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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

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Ergosterol and 3-hydroxy fatty acids, chemical markers for fungal biomass and the endotoxin of gram-negative bacteria, respectively, may be useful in studies of health effects of organic dusts, including domestic house dust. This paper reports a method for the combined determination of ergosterol and 3-hydroxy fatty acids in a single dust sample and a comparison of these chemical biomarkers determined by gas chromatography-mass spectrometry with results from fungal culture and *Limulus* assay. Analyses of replicate house dust samples resulted in correlations of 0.91 (ergosterol in six replicates; $P < 0.01$) and 0.94 (3-hydroxy fatty acids in nine replicates; $P < 0.001$). The amounts of ergosterol (range, 2 to 16.5 ng/mg of dust) correlated with those of total culturable fungi (range, 6 to 1,400 CFU/mg of dust) in 17 samples, ($r = 0.65$; $P < 0.005$). The amounts of endotoxin (range, 11 to 243 endotoxin units/mg of dust) measured with a modified chromogenic *Limulus* assay correlated with those of lipopolysaccharide (LPS) determined from 3-hydroxy fatty acid analysis of 15 samples. The correlation coefficient depended on the chain lengths of 3-hydroxy acids used to compute the LPS content. The correlation was high ($r = 0.88 \pm 0.01$; $P < 0.001$) when fatty acid chains of 10 to 14 carbon atoms were included; the correlation was much lower when hydroxy acids of 16- or 18-carbon chains were included. In conclusion, the results of the described extraction and analysis procedure for ergosterol and 3-hydroxy fatty acids are reproducible, and the results can be correlated with fungal culture and endotoxin activity of organic dust samples.

Various allergic reactions and respiratory symptoms have been linked to the inhalation of microbe-contaminated air. Cultural analysis of air or dust samples is the most commonly used method for exposure assessment. However, it has been estimated that only 0.1 to 10% of the total number of microorganisms in environmental samples are detectable by culture (32), and it is widely recognized that the stress imposed by some sampling methods (e.g., filtration) further reduces the culturability of bacteria. Although fungi are resistant to sampling stress, recoveries are strongly dependent on culture media, conditions of incubation, and the density of colonies on each plate. Alternative methods of quantifying microorganisms present in environmental samples may be appropriate, especially if health effects do not depend on variability and/or specific identification of a particular microbial genus or species.

Analysis of chemical markers in environmental samples can provide information about microbiological composition (32). During recent years, this approach has been used to study organic dust and has included determination of muramic acid (4, 5, 13, 23, 35) and diaminopimelic acid (27) for measuring peptidoglycan and determination of 3-hydroxy fatty acids (3-OH FAs) (12, 23, 27, 28, 35) and ergosterol (1, 14, 23) for measuring endotoxin and fungal biomass, respectively. Due to its high detection selectivity and sensitivity, gas chromatography-mass spectrometry (GC-MS) is the preferred method for this type of analysis.

Endotoxin (lipopolysaccharide [LPS]) is a collective term designating a characteristic group of constituents of the outer membranes of gram-negative bacteria. Inhalation of endotoxin has been correlated with workplace-related illness in both agricultural (7, 25) and nonagricultural (3, 16, 17) environments. It is also suspected to play a role in the development of the "sick building syndrome" (6, 30) and in the severity of community-acquired asthma (11). *Limulus*-based bioassays, which rely on the capacity of endotoxin to activate proteolytic enzymes from horseshoe crab amebocytes, are frequently used to measure endotoxin in environmental samples. These methods measure bioactivity rather than absolute amounts of LPS, and results correlate with some human health effects. However, *Limulus* methods are susceptible to cross-reactions and inhibition of the enzyme cascade by other environmental contaminants. The GC-MS method for analysis of the LPS-characteristic 3-OH FAs detects LPS with a low likelihood of interference and also provides some information about the bacteria that are sources of the LPS, because the relative distributions of the individual 3-OH FAs differ among species of gram-negative bacteria (33). However, measurement of 3-OH FAs detects LPS regardless of bioactivity. Various degrees of correlation have been found between the results of 3-OH FA analysis and *Limulus*-based assays for determination of endotoxin in environmental samples (27, 28, 31).

Inhalation of fungal-source particles is also suspected to

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cause toxic reactions related to structural cell wall components (β-1-3-D-glucans) and to secondary metabolites (mycotoxins) (19, 22). Fungi are also well-recognized allergen sources (9). Cultivation of fungi from environmental samples (the usual approach to exposure assessment) provides some quantitative information on culturable fungi and on species identity, but the method does not detect nonculturable or disrupted spores or hyphal elements. The specific membrane lipid ergosterol has been widely used as a chemical marker for the measurement of fungal biomass in agricultural products and, to a limited extent, in organic dust samples (1, 14). To the best of our knowledge, amounts of ergosterol in organic dust have not yet been related to levels of culturable fungi.

