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Characterization and Variability of Endotoxin and 3-Hydroxy Fatty Acids in an Office Building During a Particle Intervention Study

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Abstract Air and dust samples were collected on two floors of an office building during a double-blind particle intervention study to examine spatial and temporal variability of airborne endotoxin over a period of weeks, and to characterize endotoxin activity and lipopolysaccharide (LPS) content in carpet and chair dust. Air samples were collected on multiple days within and across weeks. Dust samples were collected from carpets and chairs one day per week for three weeks. Endotoxin was measured using a *Limulus* assay. Dust samples were analyzed for LPS by determination of 3-hydroxy fatty acids (3-OHFAs) using gas chromatography-mass spectrometry. The geometric mean (geometric standard deviation) for 96 indoor air samples was 0.24 (1.6) EU/m^3. Significant within-floor spatial variation of airborne endotoxin was found ($P<0.0001$, $n=80$). Temporal variability of airborne endotoxin was not significant across weeks. Mean (±SD) endotoxin levels in carpet dust (59±9.3 EU/mg dust, $n=12$) and in chair dust (38±7.7 EU/mg dust, $n=10$) were significantly different ($P<0.001$). Carbon chain length-dependent differences in 3-OHFA levels by dust source and floor were found. Enhanced air filtration did not significantly affect airborne endotoxin ($P=0.62$); however, total dust mass and total endotoxin in carpet dust samples increased significantly after enhanced surface cleaning ($P<0.01$). These findings suggest that spatial variability, dust source, and surface cleaning may influence building occupant exposures to endotoxin.

Key words Endotoxion; 3-hydroxy fatty acids; Indoor air; Exposure assessment.

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Introduction

Endotoxins are biologically active lipopolysaccharide (LPS) molecules that form the outer membrane of Gram-negative bacteria (GNB). Endotoxin toxicity is thought to be related to LPS composition, which varies among bacterial species. Lipid A, a component of LPS, contains characteristic 3-hydroxy fatty acids (3-OHFAs), primarily of carbon chain lengths C_{10}, C_{12}, C_{14}, C_{16}, and C_{18} (Wilkinson, 1988). These 3-OHFAs can be used as chemical markers to estimate total LPS (Sonesson et al., 1994). Endotoxin activity is typically measured using a *Limulus*-based bioassay (Milton, 1995a). LPS-associated 3-OHFAs are determined by gas chromatography-mass spectrometry (GC-MS), which provides a quantitative distribution of 3-OHFAs in each sample (Mielniczuk et al., 1993; Gradowska and Larsson, 1994; Saraf et al., 1997).

Numerous studies in office buildings, homes and other non-industrial indoor environments have investigated the relationship between endotoxin or GNB exposure and health effects among occupants. In office buildings, two large studies of endotoxin exposures and symptoms have been conducted. In the first study, a dose-response relationship was found between airborne endotoxin levels and building-related symptoms in 19 office buildings in The Netherlands (Teeuw et al., 1994). Among mechanically-ventilated buildings, airborne endotoxin levels were six times higher in buildings classified as ''sick'' as compared to those classified as ''healthy'' (254 vs. 46 ng/m^3), and seven times higher in ''sick'' buildings than in naturally-ventilated buildings (254 vs. 35 ng/m^3). The concentration of airborne viable Gram-negative rods was highest in the

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"sick" buildings, and components of the mechanical ventilation systems were also more likely to be contaminated with Gram-negative rods in the "sick" buildings than in the "healthy" buildings. In the second study, the prevalence of GNB in floor dust was significantly correlated with general symptoms and mucous membrane symptoms in 12 Danish town halls; however, endotoxin levels in dust (mean: 0.0248 EU/mg dust, range: 0.0033–0.061 EU/mg dust (note: ng per EU endotoxin not given)) were not correlated with symptoms (Gyntelberg et al., 1994).

Several studies have also been conducted in homes. Airborne endotoxin levels in 22 flats in Sweden ranged from 0 [not detected] to 18.1 ng/m^3 after disturbance of indoor surfaces to simulate human activity (Rylander et al., 1989). Dose-response relationships were reported for endotoxin and cough, breathing difficulties, itchy eyes, and tiredness. In another study, airborne endotoxin in eight homes ranged from 0.03 to 7.6 ng/m^3 under non-aggressive sampling conditions (Milton, 1995b). Michel et al. (1991) found a significant relationship between clinical severity of asthma and house dust endotoxin levels (mean($\pm$SD)=2.59 ($\pm$3.41) ng/mg dust, range: 0.12–20 ng/mg dust) in a study of 28 homes. In a subsequent study, endotoxin in house dust was related to asthma severity in house dust mite-sensitized subjects who were concurrently exposed to high levels of dust mite antigen (Michel et al., 1996). Mean ($\pm$SE) endotoxin levels for mattress dust and floor dust in this study were 1.86 ($\pm$0.1) and 1.78 ($\pm$0.1) ng/mg dust, respectively (1 ng=12 EU). Rizzo et al. (1997) also reported a significant correlation between endotoxin levels in house dust (median=10.8 EU/mg dust, range: 0.03–1100 EU/mg dust (note: ng per EU endotoxin not given)) and severity of clinical symptom scores in nine house dust mite-sensitized asthma patients. House dust endotoxin levels were significantly higher in summer than in winter. Milton et al. (1997) reported a geometric mean of 9.36 ng/mg dust for endotoxin in settled dust from 32 homes.

Evidence of a link between endotoxin exposures and health effects in other non-industrial indoor environments has also been reported. In a daycare center, airborne endotoxin levels were significantly higher in two areas where symptoms were present, as compared to an area with no symptoms (Rylander et al., 1989). Exact endotoxin air levels were not reported; however, graphed data indicated levels were less than 0.25 ng/m^3. Rylander et al. (1992) also reported mean ($\pm$SD) airborne endotoxin levels in an office building (0.06 ± 0.01 ng/m^3), day care center (0.43 ± 0.18 ng/m^3), post office (0.19 ± 0.11 ng/m^3), and two schools (0.26 ± 0.14 and 0.21 ± 0.16 ng/m^3) after aggressive sampling had been performed. A statistically significant correlation between airborne endotoxin and skin rashes was found in these settings.

