxin, house dust endotoxin, and TSP concentration.

| Measurement | TSP | House dust endotoxin (EU/mg) | | | |
		BB	BF	KF	Mean[b]
Airborne endotoxin[c] [EU/m^3]	0.13 (15)[d]	−0.23 (11)	0.18 (14)	0.33 (14)	0.04 (15)
TSP (mg/m^3)	—	0.21 (11)	0.03 (14)	−0.24 (14)	0.26 (15)
Bedroom bed (EU/mg)	—	— (16)	0.45 (15)	0.00 (15)	—
Bedroom floor (EU/mg)	—	—	— (19)	0.46* (19)	—
Kitchen floor (EU/mg)	—	—	—	— (19)	—

Abbreviations: BB, bedroom bed; BF, bedroom floor; KF, kitchen floor.
[a]Average over all repeated measurements during the study period by each sample type (BB, BF, KF, or air) within a home. [b]Mean overall dust endotoxin measurements within a home during the study period. [c]Airborne endotoxin was sampled in bedroom. [d]Number of homes. *$p < 0.05$.

Table 4. Spearman correlation coefficient (number of homes) between average[a] values of airborne endotoxin and house dust endotoxin, TSP concentration, and indoor climate.

| Home climate parameters | Airborne[b] endotoxin | TSP | House dust endotoxin level (EU/mg) | | | |
			BB	BF	KF	Mean[c]
Percent relative humidity	0.18 (15)	−0.38 (15)	0.36 (16)	0.19 (19)	0.25 (19)	0.01 (20)
Absolute humidity	−0.08 (15)	−0.39 (15)	0.31 (16)	−0.03 (19)	0.03 (19)	−0.23 (20)
Water activity of bed	0.26 (15)	−0.48 (15)	0.18 (16)	0.31 (19)	0.32 (19)	−0.02 (20)
Water activity of BF	0.13 (15)	−0.43 (15)	0.35 (16)	0.31 (19)	0.42 (19)	0.10 (20)
Dry-bulb temperature of air	−0.19 (15)	0.11 (15)	−0.48 (16)	−0.36 (19)	−0.41 (19)	−0.42 (20)
Wet-bulb temperature of air	0.08 (15)	−0.01 (15)	−0.09 (16)	−0.22 (19)	−0.31 (19)	−0.59* (20)
Surface temperature of bed	−0.21 (15)	0.28 (15)	−0.22 (16)	−0.40 (19)	−0.51* (19)	−0.28 (20)
Surface temperature of BF	−0.22 (15)	0.29 (15)	−0.31 (16)	−0.34 (19)	−0.46* (19)	−0.34 (20)

Abbreviations: BB, bedroom bed; BF, bedroom floor; KF, kitchen floor.
[a]Average overall repeated measurements during the study period by each sample type (BB, BF, KF, or air) within a home. [b]Airborne endotoxin was sampled in the bedroom. [c]Mean overall dust endotoxin measurements within a home during the study period. *$p < 0.05$.

Table 5. Seasonal variation in home endotoxin level—mixed-effect regression models.

| Model[a] | Response var[b] log (endotoxin) | No. | Season: main effect | | Covariates[c] | Least squares mean by season | | | |
			Variable[c]	p-Value		Spring	Summer	Fall	Winter
A	Bed dust	118	Season (F)	0.75	Home (R)	40.3	46.2	48.9	42.0
B			Season (F)	0.85	Home (R) + HC (F)	40.7	46.7	45.0	37.8
A	Bedroom floor dust	200	Season (F)	0.20	Home (R)	83.5	68.0	67.3	71.2
B			Season (F)	0.14	Home (R) + HC (F)	86.5	73.9	66.4	69.3
A	Kitchen floor dust[c]	128	Season (F)	< 0.01	Home (R)	154.0	97.4	58.0	93.1
B			Season (F)	< 0.01	Home (R) + HC (F)	155.8	82.9	49.4	106.4
A	Airborne endotoxin[c]	139	Season (F)	0.01	Home (R)	0.94	0.59	0.77	0.51
B			Season (F)	0.02	Home (R) + HC (F)	0.98	0.50	0.64	0.60

Abbreviations: R, random effect covariate; F, fixed effect covariate; HC, time-varying home characteristics within home.
[a]A: mixed-effect model with a random home effect and a fixed season effect (spring: April–May, summer: June–August, fall: September–October, winter: November–March). B: Mixed-effect model A is additionally adjusted for time-varying home characteristics within home (windows open, number of layers of bedding, and home climate parameters: humidity and temperature). [b]Airborne endotoxin, EU/m^3; dust endotoxin, EU/mg. [c]Significant seasonal effect.

however, the difference was only significant during the winter ($p = 0.01$). Indoor levels tended to be somewhat but not significantly ($p = 0.26$) lower than outdoor levels during the summer (June through August).