Our studies were designed to (i) develop a method for measuring both endotoxin and ergosterol in the same dust sample, (ii) compare our GC-MS-based methods for endotoxin and ergosterol with *Limulus* analysis and culture, respectively, and (iii) evaluate the relationship of 3-OH FA chain length and strain source to endotoxin bioactivity.

MATERIALS AND METHODS

Dust samples. Samples of floor dust were collected from homes by vacuuming a 2-m^2 area of carpeting for 5 min with a Eureka Mighty Mite vacuum cleaner that had been modified so that dust was trapped in a Soxhlet cellulose extraction thimble. Each sample was sieved, and the fine-dust fraction (particle diameter, <400 μm) was weighed and divided into aliquots for analysis. The house dust samples were obtained from ongoing studies of domestic exposures and asthma being conducted in the Boston, Mass., area. Some samples came from homes of Harvard University faculty, staff, and students participating in a study of the seasonal variation of allergen levels. Other samples came from Boston area homes whose residents were participating in a prospective study of asthma. In some cases, more than one sample (e.g., living room and bedroom) from one home was used. Houses were sampled without respect to their individual characteristics.

Cultivation and identification of fungi in dust. Dust samples were dilution cultured on dichloran glycerol medium (DG 18; Difco Laboratories, Detroit, Mich.) (20). Fungal colonies (including yeasts) were counted after 10 days of incubation at room temperature and classified according to morphological criteria as either yeast-like or filamentous. Data are expressed as CFU per milligram of fine dust.

Reference bacterial preparations. Cultures of gram-negative bacteria with a known variety of 3-OH FA composition (33) were studied. *Escherichia coli* (clinical isolate), *Branhamella catarrhalis* (clinical isolate), *Pseudomonas aeruginosa* (ATCC 27853), and *Pseudomonas cepacia* (CCUG 13226) were cultivated on Progressive Diagnostics Manufacturers agar (Biodisk AB, Solna, Sweden) overnight at 37°C. *Helicobacter pylori* (CCUG 17874) was cultivated under microaerophilic conditions for 3 days at 37°C on selective blood-containing hematin agar plates prepared as previously described (26). The bacteria were suspended in 5 ml of pyrogen-free water, heated at 80°C for 15 min, and centrifuged at 3,000 × *g* for 15 min. Each supernatant was passed through a disposable sterile cellulose acetate filter (0.45-μm pore size; Millipore SA, Molsheim, France), autoclaved, and analyzed for both 3-OH FAs and *Limulus* activity as described below.

***Limulus* assays.** Dust samples (25 mg) were sonicated in 5 ml of 0.05 M potassium phosphate buffer containing 0.01% triethylamine (pH 7.5). Aliquots (50 μl) of these samples and of the bacterial extracts (see above) were analyzed for endotoxin by the kinetic *Limulus* assay with resistant parallel-line estimation (KLARE) method described in previous publications (15, 31). Kinetic chromogenic *Limulus* amebocyte lysate (LAL) preparations were obtained from BioWhittaker (Walkersville, Md.) for analysis of dust samples and bacterial extracts. Additional chromogenic lysate was obtained from Endosafe (Charlestown, S.C.), and kinetic turbidimetric LAL was obtained from Associates of Cape Cod (Woods Hole, Mass.) for analysis of the bacterial samples. Reference standard endotoxin (EC6) came from the United States Pharmacopoeia (Rockville, Md.). Tests were run in polystyrene microplates which were incubated at 37°C and monitored at 405 nm for changes in optical density (OD). The maximal rate of change in OD (V_{max}) and the log of the time required to reach a 200-milli-OD unit (chromogenic) or 20-milli-OD unit (turbidimetric) increase over the initial OD (on set time) were used as response indicators; these values were analyzed by resistant parallel-line regression to compute endotoxin potency as previously described (15).