These findings suggest that further characterization of endotoxin exposures in indoor environments is warranted. In particular, limited information is available regarding the spatial and temporal variability of endotoxin in office buildings. Such information is important in order to properly assess the exposures of building occupants. Characterization of the LPS content of dust in occupied spaces may also provide insight into endotoxin sources and potency. In the present study, air and dust samples were collected over a period of weeks at multiple locations on two floors of an office building during an intervention study that involved the controlled removal of particles from air and surfaces at specific times. The sampling objectives were to determine endotoxin levels in air and settled dust, to describe the spatial and temporal variability of airborne endotoxin, to measure 3-OHFA levels in settled dust, and to examine the relationship between endotoxin and 3-OHFAs in the dust. Results of the intervention study are reported elsewhere (Mendell et al., 1999a; Mendell et al., 1999b).

Material and Methods

Office Building

A four-story office building in St. Louis, MO, USA, that did not have a history of health complaints nor obvious sources of microbiological contamination was selected for the intervention study. Floor 2 (4,130 m^2, 165 persons) and floor 4 (4,840 m^2, 280 persons) were sampled during a nine-week period from July to September (Table 1). The two floors had independent ventilation systems, sealed windows, an open cubicle design, wall-to-wall carpeting and fabric-covered chairs. Each floor was divided into four subspaces (floor 2: 21, 22, 23, 24; floor 4: 41, 42, 43, 44), and each worker was considered to work in one subspace. Ventilation air handlers supplied air to pairs of subspaces (21&22, 23&24, 41&42, and 43&44). Two interventions aimed at reducing particles in the building were conducted according to the schedule shown in Table 1 (Fisk et al., 1998). The first intervention, enhanced air filtration, was achieved by installing highly efficient particle filters (estimated $\geq$95% efficient at 0.3 μm). The second intervention, enhanced surface cleaning of carpets and chairs, was done using two custom-built vacuum cleaners with high-efficiency particulate aerosol (HEPA) filters (99.97% for 0.3 m particles) and unusually high flow rates (manufacturer's data: 1840–2640 L/min, floors; 1560–2040 L/min, chairs). Each vacuum

Table 1 Sequence of Interventions and Sample Collection

Week (Mon–Sun)	Dates (1996)	Condition: Floor 2[a]	Condition: Floor 4[a]	Sample collection	
				Air	Dust
1	July 15–21	Normal efficiency filters	Normal efficiency filters	Mon[b], Wed, Fri[b]	Sat
2	July 22–28	Normal efficiency filters	Normal efficiency filters	none	none
3	July 29–Aug 4	Normal efficiency filters	High efficiency filters	Thur	none
4	Aug 5–11	High efficiency filters	Normal efficiency filters	Thur	none
5	Aug 12–18	Normal efficiency filters	High efficiency filters	none	none
6	Aug 19–25	High efficiency filters	Normal efficiency filters	none	Fri, Sat
7	Aug 26–Sept 1	Normal efficiency filters	Normal efficiency filters	Thur	none
8	Sept 2–8	Enhanced carpet and chair cleaning[c] Normal efficiency filters	Normal efficiency filters	Thur	Fri, Sat
9	Sept 9–15	Normal efficiency filters	Normal efficiency filters	none	none

[a] Refers to particle filters installed in the building's heating, ventilating, and air conditioning system, which filter the mixture of incoming outdoor and recirculated indoor air
[b] Floor 2 only
[c] Enhanced cleaning of carpets and chairs was done Sat (8/31) and Sun (Sept 1) preceding week 8

cleaner was equipped with a settling chamber and cyclone separator to prevent plugging of the HEPA filter and an associated reduction in cleaning performance from a diminished vacuum cleaner flow rate. Average relative humidity and temperature during the study period ranged from 42–58% and 22–26°C, respectively.

Collection of Air Samples

Air sampling was performed according to the schedule in Table 1. Two area samples (not side-by-side) were collected from each subspace. Samples were taken approximately 4 feet above the floor. Two outdoor air samples were also collected each sampling day in an air intake room upstream from the building filters. Air samples were collected on 0.4-μm polycarbonate filters in closed-faced 3-piece polystyrene filter cassettes at a nominal flow rate of 3 Lpm for 8 h using personal sampling pumps (Model 224-PCXR8; SKC, Eighty Four, PA, USA). Pump calibration was checked pre- and post-sampling, and periodically during the day with a precision rotameter calibrated against a primary standard. Eleven field blanks (no air pulled through the filter), and two rotameter blanks (air pulled through the filter for 30 s with the rotameter upstream from the filter) were collected.

Collection of Dust Samples

Dust samples from carpeted floors and fabric-covered chairs were collected in 37-mm glass fiber closed-faced filter cassettes according to the schedule in Table 1. AirCon2 pumps (Sensidyne Inc., Clearwater, FL, USA) were used for all dust sampling.

Carpet dust samples were collected by pulling an apparatus designed to maintain a controlled flow rate (approximately 8.3 Lpm) and a fixed height between the inlet and carpet (approximately 1 mm below the surface of the carpet) at a constant rate for 10 min over a 240-m path (approximately two-thirds office cubicle and one-third aisle) intended to represent the entire floor (Fisk et al., 1998).

Chair dust samples were collected at 20 Lpm using a hand-held nozzle (15.9 mm I.D.) attached to the inlet of a filter cassette and a plastic sampling template with holes 5.1 cm in diameter (Fisk et al., 1998). A composite dust sample of 12 or 20 chairs was collected by placing the template on the seat of a chair and manually vacuuming four holes in the template for 3 s each.

A tube filled with the desiccant Drierite™ was attached to the outlet of each air and dust cassette after sampling. Cassettes were refrigerated and then shipped cold (not frozen) to the laboratory. Air sample filters were removed from the cassettes and placed in separate borosilicate glass tubes. Total mass for dust samples was determined by weighing the dust harvested from the cassettes, except for six samples where the difference in cassette weight before and after sampling was used. An aliquot of dust (approximately 25 mg) from each sample was placed in a borosilicate glass tube. All glass tubes were covered with parafilm, and the parafilm was poked with a pin. Air filters and dust aliquots were stored dessicated at 4°C (Walters et al., 1994) and −20°C (Milton et al., 1997), respectively, until analysis.

Endotoxin Analysis

Endotoxin was quantified by the kinetic *Limulus* assay with resistant-parallel-line estimation (KLARE) method (Milton et al., 1997; Milton et al., 1992; Walters et al., 1994). Filters and dust aliquots were extracted in 5 mL of buffer (0.05 M potassium phosphate, 0.01% triethylamine, pH 7.5) for 60 min at 20°C in a bath sonicator. Dust extracts were vortexed every 15 min.