Analysis of the association between season and outdoor airborne endotoxin level, adjusted for multiple comparisons, indicated that the level during winter [least squares mean (LSM) = 0.19 EU/m^3] was significantly lower than any other season ($p < 0.05$ for all pairwise comparisons). The level was highest during summer (LSM = 0.92 EU/m^3). Summer levels were significantly greater ($p = 0.004$) than levels during the fall (LSM = 0.42 EU/m^3), but not significantly different from spring levels (LSM = 0.64 EU/m^3).

Overall mean airborne endotoxin levels at the urban sampling location ($n = 32$, GM = 0.51 EU/m^3, GSD = 2.1) appeared to be higher than those at the suburban location ($n = 35$, GM = 0.39 EU/m^3, GSD = 3.1). However, the location effect was not significant in a regression model with a fixed location effect controlled for a fixed effect of season; we found no statistical evidence for an urban/suburban location by season interaction effect.

Discussion

Variance of and relationship between airborne and dust endotoxin. Rappaport and colleagues (*11,12,20,21*)and others (*13,14*) have used variance components (between- and within-person variance) to examine assumptions about homogeneity of exposure within groups and the utility of exposure assessment strategies in occupational epidemiology. In this study we applied a similar approach to examine how well various measures of home endotoxin distinguish domestic exposure

among a group of faculty, staff, and students at the Harvard School of Public Health. We found that the ratio of within-home to between-home variance was less than 1 for bed dust, but not for bedroom or kitchen floor dust endotoxin. The reproducibility of repeated endotoxin measurements within homes (within-home correlation coefficient) was greater for bed dust than for either bedroom or kitchen floor dust. This implies that if house dust is to be used for endotoxin exposure assessment, bed dust may provide better discrimination of exposure between individuals than can be achieved (*24*) with bedroom or kitchen floor dust. Our finding is consistent with the observation by Michel et al. (*4*) that endotoxin in dust collected from beds was significantly associated with asthma severity in house dust mite–sensitized adults. However, our data may also be consistent with the observation by Douwes et al. (*24*) that peak flow variability was not significantly associated with endotoxin in living room floor dust, and may help to explain these otherwise apparently discrepant results. The largest within-home variance for endotoxin in dust was observed in kitchen floor samples; water and organic material in the kitchen environment may provide more variable conditions for bacterial growth or accumulation of endotoxin than do the bed or bedroom floor.

The within-home geometric standard deviation (GSD$_w$) and variance ratio were larger for airborne endotoxin, and the reproducibility was poorer than for house dust endotoxin (Table 2). Thus, merely on statistical grounds, it is clear that much greater effort would be required to discriminate domestic exposures using measurement of airborne rather than house dust endotoxin.

This finding suggests that, rather than a single 24-hr air sample, multiple samples or a longer sampling period may be required to accurately assess airborne endotoxin exposure.

Whether dust endotoxin can be appropriately considered an indicator of endotoxin exposure, however, depends on some assumptions about how individuals are exposed. We found no significant association between dust endotoxin and airborne endotoxin in either crude correlation analyses or mixed regression models taking account of the repeated measures design of the study. Thus, dust endotoxin alone is a weak surrogate for airborne endotoxin levels. If airborne endotoxin at home represents true exposure, then use of dust endotoxin as a surrogate will result in nondifferential misclassification of exposure. On the other hand, endotoxin in dust may be a significant direct source of exposure for infants, and endotoxin in the bed may be a significant source of exposure for both children and adults.

Therefore, two sources of bias toward the null in analysis of exposure–response relationships should be considered when single measurements of house dust endotoxin are used for exposure assessment. The first source of bias arises if dust endotoxin is considered a surrogate measure for airborne endotoxin (*25*), and the second when the within-home variance is larger than the between-home variance (*13*). Bias due to these sources of error may be reduced through use of internal validation study designs.