Determination of 3-OH FA GC-MS response factors. The response factors of the GC-MS to methyl ester-trimethylsilyl (TMS) derivatives of 3-OH-$C_{10:0}$, 3-OH-$C_{12:0}$, 3-OH-$C_{14:0}$, 3-OH-$C_{16:0}$, and 3-OH-$C_{18:0}$ were studied as follows. Methyl esters of these acids (i.e., a mixture of the esters, purchased from Larodan Lipids AB, Malmö, Sweden) were converted to TMS derivatives (12); the internal standard (deuterated 3-OH-$C_{14:0}$, 10 ng/μl) was esterified and TMS derivatized separately. The derivatized methyl ester mixture (1 ng/μl) was diluted in several steps (in duplicate), and 50 ng of the internal standard derivative was added to each dilution. To obtain calibration graphs, 0- to 1,000-pg aliquots (1 μl) of the dilutions of the derivatized mixture were injected into the GC-MS, and the amount of each of the acids in the injected aliquots was plotted against the ratio between the areas of the derivatized acids and the areas of the internal standard; the ion *m/z* 175 (*m/z* 178 for the derivative of the internal standard) was monitored as previously described (12).

Dust extraction and GC-MS analysis. Fine-dust samples (11 to 59 mg) were heated at 80°C for 90 min in 3 ml of 10% methanolic KOH. After cooling, dehydrocholesterol (500 ng) was added as an internal standard. Next, 1 ml of water was added and the samples were extracted twice with 2 ml of hexane. The hexane phases were used for analysis of ergosterol, and the aqueous phases were used for analysis of 3-OH FAs.

For analysis of ergosterol, the hexane phases were pooled, dried under a stream of nitrogen, dissolved in 1 ml of dichloromethane-hexane (1:1, vol/vol), and applied to a disposable silica gel column (100 mg of Si/ml; Analytichem, Harbour City, Calif.) that had been preconditioned with 1 ml of ether and 1 ml of dichloromethane-hexane. The column was washed with two 1-ml portions of dichloromethane-hexane before elution of sterols and other polar lipids with two 1-ml portions of diethyl ether. After evaporation of the solvent, TMS derivatives were formed and analyzed as previously described (1).

For analysis of 3-OH FAs, the aqueous phases were acidified to pH 1 to 2 with 4 M HCl and then partially evaporated under a stream of nitrogen and freeze-dried. The dried preparations were heated in 4 M methanolic HCl for 18 h at 100°C. After cooling, 500 ng of the internal standard (deuterated 3-OH-$C_{14:0}$ methyl ester) was added. The preparations were extracted with hexane, dried, dissolved in 1 ml of dichloromethane-hexane (1:1, vol/vol), and applied to a silica gel column (100 mg of Si/ml; Varian, Harbour City, Calif.) that had been preconditioned with 1 ml each of diethyl ether and dichloromethane-hexane. The column was then washed twice with 1 ml of dichloromethane-hexane and then twice with 1 ml of diethyl ether. The eluates were dried under a stream of nitrogen, and 3-OH FAs were analyzed as methyl ester-TMS derivatives as previously described (12). LPS was quantitated by using the calibration graphs for the individual 3-OH FAs (chain lengths of 10 to 18 carbon atoms) and assuming that 4 mol of 3-OH FAs corresponded to 1 mol of LPS (33) and that the average molecular mass of environmental LPS was 8,000 Da (unpublished results). The reproducibility of the GC-MS assay was examined by comparing the amounts of 3-OH FAs found in nine replicate dust samples, as well as those in seven equal volumes of a hydrolysate of a separate dust sample (10.5 mg, from a home vacuum cleaner).

To determine the efficiency of the TMS derivatization of the 3-OH FA methyl esters, a separate house dust sample (approximately 20 mg) was heated in 4 M methanolic hydrogen chloride overnight at 100°C (12), and the resulting hydrolysate was centrifuged and divided into two equal parts. The internal standard was added, and the two samples were extracted with hexane, evaporated to dryness, and TMS derivatized (12) at 80°C for 20 and 30 min, respectively. Aliquots (1 μl) of the preparations (each 1,000 μl) were analyzed immediately after the derivatization and after 1, 3, 6, 24, and 48 h of storage at room temperature.

Statistical analysis. Based on graphical analysis, the distribution of data for ergosterol and culturable fungi in the dust samples was approximately log normal. Therefore, these data were log transformed for computation of Pearson correlation coefficients (34). The distribution of data for the endotoxin and LPS data was approximately normal, and these data were analyzed on the arithmetic scale. Reproducibility of the assays was determined by computing the appropriate Pearson correlation coefficient and by computing the coefficient of variation for replicate analyses of a single dust sample.