Initial dilutions for dust extracts (1:25) and control standards (*Escherichia coli* 0113:H10:K0; Associates of Cape Cod, Woods Hole, MA, USA) brought them into the upper endotoxin concentration range (0.03–50 EU/mL) of the assay, followed by serial dilution in buffer of all samples and standards. *Limulus* amebocyte lysate was obtained from BioWhittaker (Walkersville, MD, USA). The V_{max} response was used as the outcome measure and endotoxin activity was determined after correction for dilution-dependent interference. No samples above the limit of quantification (0.1 EU/mg dust) showed dilution-independent interference. Results were reported in endotoxin units (EU) with reference to EC6 (USP, Reference Standard Endotoxin Lot G, 1 ng=10 EU). Potency of the control standard endotoxin was determined by direct assay with the reference standard, with both standards diluted in buffer. In the present paper, the term ''endotoxin level'' means the biological activity of the sample in EU/m^3 or EU/mg dust, and the term ''endotoxin potency'' means the level of endotoxin activity in EU per picomole of total LPS.

3-OHFA Analysis

Dust samples (3–8 mg) were heated in 3.6 M methanolic HCl at 100°C for 4 h after which 50 ng of deuterated 3-OH 14:0 methyl ester (internal standard) was added. The preparations were extracted and purified as previously described (Mielniczuk et al., 1993). Trimethylsilyl (TMS) derivatives were prepared by adding 50 μL of bis(trimethylsilyl)trifluoroacetamide (BSTA) and 5 L of pyridine to the purified extracts, and then heating at 80°C for 20 min. The final volume was brought up to 100 μL with n-heptane; 1 μL was used for GC-MS. Analytical conditions were as previously described (Mielniczuk et al., 1993), except the fused-silica capillary column (30 m×0.25 mm I.D.) contained CP-SIL 8 as the stationary phase (Chrompack, Middelburg, The Netherlands), the column temperature was programmed from 90 to 280°C, and the injector and interface (between GC and MS) were kept at 280°C. LPS was quantified by assuming that 4 moles of 3-OHFA corresponded to 1 mole of LPS (Wilkinson, 1988; Rietschel et al., 1984). Quantitation of 3-OH-$C_{10:00}$ may have been affected by an unidentified compound that partly co-eluted with this acid.

Statistical Analysis

Air Data

Indoor endotoxin air data appeared to fit an approximately lognormal distribution based on a graphical evaluation of the data, and a goodness-of-fit measure, the ratio metric (RM=1.00, n=96, CV=0.5) (Waters et al., 1991). Outdoor endotoxin air data were assumed to be described by an approximately lognormal distribution. Values for samples below the limit of detection were estimated by dividing the limit of detection (LOD) by the square root of two (Hornung and Reed, 1990). Analysis of variance (ANOVA) was performed on natural log-transformed endotoxin air levels.

Between-week spatial and temporal variability was evaluated using samples collected on Thursday (weeks 3, 4, 7 and 8) and Wednesday (week 1) from both floors (n=80). The effects of the independent variables week, floor, subspace, and replicate (location within subspace) on airborne endotoxin were tested in a nested multi-factorial ANOVA model. Within-week spatial and temporal variability were evaluated using samples collected on Monday, Wednesday, and Friday during week 1, on floor 2 (n=24). The effects of the independent variables subspace, day of week, and replicate on airborne endotoxin were also tested by ANOVA.

To test the effect of enhanced air filtration on airborne endotoxin, only data for weeks 3 and 4 (before and after filtration) were used. Since the intervention was conducted within a crossover design, the effect of enhanced filtration was completely confounded with the week by floor interaction. By using the between-week ANOVA model, restricting the analysis to weeks 3 and 4, and assuming no week by floor interaction, the test for enhanced filtration was the same as the test for week by floor.

Dust Data

Dust sample data appeared to fit an approximately normal distribution based on a graphical evaluation of the data. The effects of the independent variables source (carpet or chair), week, and floor on the endotoxin level (EU/mg dust) and the endotoxin potency (EU/pmole LPS) of the dust samples were tested in a multi-factorial ANOVA model. The correlation between endotoxin level and 3-OHFA (pmole LPS/mg dust) was evaluated for each carbon chain length alone and for all combinations of carbon chain lengths using Pearson's correlation coefficient (31 correlations). The Pearson correlation matrix for individual 3-OHFAs was also computed.

The main effects of source, floor, week, chain length, and the first and second orders interaction terms involving floor, source, and chain length on the concentration of individual 3-OHFAs were tested using a split-plot analysis. Interactions involving week and other main whole plot factors (floor and source) were combined with replicate (samples collected within the same floor and the same source) to form the whole plot error. All interactions involving week and chain length

were pooled to form the sub-plot error. The second-order interaction term was tested first, found not significant (P=0.90) and removed from the model. The three first-order interaction terms were then tested and non-significant interaction terms were removed from the model. The model was then re-run and other non-significant terms were removed from the model. At this point, all insignificant terms had been removed, except those required to make the model hierarchical.

A hierarchical cluster analysis was performed using the 3-OHFA data to explore patterns in the data. The clustered objects were the dust samples, and the attributes used to sort the samples were the five 3-OHFAs converted to mole-percent. Euclidean distance was used as the similarity metric, and the average, compact (furthest neighbor) and connected (nearest neighbor) linkage methods were used as rules for merging clusters into hierarchical trees.

A two-group before-after design was used to separately test the effect of the enhanced cleaning intervention on endotoxin level (EU/mg dust), total endotoxin (EU/sample or EU/chair), and total dust mass (mg/sample) in the carpet and chair samples. Total endotoxin was computed by multiplying the endotoxin level by the total dust mass; for chairs, total endotoxin was then divided by the number of chairs in the composite sample. A three-way main effects ANOVA was performed using week, floor, and enhanced cleaning as independent variables.

Univariate and cluster analyses were performed using S-PLUS, v. 3.3 for Windows (MathSoft, Seattle, WA, USA). ANOVA models were run using PROC GLM in SAS, v. 6.12 (SAS Institute, Inc., Cary, NC, USA). All significance testing was done at the $\alpha=0.05$ level.

Results

Air Sampling

Of 96 indoor air samples collected, 22 (23%) had endotoxin levels below the LOD of 0.17 EU/m^3; endotoxin in all 14 outdoor air samples was above the LOD. The LOD was corrected (increased) for endotoxin found on one rotameter blank. All field blanks were below the LOD.

The geometric mean (GM) and geometric standard deviation (GSD) for the indoor and outdoor air samples are given in Table 2. For the seven air sampling days, the ratio of outdoor-to-indoor endotoxin GM air levels ranged from 3.8 to 7.2. No effect of enhanced air filtration on airborne endotoxin was found (P=0.62).

