Comparison of the *Limulus* Amebocyte Lysate Test and Gas Chromatography-Mass Spectrometry for Measuring Lipopolysaccharides (Endotoxins) in Airborne Dust from Poultry-Processing Industries

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The lipopolysaccharide (endotoxin) content in airborne dust samples from three different poultry slaughterhouses was determined with both the chromogenic *Limulus* amebocyte lysate assay and gas chromatography-mass spectrometry analysis of lipopolysaccharide-derived 3-hydroxy fatty acids. Gram-negative cell walls were also measured by using two-dimensional gas chromatography/electron-capture analysis of dianmopimelic acid originating from the peptidoglycan. The correlation between the results of the *Limulus* assay and those of gas chromatography-mass spectrometry for determination of the lipopolysaccharide content in the dust samples was poor, whereas a good correlation was obtained between lipopolysaccharide and dianmopimelic acid concentrations with the gas chromatographic methods. The results suggest that it is predominantly cell-wall-dissociated lipopolysaccharides that are measured with the *Limulus* assay, whereas the gas chromatographic methods allow determination of total concentrations of lipopolysaccharide, including *Limulus*-inactive lipopolysaccharide, gram-negative cells, and cellular debris.

Workers in agricultural environments are exposed to a wide variety of airborne organic dusts containing different toxic products of microbial origin (15, 16, 22). The development of respiratory diseases, e.g., allergic alveolitis, has been associated with inhalation of airborne microorganisms (22). For example, lipopolysaccharides (LPS; endotoxins) of gram-negative bacteria have been proposed as major causative agents of lung disorders among agriculture workers (9). Inhalation of airborne LPS has also been associated with such symptoms as cough, headache, diarrhea, and fever (15). An airborne LPS concentration of 0.1 to 0.2 μg/m³ has been suggested as a critical level for offset shift decrease in lung function (23).

The LPS content of organic dust is usually determined with the *Limulus* amebocyte lysate (LAL) test. Dusts collected on filters are extracted with pyrogen-free water, and the extracts are then analyzed (1, 5, 8, 15, 23). The presence of LPS in the extracts induces gelation of the LAL. However, it is known that several substances including peptidoglycan (from bacterial cell walls), dextrans, certain proteins, and polynucleotides also activate the LAL reaction (17, 37), whereas certain other substances, e.g., electrolytes, hormones, and antibiotics, may inhibit the test (35). An alternative approach to measuring LPS is based on the detection of specific structural components (biomarkers) of the LPS molecule (10). Lipid A, the lipid component of LPS and responsible for the endotoxic effects of the molecule (20), contains a certain molar fraction of 3-hydroxy fatty acids (38). These fatty acids may serve as markers for the amount of LPS. A method utilizing gas chromatography (GC)-mass spectrometry (MS) analysis of 3-hydroxy fatty acids in LPS as 3-O-pentafluorobenzoyl (PFBO)-methyl ester derivatives was developed (29). By using negative-ion chemical ionization, selected-ion-monitoring detection, a detection limit of 1 ng of LPS of *Escherichia coli* per ml in aqueous solutions was obtained (29).

In the present study, a chromogenic LAL test and the GC-MS method were used in a comparative study to measure LPS in airborne dust sampled in poultry slaughterhouses. In addition, we measured dianmopimelic acid (DAP), which has been shown to be a useful marker of peptidoglycan in gram-negative bacteria (33). The DAP analyses were performed with two-dimensional GC with electron-capture detection (ECD) (28). It was found that it is predominantly cell-envelope-dissociated LPS that are measured with the LAL assay, whereas the GC methods measure the total amounts of gram-negative cells, debris, and free LPS in the sample.

MATERIALS AND METHODS

Chemicals and solutions. Analytical-grade DAP was obtained from Sigma Chemical Co., St. Louis, Mo.; stock solutions were prepared in 0.1 M hydrochloric acid and stored at 4°C. Heptafluorobutyric anhydride and PFBO-chloride were purchased from Fluka, Buchs, Switzerland; acetyl chloride was from E. Merck AG, Darmstadt, Federal Republic of Germany; isobutyl alcohol was from Janssen, Beerse, Belgium; and a phenol-water-extracted LPS of *E. coli* O55:B5 was from Sigma. All solvents used were of analytical reagent grade and purchased from May & Baker, Dagenham, United Kingdom. The 3-hydroxy fatty acid standards 3-hydroxyoctanoic acid (3-OH-8:0) and 3-hydroxytetradecanoic acid (3-OH-14:0) were from our laboratory collection of standards, and 3-hydroxyhexadecanoic acid (3-OH-16:0) was a generous gift from Erik Jantzen, Statens Institut for Folkehelse, Oslo, Norway. 3-Hydroxydode-
canonic acid (3-OH:12:0) and 3-hydroxoyctadecanoic acid (3-OH:18:0) were isolated from lyophilized cells of *Pseudomonas aeruginosa* and *Helicobacter pylori* (both clinical isolates), respectively.