RESULTS

Ergosterol and fungi. One aliquot of each of 11 dust samples and two duplicate aliquots of each of 10 dust samples were analyzed, resulting in a total of 31 separate GC-MS analyses for ergosterol and 21 for which ergosterol and fungal levels can be compared. Ergosterol in 4 of the 31 analyzed preparations could not be accurately determined due to interference from an unknown, partly coeluting substance. The correlation between the amounts of ergosterol in the remaining six replicate samples, with a chromatographically well-resolved peak of ergosterol in both aliquots, was 0.91 ($P < 0.01$). The amounts of ergosterol in the 17 dust samples with valid measurements ranged from 2 to 16.5 ng/mg of dust, with a geometric mean (GM) of 5.1 and a geometric standard deviation of 1.7.

Levels of total culturable fungi in these 17 dust samples ranged from approximately 6 to 1,400 (GM, 77) CFU/mg of fine dust, including 4 to 530 (GM, 49) CFU of filamentous

TABLE 1. Fungi found in samples used for ergosterol analysis ($n = 17$)

Fungal taxon	CFU/g of dust			Frequency observed (no. of samples)
	Median	Minimum	Maximum	
Alternaria	1,600	0	21,869	12
Aspergillus (other)	0	0	478,203	8
Aspergillus state of *Eurotium* spp.	0	0	7,290	8
Aspergillus niger	0	0	9,524	6
Aspergillus ochraceus	0	0	2,381	3
Aspergillus versicolor	389	0	21,875	9
Aureobasidium	9,063	0	105,769	14
Cladosporium	1,800	0	98,413	12
Coelomycetes	0	0	10,526	5
Epicoccum	0	0	3,125	1
Fusarium	0	0	400	1
Paecilomyces	0	0	9,091	1
Penicillium	3,491	0	28,571	14
Ulocladium	0	0	0	0
Wallemia	0	0	39,623	4
Yeast	9,615	0	1,363,636	13
Zygomycetes	0	0	5,263	3
Nonsporulating	5,263	0	150,000	15
Unknown	0	0	12,500	8
Total	81,579	9,737	1,386,364	17

fungi and 0 to 1,360 (GM, 15) CFU of yeasts per mg of dust (Table 1). A correlation coefficient of 0.65 ($P < 0.005$) between the levels of ergosterol (nanograms per milligram of dust) and total culturable fungi (CFU per milligram) was found when natural-log-transformed data were used (Fig. 1); separate computation of correlation coefficients between levels of ergosterol and yeasts and between levels of ergosterol and filamentous fungi resulted in values of 0.25 and 0.57, respectively.

3-OH fatty acids. The dose-response graphs for the individual 3-OH FA methyl ester-TMS derivatives were linear over the studied range, and the slope did not differ significantly between the individual acids. The correlation between the nine replicate dust samples was 0.94 ($P < 0.001$). In the assay reproducibility experiment, in which seven portions of a hydrolysate of a single dust sample were compared, the coefficient of variation for LPS was 5.3%. In the same sample, the relative amounts (mole percentages; average for the seven analyses) of the different 3-OH FAs were 13.1% (3-OH-$C_{10:0}$), 13.9% (3-OH-$C_{12:0}$), 19.6% (3-OH-$C_{14:0}$), 28.2% (3-OH-$C_{16:0}$), and 25.2% (3-OH-$C_{18:0}$). No trace of 3-OH FAs was found in any of the hexane phases of nine studied dust hydrolysates.

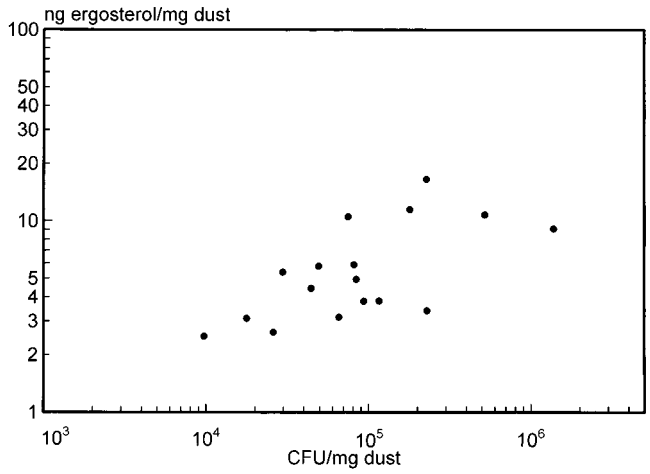

FIG. 1. Amounts of ergosterol versus level of culturable fungi in 17 house dust samples.

The TMS derivatization (at 80°C) was complete after 6 h of storage at room temperature regardless of whether the methyl ester preparations had been heated for 20 or 30 min. The yields of the derivative obtained from the preparations heated for 20 and 30 min were, respectively, 71 and 82.8% immediately after cooling to room temperature, 90 and 93% after storage for 1 h, and 98 and 99% after 3 h of storage.