In the between-week analysis, the effect of subspace on airborne endotoxin was significant (P<0.0001), while floor, week and replicate were not significant. Significant variation in airborne endotoxin by subspace is evident in the boxplot of this factor (Figure 1). No significant interactions were found. The eight subspace means were further examined using the least significant difference test. Three significantly different groups

Table 2 Endotoxin Air Levels: Means and Standard Deviations

Source	n	AM (EU/m^3)	SD (EU/m^3)	GM (EU/m^3)	GSD	Range (EU/m^3)
Indoors	96	0.26	0.13	0.24	1.6	<0.17–0.75
Outdoors	14	1.3	0.43	1.2	1.4	0.73–2.1

AM=arithmetic mean, SD=standard deviation, GM=geometric mean, GSD=geometric standard deviation

Table 3 Between-Week Endotoxin Air Levels: Means and Standard Deviations by Subspace, excluding Monday and Friday during Week 1

Subspace	n	AM (EU/m^3)	SD (EU/m^3)	GM (EU/m^3)	GSD	LSD Group
22	10	0.46	0.17	0.43	1.5	A
23	10	0.27	0.089	0.26	1.3	B
21	10	0.18	0.062	0.17	1.4	C
24	10	<0.17[a]	0.054	<0.17	1.4	C
42	10	0.29	0.053	0.28	1.2	D
43	10	0.28	0.057	0.28	1.2	D
44	10	0.29	0.12	0.27	1.5	D
41	10	0.19	0.094	0.17	1.6	E

AM=arithmetic mean, SD=standard deviation, GM=geometric mean, GSD=geometric standard deviation, LSD=least significant difference
[a]LOD=0.17 EU/m^3

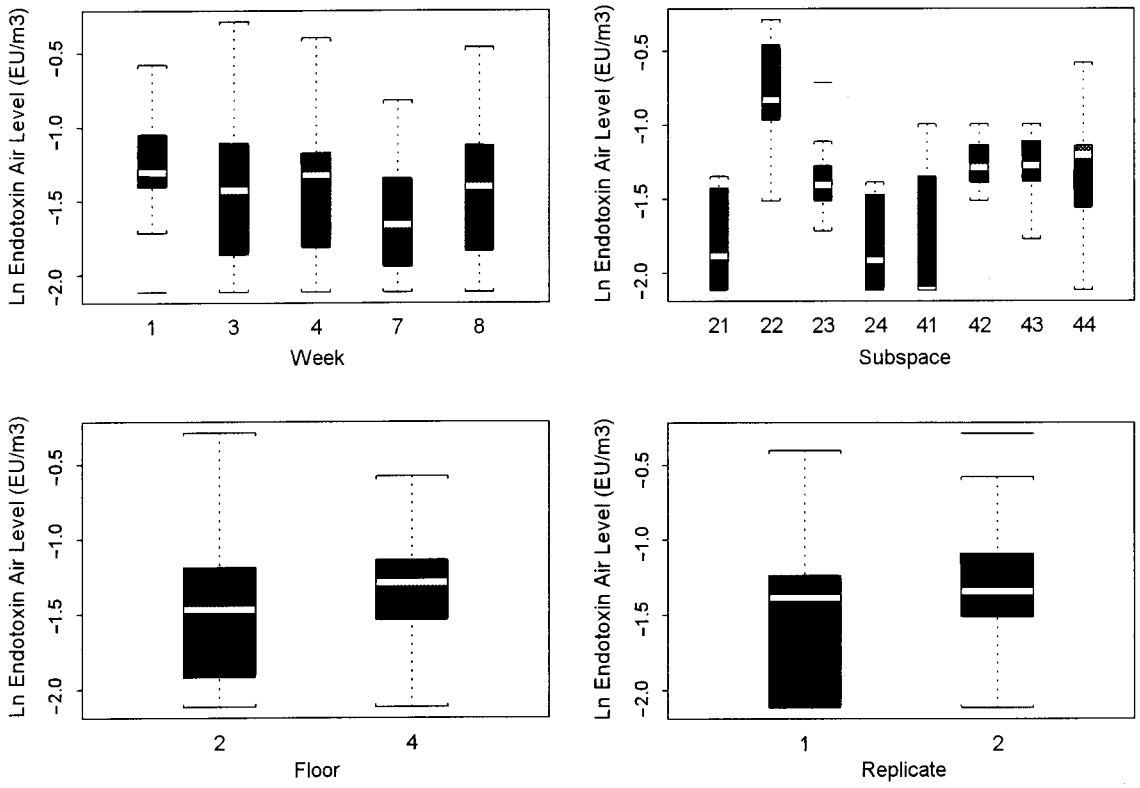

Fig. 1 Between-week ANOVA model: Tukey boxplots of the natural log of endotoxin air levels by week, subspace, floor and replicate