The overall distribution (Table 1) of home endotoxin levels in this repeated measurement study of a small number of homes was comparable to the distribution we observed in a cohort study involving 499 homes (data not shown), most visited only once. Therefore, the data used in this analysis appear to be representative, although due to the small number of homes, weak associations between air and dust endotoxin levels were not significant in the present study.

Endotoxin levels and temperature and humidity. Home airborne endotoxin may be more closely related to moisture (dampness) than to temperature. Simard et al. (*26*) reported that the level of bacteria measured inside the duct of an apartment building was associated with relative humidity in the duct. In numerous studies (*27–31*), home dampness has been significantly associated with children's respiratory disease and symptoms. Our findings suggest that home endotoxin may be among the exposures responsible for the association, and may be a reasonable objective measure of the biological burden resulting from dampness.

Seasonal variability of home and outdoor airborne endotoxin. Little data is available on seasonal variation of endotoxin in homes.
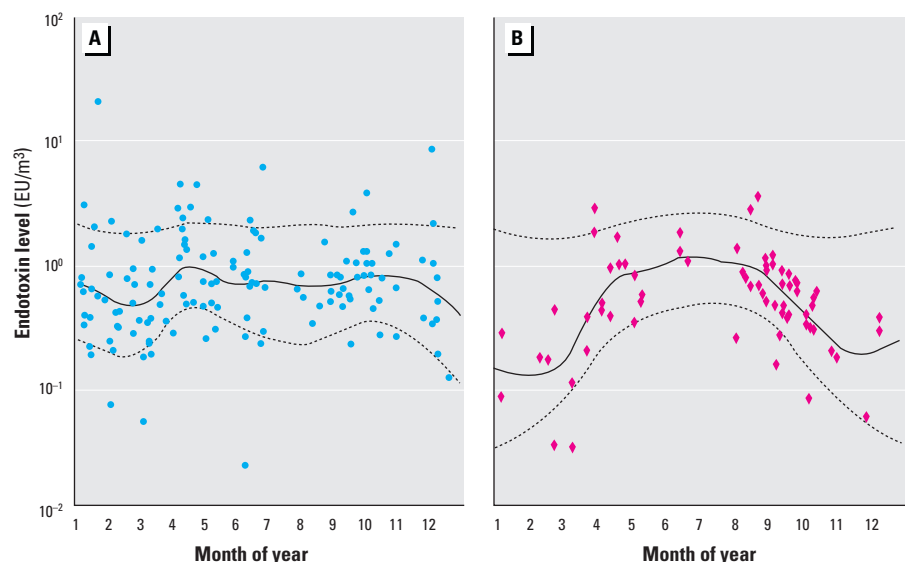

Figure 5. Smoothed plots of indoor (*A*) and outdoor (*B*) airborne endotoxin level over time. Dashed lines denote the standard error of smoothing spline (with 8 degrees of freedom).

Rizzo et al. (*3*) reported seasonal variation in house dust endotoxin with significantly lower endotoxin levels during the winter in 20 homes sampled 13 times during a 1-year study in Brazil. Our data from Massachusetts showed significant seasonal variation in kitchen dust and airborne endotoxin levels, but not in bed and bedroom dust endotoxin. The air and floor dust samples had their highest endotoxin levels in the spring, whereas endotoxin in bed dust was relatively constant. However, the range of mean endotoxin variation across seasons was small, ≤ 2-fold for all samples except for the kitchen floor. Thus, evidence to suggest an important seasonal pattern in home endotoxin is weak.

On the other hand, we observed a significant seasonal pattern in outdoor airborne endotoxin level; mean outdoor endotoxin levels varied by more than a factor of four across seasons. There was a decline of outdoor airborne endotoxin beginning at the end of summer or early in the fall. Outdoor endotoxin remained low during the winter and started to increase with the beginning of growing season. Our observations are consistent with the data suggesting that outdoor gram-negative bacteria, and thus airborne endotoxin, are shed from leaves of growing plants (*32,33*). The difference between indoor and outdoor airborne endotoxin levels varied with season. Our data indicate that indoor airborne endotoxin levels are significantly higher than outdoor levels during the winter, but similar to outdoors during the spring, summer, and fall. Thus, while indoor sources clearly predominate during the winter, outdoor airborne endotoxin may contribute to indoor airborne endotoxin, especially during the spring, summer, and fall when endotoxin levels indoors are relatively constant and homes are not tightly sealed.

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