Endotoxin and 3-OH FAs in dust and bacteria. Endotoxin in 15 dust samples was measured by the *Limulus* assay (KLARE method). The amounts of endotoxin in the samples varied between 11 and 243 endotoxin units (EU)/mg of dust. The amounts of LPS in these samples as calculated from 3-OH FA analysis (mean values for replicate aliquots) varied between 59 and 1,399 ng/mg of dust when all of the studied 3-OH FAs (ranging from 10 to 18 carbon atoms) were considered.

A direct comparison of results from the KLARE (EU per milligram) and GC-MS (nanograms of LPS per milligram) assays of the dust samples, including all of the measured 3-OH FAs, resulted in a correlation coefficient of 0.59 ($P < 0.05$). When individual 3-OH FAs were used to compute amounts of LPS (nanograms) in the sample, the correlation coefficients were 0.89 (3-OH-$C_{10:0}$), 0.90 (3-OH-$C_{12:0}$), 0.77 (3-OH-$C_{14:0}$), 0.49 (3-OH-$C_{16:0}$), and 0.30 (3-OH-$C_{18:0}$). A correlation coefficient of 0.88 ± 0.01 ($P < 0.001$) was obtained when any of the 10- to 14-carbon 3-OH FAs were combined (Fig. 2); markedly lower coefficients were computed when either 3-OH-$C_{16:0}$ or 3-OH-$C_{18:0}$ was included in the calculations.

The potency of the dust samples (EU per nanogram of LPS, with all 3-OH FAs measured) was also related to relative amounts of individual 3-OH FAs in the dust samples. This calculation resulted in correlation coefficients of 0.47 (3-OH-$C_{10:0}$), 0.59 (3-OH-$C_{12:0}$), 0.80 (3-OH-$C_{14:0}$), −0.25 (3-OH-$C_{16:0}$), and −0.74 (3-OH-$C_{18:0}$). The best correlations were obtained when the sum of 3-OH-$C_{10:0}$, 3-OH-$C_{12:0}$, and 3-OH-$C_{14:0}$ ($r = 0.84$; $P < 0.001$) was used or when only 3-OH-$C_{12:0}$

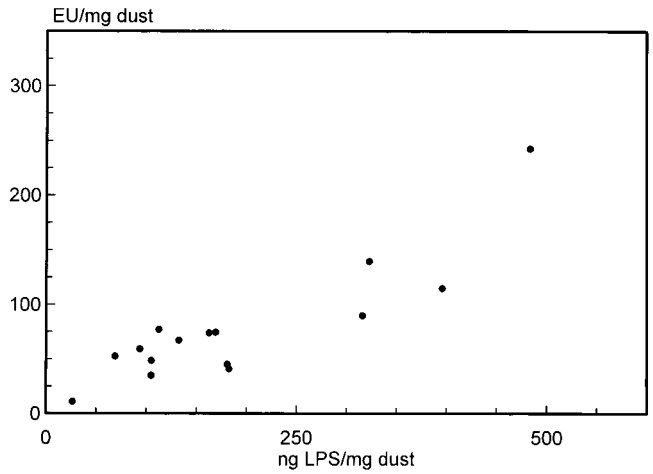

FIG. 2. Amounts of endotoxin versus LPS. The endotoxin values were obtained by *Limulus* analysis, and the LPS values were calculated from the levels of 3-OH-$C_{10:0}$, 3-OH-$C_{12:0}$, and 3-OH-$C_{14:0}$ found in 15 house dust samples.

and 3-OH-$C_{14:0}$ ($r = 0.80$; $P < 0.001$) or all acids except 3-OH-$C_{18:0}$ ($r = 0.78$; $P < 0.001$) were analyzed.

The supernatants of suspensions of the reference bacteria exhibited various potencies (per microgram of LPS) in the *Limulus* assay (Table 2). The *P. cepacia* supernatant was the most potent, followed by *P. aeruginosa*, *E. coli*, and *B. catarrhalis*, all with roughly similar potencies. *H. pylori* had a very low potency, i.e., 10^5 times lower than that of *P. cepacia*. In general, the three LALs (A, B, and C) used in the assay produced the same rank order of potencies among the bacterial species. However, the values obtained with lysate A were 10- to 100-fold lower than the values noted with lysates B and C; this was true for all species except *P. cepacia*. The LAL preparations were designed to be used in water rather than in the buffer that we used. Therefore, to determine whether the buffer was interfering with lysate A, we retested the *E. coli* supernatant with LAL A after dilution of the supernatant in water. The reaction rates in response to the *E. coli* supernatant remained very low. However, when assayed in water, the dose-response curve for the supernatant was not parallel with the standard curve (EC6 diluted in water); hence, potency could not be determined.