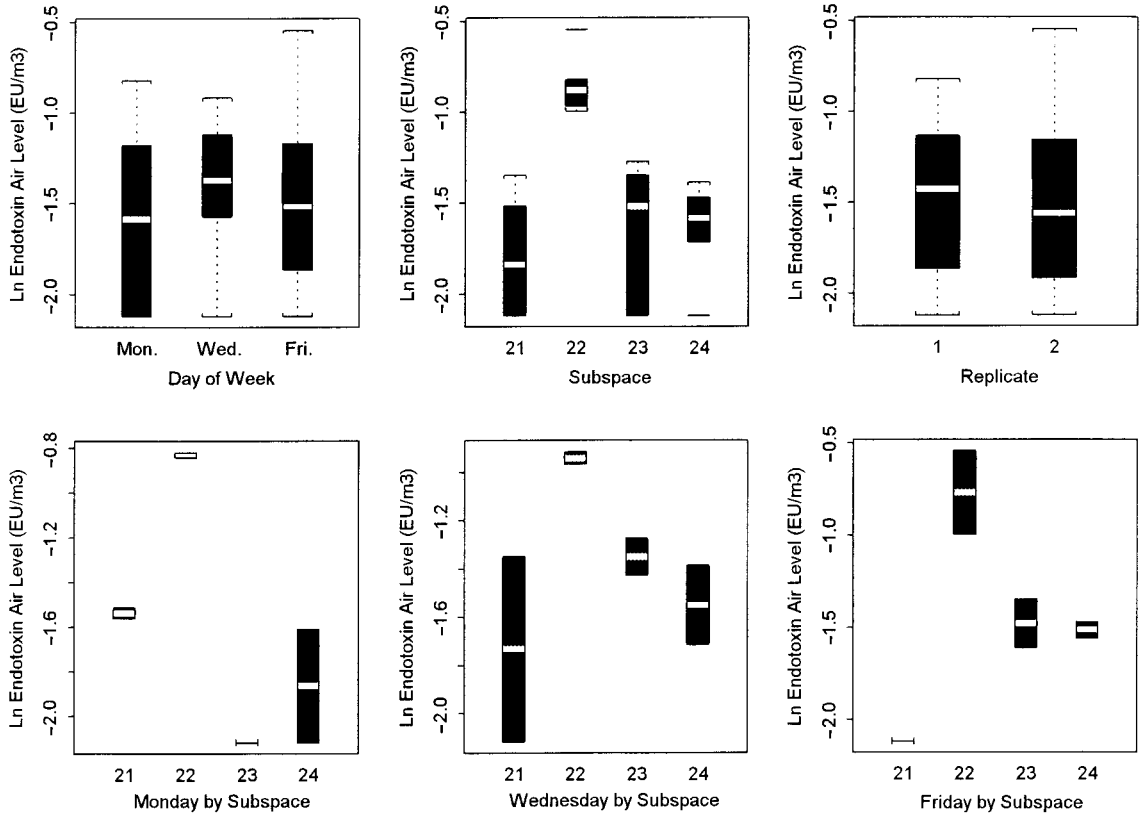

Fig. 2 Within-week ANOVA model: Tukey boxplots of the natural log of endotoxin air levels by day of week, subspace, replicate and day-by-subspace

were found on floor 2 and two significantly different groups were found on floor 4 (Table 3).

In the within-week analysis, a significant interaction (P=0.04) was found between day of week and subspace; that is, the effect of day of week depended on the subspace sampled and vice-versa. Mean endotoxin air level by subspace was examined separately for Monday, Wednesday, and Friday with the least significant difference test (Table 4). Boxplots of the main effects in the model and endotoxin levels by day are shown in Figure 2.

Settled Dust Sampling

Two carpet and two chair dust samples were collected per floor for each of the three weeks, except during week 1, when only one chair dust sample was collected per floor (n=12, carpets; n=10, chairs). The mass of dust collected ranged from 136–515 mg per sample for carpets, and 103–400 mg per sample for chairs (or 9–22 mg/chair). All settled dust samples had detectable levels of endotoxin (LOD=0.01 EU/mg dust) and 3-OHFAs (LOD=~0.2 ng LPS/mg dust).

Mean (±SD) endotoxin levels in carpet and chair samples were 59±9.3 (range 46–75), and 38±7.7 (range 25–52) EU/mg dust, respectively. The mean endotoxin level for carpet dust was significantly higher than for chair dust (P<0.001). Endotoxin levels in the carpet and chair dust did not vary significantly by floor or by week, and no interaction terms were significant.

ANOVA analysis of 3-OHFA concentrations with main effects of floor, source, week and chain length resulted in two significant first-order interactions: chain length by floor and chain length by source, indicating different LPS composition by floor and by source. Least square (LS) mean 3-OHFA concentrations in pmoles LPS/mg dust by floor and source for each chain length are given in Table 5. A significant difference in LS means between floors 2 and 4 was found for 3-OH-$C_{16:00}$ (P<0.01). Significant differences in LS means between carpets and chairs were found for 3-OH-$C_{10:00}$ (P<0.05), 3-OH-$C_{12:00}$ (P<0.01), 3-OH-$C_{16:00}$ (P<0.01), and 3-OH-$C_{18:00}$ (P<0.01). Week was not significant (P=0.841).

The correlation matrix of individual 3-OHFAs and endotoxin is shown in Table 6. Eighteen out of 31 correlations between endotoxin and 3-OHFAs (alone or in combination) were statistically significant (P<0.05). The strongest *positive* correlation coefficients were found for 3-OH-$C_{12:00}$ (r=0.61, P<0.01), and for three chain length combinations that included 3-OH-$C_{12:00}$,

Table 4 Within-Week Analysis of Endotoxin Air Levels: Results of Least Significant Difference Test

Day of week	Subspace	n	GM (EU/m^3)	GSD	Significant differences
Monday	21	2	0.21	1.0	$GM_{21}{}^{B}>GM_{23}$
	22	2	0.43	1.0	$GM_{22}>GM_{21}$, GM_{23}, and GM_{24}
	23	2	<0.17[a]	1.0	
	24	2	<0.17	1.4	
Wednesday	21	2	0.18	1.7	
	22	2	0.39	1.0	
	23	2	0.26	1.1	
	24	2	0.21	1.3	
Friday	21	2	<0.17	1.0	
	22	2	0.46	1.4	$GM_{22}>GM_{21}$, GM_{23}, and GM_{24}
	23	2	0.23	1.2	$GM_{23}>GM_{21}$
	24	2	0.22	1.1	$GM_{24}>GM_{21}$

GM=geometric mean, GSD=geometric standard deviation
[a]LOD=0.17 EU/m^3. [b] Subspace

Table 5 3-OHFA Levels in Dust: Least Squares Means by Chain Length, Floor, and Source

Chain length	Floor		Source	
	2 (pmole LPS/mg dust)	4 (pmole LPS/mg dust)	Chair (pmole LPS/mg dust)	Carpet (pmole LPS/mg dust)
$C_{10:00}$	3.5	4.6	2.5*	5.6*
$C_{12:00}$	10	11	8.1**	13**
$C_{14:00}$	7.4	7.1	7.3	7.2
$C_{16:00}$	35**	31**	41**	26**
$C_{18:00}$	18	18	20**	16**

* P<0.05. ** P<0.01

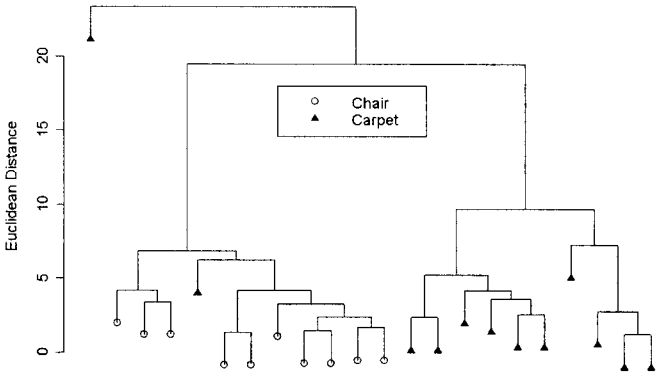

Fig. 3 Cluster analysis of carpet and chair dust samples using mole percent of the 3-OHFAs of chain lengths C_{10}, C_{12}, C_{14}, C_{16}, and C_{18} as the clustering variables, Euclidean distance as the similarity metric and the average linking method

$C_{12:00}+C_{14:00}$, $C_{12:00}+C_{10:00}$, and $C_{12:00}+C_{10:00}+C_{14:00}$ (r=0.60, r=054, r=0.54, respectively, P<0.01). The strongest *negative* correlation coefficients were found for 3-OH-$C_{16:00}$ (r=−0.67, P<0.001), and for three chain length combinations that included 3-OH-$C_{16:00}$, $C_{16:00}+C_{14:00}$, $C_{16:00}+C_{10:00}$, and $C_{16:00}+C_{18:00}$ (r= −0.65**, −0.59*, −0.59*, respectively, **p≤0.001, *P<0.01). The finding that 3-OH-$C_{12:00}$ and 3-OH-$C_{16:00}$ correlate with endotoxin in opposite directions was not surprising given that the individual 3-OHFAs were negatively correlated.