The 4 M hydrochloric acid in methanol was prepared by adding 30 ml of acetyl chloride to 75 ml of methanol, and the 3 M hydrochloric acid in isobutyl alcohol was prepared by adding 20 ml of acetyl chloride to 80 ml of isobutyl alcohol.

All glassware were heated at 400°C for 10 h before use. The test tubes used had Teflon-lined screw caps.

**Bacterial suspensions and LPS standards.** A strain of *E. coli*, isolated from a clinical sample, was used to prepare bacterial suspensions of different concentrations (in glass tubes). The concentration of the stock suspension was 1.3 × 10⁹ CFU/ml as determined by viable count. Two 1-ml samples were taken from each suspension and lyophilized, one sample being subjected to LAL assay (polypropylene tubes) and the other to GC-MS analysis (glass tubes).

The *E. coli* O55:B5 LPS was used for constructing the standard curve (1 to 2,000 ng) in aqueous solution for use with the GC-MS method. The 3-OH:14:0 content of the LPS was determined by GC as trifluoroacetyl-methyl ester (7, 26). LPS standard preparations were also analyzed with the LAL assay.

**Dust samples.** Airborne dust samples from three different poultry slaughterhouses were collected on conditioned pre-weighted membrane cellulose acetate filters (37-mm diameter, 0.8-μm pore size; Millipore Corp., Bedford, Mass.) by means of personal samplers (2 to 3 liters/min; Casella AFC 123) at breathing zone level. Sampling periods were of 2 to 3 h in duration. After determination of the total aerosol mass, the filters were eluted in 10 ml of pyrogen-free water for 60 min (23). Samples, 1 ml, were subjected to the LAL assay, whereas the remaining volumes were transferred to glass tubes and lyophilized for GC-MS and GC-ECD analyses (see below). Extracts of blank filters were also analyzed. In addition, five polyvinyl chloride filters (37-mm diameter, 0.8-μm pore size; Nuclepore Corp., Pleasanton, Calif.) were also used. These filters were cut in half after sampling. One-half was eluted in water as above and subjected to the LAL assay, and the other was directly subjected to acid hydrolysis for GC-MS analysis (see below).

**Sample preparation.** The freeze-dried filter extracts, with the internal standard (35 ng of 3-0H-9:0) added, were heated in 1 ml of 4 M methanolic hydrochloric acid at 100°C for 18 h. After cooling, 1 ml of water and 1.5 ml of hexane were added, and the sample was extracted. The hexane phase was transferred to a new tube and evaporated under reduced pressure, and the methanolic phase was saved for DAP analysis. The methyl esters in the evaporated hexane phase were redissolved in 0.1 ml of acetonitrile and 20 μl of PFBO-chloride was added, the mixture then being heated at 150°C for 1 h. After cooling, 0.5 ml of heptane and 1 ml of 1 M phosphate buffer (pH 7.0) were added. The tube was shaken and centrifuged (about 1,000 × g), and the organic phase was evaporated. This procedure was a modification of a method described earlier (27). The sample was made up with heptane prior to GC-MS analysis.

For measurement of DAP, the aqueous methanolic solution was lyophilized and the residue was subjected to hydrolysis in 6 M hydrochloric acid at 150°C for 6 h. The acid was removed in a lyophilizer. The carboxyl groups of the amino acid were esterified by heating in 3 M isobutanolatic hydrochloric acid, and the amino groups were acylated with heptanuflorobutyric anhydride as described elsewhere (27).

The preparation was made up with ethyl acetate prior to analysis with GC-ECD. The yield of DAP obtained after analyzing standard suspensions of *E. coli* cells (10¹⁵ CFU) by this modified method was compared with that of an earlier-described method (27).

**GC.** A model 4160 GC (Carlo Erba, Rodano, Italy) equipped with a flame ionization detector, an all-glass splitless injection system, and a fused-silica capillary column (30 m by 0.32-mm inside diameter) coated with cross-linked SE-52 (film thickness, 0.2 μm; J&W, Folsom, Calif.) was used to determine the amount of 3-OH:14:0 in the *E. coli* LPS standard and the yield in the PFBO derivatization step.

The temperature of the injector was 280°C and that of the detector was 300°C. The initial temperature of the column was 80°C, programmed to increase by 5°C/min (starting 1 min after injection) to a final temperature of 280°C. Injections were made in the splitless mode, the split valve being opened 1 min after injection. Hydrogen served as the carrier gas at a flow rate of 1 ml/min. An SP4270 Integrator (Spectra Physics, San Jose, Calif.) was used for integration of peak areas.