DISCUSSION

The sample analysis approach reported here provides an extraction procedure that allows measurement of both ergosterol and 3-OH FAs in a single dust sample. Ergosterol has previously been widely used as a measure of fungal biomass in the food-agriculture industry. In the early stages of development of the assay, comparisons between levels of ergosterol (usually measured by high-performance liquid chromatography) and culturable fungi were made (2, 24). It has been shown that the amounts of ergosterol in fungi may vary considerably according to species and culture conditions (18, 24). However, our studies demonstrate, for the first time, a relationship between levels of ergosterol in settled house dust and levels of culturable fungi ($r = 0.65$; $P < 0.005$). Future studies are needed to determine whether nonspecific measures of fungal biomass, such as ergosterol, are useful predictors of health outcomes in the indoor environment.

Early comparisons of endotoxin measurements by *Limulus* tests and LPS content by GC-MS were focused on detecting false-positive reactions of the bioassay with clinical samples (10). More recently, attention has focused on possible underestimation of endotoxin exposure from organic dusts by *Limulus* assays (21, 27, 28). The level of agreement between endotoxin and LPS measurements has varied and may be affected by collection and extraction methods, especially when air samples collected on filter media are analyzed (27, 28, 31). The types of 3-OH FAs in the LPS may also play an important role in determining the extent of correlation between bioactivity and total LPS mass. The only previous finding of highly correlated *Limulus* bioactivity and LPS measurements for air samples was in an environment where the majority of the LPS was derived from a limited number of species containing almost exclusively 3-OH-$C_{10:0}$ and 3-OH-$C_{12:0}$ (31).

In this study, we found a wide distribution of 3-OH FA chain

TABLE 2. Endotoxin potencies of bacterial supernatants determined in three LAL preparations compared with LPS content and characterization of the supernatants determined from 3-OH FA analysis

Sample	Mol% of 3-OH fatty acids with chains with the following no. of carbons:					LAL	EU/μg of LPS	
	10	12	14	16	18		Onset	V_{max}
P. cepacia	0.2		34.8	65.0		A	2,930	2,040
						B	3,130	4,320
						C	4,740	2,450
P. aeruginosa	22.4	76.4	1.2			A	64.9	34.4
						B	822	885
						C	1,260	904
B. catarrhalis	12.2	74.8	13.0			A	28.7	17.9
						B	46.6	495
						C	540	347
E. coli		0.9	98.4	0.7		A	3.39	2.16
						B	416	272
						C	367	113
H. pylori	1.0		2.3	46.2	50.5	A	0.002	0.003
						B	0.043	0.033
						C	0.182	0.471

lengths in house dust. We also found that the correlation between results of the *Limulus* test and 3-OH FA analysis varied considerably when different 3-OH FAs were taken into account. In general, if 3-OH-$C_{18:0}$ and, to some extent, 3-OH-$C_{16:0}$, were excluded from the calculations, the resulting correlation coefficient exceeded 0.8, i.e., was much higher than has been noted in some previous studies (27, 28). This observation, that certain 3-OH FAs were not associated with biologically active LPS, was buttressed by our experiments with bacterial supernatants. The potencies of the supernatants of the five bacteria (per microgram of LPS) in the *Limulus* assays varied, and the supernatant where 3-OH-$C_{18:0}$ dominated (*H. pylori*) showed extremely low potency. Previous experiments with chemically synthesized variants of lipid A (29) have shown that the potency may vary considerably, with chemical composition. In our study we found a correlation between *Limulus* activity and the chain lengths of the 3-OH FAs in the dust samples. Given that differences in bioactivity between LPSs from different bacteria have been found in inhalation studies (8), this finding suggests that future studies measuring 3-OH FAs as markers of endotoxin in environmental samples should not rely on LPS estimates based on all 3-OH FAs.