Endotoxin potency estimates of carpet and chair dust in EU/pmole LPS were determined by dividing the endotoxin level in EU/mg dust by the total estimated LPS concentration in pmole/mg dust (sum of 3-OH-$C_{10:00}$ through 3-OH-$C_{18:00}$) for each sample. Carpet and chair dust mean (±SD) endotoxin potency estimates were 0.88±(0.16) and 0.50±(0.11) EU/pmole LPS, respectively. These means were significantly different (P<0.001). Floor, week, and all interaction terms were not significant.

The hierarchical tree produced using mole percent of 3-OHFA for the 5 chain lengths as the clustering variables, Euclidean distance as the similarity metric, and the average linking method is shown in Figure 3. Each branch of the tree represents a sample. Of 22 dust samples, all 10 chair samples clustered on the left side, and 10 out of 12 carpet samples clustered on the right side of the tree. One carpet sample was not very similar to all other samples (top left of Figure 3). No obvious clusters were noted by floor or week (results not shown). The compact and connected linking methods produced similar results.

For chair samples, the enhanced surface cleaning intervention did not significantly change endotoxin level, total endotoxin, or total dust mass (P>0.05); however, for carpet samples, total dust mass and total endotoxin per sample increased significantly after the intervention (Table 7).

Table 6 Pearson's Product-moment Correlation Matrix for Carpet and Chair Dust Samples: Endotoxin (EU/mg dust) and 3-OHFAs (pmoles LPS/mg dust) by Carbon Chain Length, n=22

	Pearson correlation coefficients					
	Endotoxin	$C_{10:00}$	$C_{12:00}$	$C_{14:00}$	$C_{16:00}$	$C_{18:00}$
Endotoxin	1.0					
$C_{10:00}$	0.43*	1.0				
$C_{12:00}$	0.61**	0.83***	1.0			
$C_{14:00}$	0.11	0.098	0.17	1.0		
$C_{16:00}$	−0.67***	−0.52*	−0.68***	0.28	1.0	
$C_{18:00}$	−0.23	−0.43*	−0.34	0.29	0.58**	1.0

*P<0.05. **P<0.01. ***P<0.001

Table 7 Results of Enhanced Surface Cleaning Intervention

		Chairs				Carpets			
		n	LS Mean	SE	P-value	n	LS Mean	SE	P-value
Endotoxin level (EU/mg dust)	Before	2	42	4.2	0.39	2	51	3.2	0.17
	After	2	30	9.5		2	66	7.0	
Total endotoxin (EU/sample or EU/chair)	Before	2	730	100	0.11	2	11000	920	0.0015
	After	2	120	240		2	31000	2100	
Total dust mass (mg/sample)	Before	2	18	1.9	0.092	2	220	3.5	0.0001
	After	2	5.9	4.2		2	510	7.8	

LS Mean=Least Squares Mean, SE=standard error

Discussion

Endotoxin air levels on the two floors of the office building were over 1,000-fold *lower* than levels reported by Teeuw et al. (1994) in offices. The levels are similar to the lower range reported for homes (Milton, 1995b). While direct comparisons to indoor endotoxin air levels obtained when using aggressive sampling are not appropriate, the present study results, although lower, are consistent with office building levels reported by Rylander et al. (1992). By contrast, endotoxin settled dust levels in this study were approximately 1,000-fold *higher* than levels reported in offices in Denmark (Gyntelberg et al., 1994). When compared to reported endotoxin levels in house dust, the endotoxin dust levels in this office building were approximately half the GM level reported by Milton (1995), approximately twice the mean levels reported by Michel et al. (1991, 1996), and approximately four times the median level reported by Rizzo et al. (1997).

Significant within-floor spatial variation was found for airborne endotoxin, even though each floor was a single ventilation air handling zone. Subspace 22 had higher endotoxin air levels than other subspaces on the two floors. While no visible source of bacterial contamination was found in this subspace, the most probable explanation for higher endotoxin levels in this area was the presence of an unidentified endotoxin source within the subspace. The higher level of endotoxin in subspace 22 is unlikely to be a consequence of a higher entry rate of endotoxin in outdoor air, because the same air handling system supplied air to subspaces 21 and 22. The building envelope appeared to be well-sealed; however, the possibility of a higher rate of outdoor air leakage into subspace 22 cannot be ruled out.

Temporal variability of airborne endotoxin was not significant across weeks. Data to examine within-week variability was limited and should be interpreted with caution. The effect of day of week on airborne endotoxin depended on the subspace sampled (significant interaction of day and subspace). The explanation for this finding is not readily apparent and additional sampling should be done to further describe day-to-day variability.

Carpet and chair dust differed in composition based on endotoxin levels and 3-OHFA profiles. Endotoxin levels in carpet dust were significantly higher than in chair dust. Mean levels of 3-OH-$C_{10:00}$ and 3-OH-$C_{12:00}$ were greater in carpet dust than in chair dust, while mean levels of 3-OH- $C_{16:00}$ and 3-OH-$C_{18:00}$ were greater in chair dust than in carpet dust. Similarity analysis of the dust samples also indicated that the 3-OHFA profiles of the two floors were substantially different for carpets and chairs. Significant variation in dust composition between floors was found for 3-OH-$C_{16:00}$ These carbon chain length-specific differences in 3-OHFAs suggest that different microbial populations were present in carpets and chairs, and on each floor. The absence of significant variation in 3-OHFA levels by week suggests that LPS composition was stable over the short duration of this study.

The predominant 3-OHFAs in the dust samples were 3-OH-$C_{16:00}$ and 3-OH-$C_{18:00}$ (69% of total). Species identification of the putative bacteria is not possible without additional analyses; however, bacteria containing 3-OH-$C_{16:00}$ and 3-OH-$C_{18:00}$ fatty acids include some species of *Pseudomonas*, *Rhizobium*, *Agrobacterium*, *Azospirillium*, *Bacteroides*, *Fusobacterium*, *Methylomonas* and *Methylosinus* (Wilkinson, 1988).