The main advantage of using MS for characterization of microorganisms in organic dust is the well-proven high inter- and intralaboratory reproducibilities of this analytical method. In the present investigation we found that the MS detector responded in essentially the same way to the methyl-TMS derivatives of the different 3-OH FAs. Thus, although the amounts of the 3-OH FAs were determined by using the calibration graphs, direct comparison of peak areas is also applicable: a difference in LPS levels of less than 1.5% between the two methods was noted when analyzing the house dust samples (data not shown). The main disadvantage is the relatively low sensitivity and possibility of detecting inactive compounds, as compared with the *Limulus* assay. However, our data suggest that biological activity can be predicted based on the types of 3-OH FAs, so that GC-MS methods may be useful in predicting endotoxin-related health effects. Studies are now in progress to test new markers, as well as to improve the analytical selectivity by using tandem MS (23) and to extend the investigations focused on the relation between chemical markers, bioactivity, and health effects (35).

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REFERENCES

1. **Axelsson, B., A. Saraf, and L. Larsson.** 1995. Determination of ergosterol in organic dust by gas chromatography-mass spectrometry. J. Chromatogr. B **666:**77–84.
2. **Börjesson, T., U. Stöllman, and J. Schnürer.** 1992. Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal grains. Appl. Environ. Microbiol. **58:**2599–2605.
3. **Flaherty, D. K., F. H. Deck, J. Cooper, K. Bishop, P. A. Winzenburger, L. R. Smith, L. Bynum, and W. B. Witmer.** 1984. Bacterial endotoxin isolated from a water spray air humidification system as a putative agent of occupation-related lung disease. Infect. Immun. **43:**206–212.
4. **Fox, A., and R. Rosario.** 1993. Quantitation of muramic acid, a marker for bacteria, in dust from hospital and home air conditioners using gas chromatography-mass spectrometry, p. 267–272. *In* P. Kalliokoski, M. Jantunen, and O. Seppänen (ed.), Proceedings of the 6th International Conference on Indoor Air Quality and Climate. Indoor air '93, Helsinki, Finland.
5. **Fox, A., R. Rosario, and L. Larsson.** 1993. Monitoring of bacterial sugars and hydroxy fatty acids in dust from air conditioners by gas chromatography-mass spectrometry. Appl. Environ. Microbiol. **59:**4354–4360.
6. **Gyntelberg, F., P. Suadicani, J. W. Nielsen, P. Skov, O. Valbjørn, P. A. Nielsen, T. Schneider, O. Jørgensen, P. Wolkoff, C. K. Wilkins, S. Gravesen, and S. Norn.** 1994. Dust and the sick building syndrome. Indoor Air **4:**223–238.
7. **Heederik, D., R. Brouwer, K. Bierstecker, and J. Boleij.** 1991. Relationship of air-borne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. Int. Arch. Environ. Health **62:**595–601.
8. **Helander, I., H. Saxen, M. Salkinoja-Salonen, and R. Rylander.** 1982. Pulmonary toxicity of endotoxins: comparison of lipopolysaccharides from various bacterial species. Infect. Immun. **35:**528–532.
9. **Horner, W., A. Helbling, J. Salvaggio, and S. Lehrer.** 1995. Fungal allergens. Clin. Microbiol. Rev. **8:**161–179.
10. **Maitra, S. K., R. Nachum, and F. C. Pearson.** 1986. Establishment of beta-hydroxy fatty acids as chemical marker molecules for bacterial endotoxin by gas chromatography-mass spectrometry. Appl. Environ. Microbiol. **52:**510–514.
11. **Michel, O., R. Ginanni, J. Duchateau, F. Vertongen, B. Le Bon, and R. Sergysels.** 1991. Domestic endotoxin exposure and clinical severity of asthma. Clin. Exp. Allergy **21:**441–448.
12. **Mielniczuk, Z., E. Mielniczuk, and L. Larsson.** 1993. Gas chromatography-mass spectrometry methods for analysis of 2- and 3-hydroxylated fatty acids: application for endotoxin measurement. J. Microbiol. Methods **17:**91–102.
13. **Mielniczuk, Z., E. Mielniczuk, and L. Larsson.** 1995. Determination of muramic acid in organic dust by gas chromatography-mass spectrometry. J. Chromatogr. B **670:**167–172.
14. **Miller, J. D., A. M. Laflamme, Y. Sobol, P. Lafontaine, and R. Greenhalgh.** 1988. Fungi and fungal products in some Canadian houses. Int. Biodeter. **24:**103–120.
15. **Milton, D. K., R. Gere, H. Feldmen, and I. Greaves.** 1990. Endotoxin measurement: aerosol sampling and application of a new *Limulus* method. Am. Ind. Hyg. Assoc. J. **51:**331–337.
16. **Milton, D. K., J. Amsel, C. E. Reed, P. L. Enright, L. R. Brown, G. L. Aughenbaugh, and P. R. Morey.** 1995. Cross-sectional follow-up of a flu-like respiratory illness among fiberglass manufacturing employees: endotoxin exposure associated with two distinct sequelae. Am. J. Ind. Med. **28:**469–488.
17. **Milton, D. K., D. Wypij, D. Kriebel, M. Walters, S. K. Hammond, and J. Evans.** 1996. Endotoxin exposure-response in a fiberglass manufacturing plant. Am. J. Ind. Med. **29:**3–13.
18. **Nout, M. J. R., T. M. G. Bonants-van Laarhoven, P. de Jong, and P. G. de Koster.** 1987. Ergosterol content of *Rhizopus oligosporum* NRRL 5905 grown in liquid and solid substrates. Appl. Microbiol. Biotechnol. **26:**456–461.
19. **Olsen, J. H., L. Dragsted, and H. Autrup.** 1988. Cancer risk and occupational exposure to aflatoxins in Denmark. Br. J. Cancer **58:**392–396.
20. **Pitt, J. I., and A. D. Hocking.** 1985. Fungi and food spoilage. Academic Press, Sydney, Australia.
21. **Rylander, R., B. Bake, J. J. Fischer, and I. M. Helander.** 1989. Pulmonary function and symptoms after inhalation of endotoxin. Am. Rev. Respir. Dis. **140:**981–986.
22. **Rylander, R., K. Persson, H. Goto, K. Yuasa, and S. Tanaka.** 1992. Airborne beta-1,3-glucan may be related to symptoms in sick buildings. Indoor Environ. **1:**263–267.
23. **Saraf, A., and L. Larsson.** 1996. Use of gas chromatography-ion trap tandem mass spectrometry for the determination of chemical markers of microorganisms in organic dust. J. Mass Spectrom. **31:**389–396.
24. **Schnürer, J.** 1993. Comparison of methods for estimating the biomass of three food-borne fungi with different growth patterns. Appl. Environ. Microbiol. **59:**552–555.
25. **Schwartz, D. A., P. S. Thorne, S. J. Yagla, L. F. Burmeister, S. A. Olenchock, J. L. Watt, and T. J. Quinn.** 1995. The role of endotoxin in grain dust-induced lung disease. Am. J. Respir. Crit. Care Med. **152:**603–608.
26. **Soltesz, V., C. Schalén, and P.-A. Mårdh.** 1988. New selective medium for *Campylobacter pylori*, p. 433–436. *In* B. Kajser and E. Falsen (ed.), Campylobacter. Goterna, Kungälv, Sweden.
27. **Sonesson, A., L. Larsson, A. Fox, G. Westerdahl, and G. Odham.** 1988. Determination of environmental levels of peptidoglycan and lipopolysaccharide using gas chromatography with negative-ion chemical-ionization mass spectrometry utilizing bacterial amino acids and hydroxy fatty acids as biomarkers. J. Chromatogr. Biomed. Appl. **431:**1–15.
28. **Sonesson, A., L. Larsson, A. Schütz, L. Hagmar, and T. Hallberg.** 1990. Comparison of the *Limulus* amebocyte lysate test and gas chromatography-mass spectrometry for measuring lipopolysaccharides (endotoxins) in airborne dust from poultry processing industries. Appl. Environ. Microbiol. **56:**1271–1278.
29. **Takada, H., and S. Kotani.** 1989. Structural requirements of lipid A for endotoxicity and other biological activities. Crit. Rev. Microbiol. **16:**477–523.
30. **Teeuw, K. B., C. M. Vandenbroucke-Grauls, and J. Verhoef.** 1994. Airborne gram-negative bacteria and endotoxin in sick building syndrome. A study in Dutch governmental office buildings. Arch. Intern. Med. **154:**2339–2345.

31. **Walters, M., D. Milton, L. Larsson, and T. Ford.** 1994. Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques. Appl. Environ. Microbiol. **60:**996–1005.
32. **White, D. C.** 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. Soc. Gen. Microbiol. **34:**37–66.
33. **Wilkinson, S.** 1988. Gram-negative bacteria, p. 299–488. *In* S. Wilkinson and C. Ratledge (ed.), Microbial lipids. Academic Press, New York, N.Y.
34. **Zar, J. H.** 1984. Biostatistical analysis, p. 276–277. Prentice-Hall, Inc., Englewood Cliffs, N.J.
35. **Zhiping, W., P. Malmberg, B.-M. Larsson, K. Larsson, L. Larsson, and A. Saraf.** 1997. Exposure to bacteria in swine house dust and acute inflammatory reactions in man. Am. J. Respir. Crit. Care Med. **154:**1261–1266.