The mean endotoxin potency of office dust in this study (0.7 EU/pmole LPS) was approximately half the endotoxin potency reported by Saraf et al. (1997) for house dust (~1.4 EU/pmole LPS), suggesting differences in the LPS composition of dust from these two environments. Differences in the chemical composition of LPS isolated from *Enterobacter agglomerans*, *Citrobacter freundii* and *Agrobacterium sp.* have been shown to be related to guinea pig pulmonary toxicity, mouse lethality and rabbit pyrogenicity (Helander et al., 1982). Therefore, the LPS composition of endotoxin within buildings and across different types of indoor environments may have important implications for human health.

Both this study of office dust and the Saraf et al. (1997) study of house dust found a significant positive correlation between endotoxin measured by KLARE and 3-OH-$C_{12:00}$ measured by GC/MS, although the correlation coefficient for office dust ($r=0.61$) was lower than for house dust ($r=0.90$). The lower correlation coefficient found in this study may be related to the narrower range of endotoxin levels found in office dust samples obtained from one building (24.9–74.9 EU/mg) than in house dust samples obtained from many different homes (11–243 EU/mg). As with house dust, the combination of 3-OH-$C_{10:00}$, 3-OH-$C_{12:00}$, and 3-OH-$C_{14:00}$ was significantly correlated with endotoxin, although less strongly in office ($r=0.54$) than in house dust ($r=0.88$).

The significant negative correlation found for 3-OH-$C_{16:00}$ contrasts with Saraf et al. (1997) where 3-OH-$C_{16:00}$ did not correlate strongly (positively or negatively) with endotoxin activity. Saraf et al. (1997) tested the supernatants of several different bacterial suspensions and found that *P. cepacia* with predominately 3-OH-$C_{14:00}$ and 3-OH-$C_{16:00}$ fatty acids had greater endotoxin potency than *E. coli* with predominately 3-OH-$C_{14:00}$, but *H. pylori* with mostly 3-OH-$C_{16:00}$ and 3-OH-

$C_{18:00}$ had very low potency compared to both *P. cepacia* and *E. coli*. If the presence of substantial amounts of 3-OH-$C_{16:00}$ and 3-OH-$C_{18:00}$ fatty acids is related to reduced endotoxin potency, then this might explain the low endotoxin potency found for office dust in this study where 3-OH-$C_{16:00}$ and 3-OH-$C_{18:00}$ were the predominant 3-OHFAs in the dust samples.

Enhanced air filtration did not significantly affect airborne endotoxin in this building. The higher efficiency filters substantially reduced airborne concentrations for small particles, e.g., by 94% for 0.3 to 0.5 μm and by 84% for 0.5–0.7 μm, while having a much smaller effect on large particles (by 16% for >2 μm) already efficiently removed by the building's normal filters. The large reduction in small particles, and the small change in the already low levels of large particles, plus the lack of an effect of filtration on endotoxin levels, suggests that endotoxin was associated with particles larger than 2 μm.

The lack of a significant change in dust endotoxin levels after enhanced surface cleaning was consistent with the expectation that higher efficiency cleaning would not preferentially remove dust with either high or low endotoxin levels. The increase in total endotoxin and total dust mass per sample after cleaning was surprising, and is not readily explained. Studies of the effect of vacuum cleaning on contaminants in carpets have produced mixed results. One study found that lead concentrations in carpets did not change after repeated vacuum cleaning; however, lead loading on carpet surfaces increased up to four-fold in some instances after the first several cleanings, possibly due to the movement of lead from deeper in the carpet to the surface (Ewers et al., 1994). Another study found a significant decrease in dust loading and lead loading after a year-long intervention that included HEPA vacuuming of carpets in homes (Lioy et al., 1998). Significant reductions in house dust mite allergen in carpets after five weeks of near daily vacuum cleaning have also been reported (Adilah et al., 1997). It may be that enhanced cleaning needs to be performed repeatedly or over several weeks or months to produce significant decreases in dust or contaminant loading.

It is possible that dust mass and total endotoxin increases observed in this study may be due to sampling technique differences (before and after samples were taken by two different persons); however, efforts were made to standardize operator-dependent aspects of sampling, and total dust per sample decreased on the floor that did not receive the cleaning intervention. Additional studies are needed on the temporal variability of carpet dust levels, and the effect of cleaning methods on the availability of surface dust for capture by sampling methods similar to those used in this study. If increases in the surface loading of contaminants after some types of cleaning are possible, then the impact on occupant exposure needs to be explored.

Conclusion

In summary, airborne endotoxin levels on the two floors of the office building in this study were similar to levels found in homes (Milton, 1995), but substantially lower than levels measured in ''healthy'' Dutch office buildings (Teeuw et al., 1994). The wide variation in office building endotoxin levels between the two studies may be due to differences in building characteristics and/or in the analytical methods used. Also, the present study sampled only one office building which may not be representative of U.S. office buildings. Significant spatial variation in airborne endotoxin was found in the study building, even within the same air handling zone, and this variation was probably due to an endotoxin source (or possibly high outside air infiltration) in one subspace. This spatial variation in endotoxin air levels suggests that lack of sufficient information about spatial variability could lead to significant occupant exposure misclassification. Significant differences in endotoxin levels and LPS composition were found for carpet and chair dust; however, the importance of this finding for occupant exposure and health is not yet clear.

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References

Adilah, N., Fitzharris, P., Crane, J. and Siebers, R.W. (1997) ''The effect of frequent vacuum cleaning on the house dust mite allergen, Der p 1 in carpets: a pilot study'', *New Zealand Medical Journal*, **110**, 438–9.

Ewers, L., Clark, S., Menrath, W., Succop, P. and Bornschein, R. (1994) ''Clean-up of lead in household carpet and floor dust'', *American Industrial Hygiene Association Journal,* **55**, 650- 657.

Fisk, W.J., Faulkner, D., Sullivan, D., Dong, M., Dabrowski, C., Thomas, J.M., Mendell, M.J., Hines, C.J., Ruder, A. and

Boeniger, M. (1998) *The Healthy Building Intervention Study: Objectives, Methods, and Results of Selected Environment Measurements*, Report LBNL-41546, Berkeley, CA, Lawrence Berkeley National Laboratory.

Gradowska, W. and Larsson, L. (1994) ''Determination of absolute configurations of 2- and 3-hydroxy fatty acids in organic dust by gas chromatography-mass spectrometry'', *Journal of Microbiological Methods*, **20**, 55–67.

Gyntelberg, F., Suadicani, P., Wohlfahrt, N.J., Skov, P., Valbjørn, O., Nielsen, P.A., Schneider, T., Jørgensen, O., Wolkoff, P., Wilkins, C.K., Gravesen, S. and Norn, S. (1994) ''Dust and the sick building syndrome'', *Indoor Air*, **4**, 223–238.

Helander, I., Saxén, H., Salkinoja-Salonen, M. and Rylander, R. (1982) ''Pulmonary toxicity of endotoxins: comparison of lipopolysaccharides from various bacterial species'', *Infection and Immunity*, **35**, 528–532.

Hornung, R.W. and Reed, L. (1990) ''Estimation of average concentration in the presence of non-detectable values'', *Applied Occupational and Environmental Hygiene*, **5**, 46–51.

Lioy, P.J., Yiin, L., Adgate, J., Weisel, C. and Rhoads, G.G. (1998) ''The effectiveness of a home cleaning intervention strategy in reducing potential dust and lead exposures'', *Journal of Exposure Analysis and Environmental Epidemiology*, **8**, 17–35.

Mendell, M.J., Fisk, W.J., Petersen, M., Dong, M.X., Hines, C.H., Faulkner, D., Deddens, J.A., Ruder, A.M., Sullivan, D. and Boeniger, M.F. (1999a) ''Enhanced particle filtration in a non-problem office environment: summary findings from a double-blind crossover intervention study'', *Proceedings of the 8th International Conference on Indoor Air Quality and Climate*, Edinburgh, Scotland, Vol. 4, pp. 974–975.

Mendell, M.J., Fisk, W.J., Dong, M.X., Petersen, M., Hines, C.H., Faulkner, D., Deddens, J.A., Ruder, A.M., Sullivan, D. and Boeniger, M.F. (1999b) ''Effects on occupants of enhanced particle filtration in a non-problem office environment: preliminary results from a double-blind crossover intervention study'', *American Journal of Industrial Medicine*, Suppl. 1, 55–57.

Michel, O., Kips, J., Duchateau, J., Vertongen, F., Robert, L., Collet, H., Pauwels, R. and Sergysels, R. (1996) ''Severity of asthma is related to endotoxin in house dust'', *American Journal of Respiratory and Critical Care Medicine*, **154**, 1641–1646.

Michel, O., Ginanni, R., Duchateau, J., Vertongen, F., LeBon, B. and Sergysels, R. (1991) ''Domestic endotoxin exposure and clinical severity of asthma'', *Clinical and Experimental Allergy*, **21**, 441–448.

Mielniczuk, Z., Mielniczuk, E. and Larsson, L. (1993) ''Gas chromatography-mass spectrometry methods for analysis of 2- and 3-hydroxylated fatty acids: application for endotoxin measurement'', *Journal of Microbiological Methods*, **17**, 91–102.

Milton, D.K., Johnson, D.K. and Park, J-H. (1997) ''Environmental endotoxin measurement: interference and sources of variation in the *Limulus* assay of house dust'', *American Industrial Hygiene Association Journal*, **58**, 861–867.

Milton, D.K. (1995a) ''Endotoxin''. In: Burge, H.A. (ed.) *Bioaerosols*, Boca Raton, FL, Lewis Publishers, pp. 77–86.

Milton, D.K. (1995b): *Bacterial endotoxins: a review of health effects and potential impact in the indoor environment* [Unpublished document], Report for the National Institute for Occupational Safety and Health, Cincinnati, OH, National Institute for Occupational Safety and Health p. 14.

Milton, D.K., Feldman, H.A., Neuberg, D.S., Bruckner, R.J. and Greaves, I.A. (1992) ''Environmental endotoxin measurement: the kinetic Limulus assay with resistant-parallelline estimation'', *Environmental Research*, **57**, 212–230.

Rietschel, E. T., Wollenweber, H.W., Brade, H., Zähringer, U., Lindner, B., Seydel, U., Bradaczek, H., Barnickel, G., Labischinski, H. and Giesbrecht, P. (1984) ''Structure and conformation of the lipid A component of lipopolysaccharides''. In: Rietschel, E.T. (ed.) *Handbook of Endotoxin*, Amsterdam, Elsevier Science Publishers, Vol. 1, pp. 187- 220.

Rizzo, M.C., Naspitz, C.K., Fernández-Caldas, E., Lockey, R.F., Mimica, I. and Solé, D. (1997) ''Endotoxin exposure and symptoms in asthmatic children'', *Pediatric Allergy and Immunology*, **8**, 121–126.

Rylander, R., Persson, K., Goto, H., Yuasa, K. and Tanaka, S. (1992) ''Airborne beta-1,3-glucan may be related to symptoms in sick buildings'', *Indoor Environment*, **1**, 263–267.

Rylander, R., Sörensen, S., Goto, H., Kazumi, Y. and Tanaka, S. (1989) ''The importance of endotoxin and glucan for symptoms in sick buildings''. In: Bieva, C.J., Coutois, M. and Govaertsm M. (eds.) *The Present and Future of Indoor Air Quality*, Amsterdam, Elsevier Science Publishers, pp. 219–226.

Saraf, A., Larsson, L., Burge, H. and Milton, D. (1997) ''Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography-mass spectrometry: comparison with fungal culture and determination of endotoxin by a *Limulus* amebocyte lysate assay'', *Applied and Environmental Microbiology*, **63**, 2554–2559.

Sonesson, H.R.A., Zähringer, U., Grimmecke, H.D., Westphal, O. and Rietschel, E.T. (1994) ''Bacterial Endotoxin: Chemical Structure and Biological Activity''. In: Brigham, K. (ed.) *Endotoxin and the Lungs*, New York, Marcel Dekker Inc., pp. 1–20.

Teeuw, K.B., Vandenbroucke-Grauls, C.M.J.E. and Verhoef, J. (1994) ''Airborne Gram-negative bacteria and endotoxin in sick building syndrome'', *Archives of Internal Medicine*, **154**, 2339–2345.

Walters, M., Milton, D., Larsson, L. and Ford, T. (1994) ''Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques'', *Applied and Environmental Microbiology*, **60**, 996–1005.

Waters, M.A., Selvin, S. and Rappaport, S.M. (1991) ''A measure of goodness-of-fit for the lognormal model applied to occupational exposures'', *American Industrial Hygiene Association Journal*, **52**, 493–502.

Wilkinson, S.G (1988) ''Gram-negative bacteria''. In: Ratledge, C. and Wilkinson, S.G. (eds.) *Microbial Lipids*, New York, Academic Press, Vol. 1, pp. 299–488.