**GC-ECD.** A model 3700 (Varian, Los Altos, Calif.) GC equipped with a 63Ni ECD operating in the frequency-pulsed mode and with a MUSIC (multiple switching intelligent controller) column-switching system (Chrompack, Middelburg, The Netherlands) was used for the measurement of DAP. A fused-silica wide-bore column (10 m by 0.53-mm inside diameter) with cross-linked CP-Sil-8 (Chrompack) as the stationary phase (film thickness, 6.4 μm) was used as a precolumn attached to a flame ionization detector, and a fused-silica capillary column (25 μm by 0.22-mm inside diameter) coated with cross-linked SE-30 (film thickness, 0.2 μm; SGE, Ringwood, Australia) served as the analytical column attached to the ECD. The nitrogen carrier gas flow rate was 4 ml/min through the precolumn and 1 ml/min through the analytical column. The makeup gas (nitrogen) flow rate through the ECD was 15 ml/min. Injections were made on-column. The temperature of the injector was 250°C and that of the detectors was 350°C. The initial temperature of the precolumn, 140°C, was programmed to increase by 10°C/min to a final temperature of 260°C. The cold trap, chilled to −70°C with liquid carbon dioxide, was heated to 250°C upon recombination of the trapped fractions into the analytical column, which was kept at a constant temperature of 260°C. Data handling was processed with the Chrompack control and integration system, using an IBM PS/2 model 30 and Chrompack BD 70 printer plotter.

**GC-MS.** An R10-10c quadrupole GC-MS system (Ribermag, Rueil-Malmaison, France) was used. The GC was used was a Carlo Erba model 4160 equipped with an all-glass splitless injector and with a fused-silica capillary column (25 m by 0.22-mm inside diameter) coated with cross-linked SE-52 (film thickness, 0.2 μm). Helium, at an inlet pressure of 0.8 kg/cm², served as carrier gas. The temperature of the injector was 250°C, that of the interface between the GC and the ion source was 250°C, and that of the ion source was 120°C. The column temperature was initially held at 140°C and after 1 min was increased to 260°C by 10°C/min. The split valve was opened 1 min after injection. The isobutane reagent gas in chemical ionization at 0.07 torr (purity, >99.95%) was ionized with electrons at an energy of 93 eV. The manual integration facility in the MS system standard software was used for peak integration.
TABLE 1. Analysis of an E. coli O55:B5 LPS standard preparation with the chromogenic LAL test and with GC-MSa

<table>
<thead>
<tr>
<th>LPS samples (ng)</th>
<th>LPS (ng) by:</th>
<th>GC-MSb</th>
<th>GC-MSc</th>
</tr>
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<tbody>
<tr>
<td>2,000</td>
<td>1,270</td>
<td>2,020</td>
<td></td>
</tr>
<tr>
<td>1,000</td>
<td>500</td>
<td>920</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>8.3</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1.1</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.002</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The data represent one representative experiment.

b Lyophilized in polypropylene tubes.
c Lyophilized in glass tubes. LPS was calculated from the amount of 3-OH-14:0; the LPS standard used contained 20% (wt/wt) 3-OH-14:0.

The hydroxy fatty acid derivatives were analyzed with selected-ion monitoring, using combinations of different ion-monitoring sets characteristic of the different derivatives. The time events and ions were 11 min, m/z 382.30 (3-OH-9:0); 1 min, m/z 396.3 (3-OH-10:0); 2 min, m/z 424.4 (3-OH-12:0); 2 min, m/z 452.4 (3-OH-14:0); 2 min, m/z 480.4 (3-OH-16:0); and 2 min, m/z 508.3 (3-OH-18:0), corresponding to the molecular radical anions of the 3-O-PFBO-methyl derivatives of the different acids (27).

RESULTS

DAP analysis. A calibration curve for DAP was prepared with standard preparations in the range 1 to 2,000 ng. A linear curve with the equation y = 1.4x + 220 (r² = 0.92) was obtained, and the detection limit was estimated to be approximately 5 ng (injected amount) at a signal/noise ratio of 3:1. Analysis of the methanolic phase after methanolation of standard suspensions of E. coli cells gave approximately the same yields of DAP (91%; standard deviation = 7%; n = 6) as did direct hydrolysis of the same bacterial cellular concentrations, using 6 M hydrochloric acid (27).

LPS analysis. The method used for preparing 3-O-PFBO-methyl esters was a modification of an earlier method (27). Use of 4 M methanolic hydrochloric acid methanolation at 100°C for 18 h has been found to quantitatively release amide-linked 3-hydroxy fatty acids of LPS (26). Further, by increasing the concentration of the PFBO-chloride, the yield in the PFBO derivatization step was increased from 65 to 90% (standard deviation = 4%; n = 6), as determined by summation of peak areas from the 3-O-PFBO-methyl esters and 3-hydroxymethyl esters (with a free hydroxyl group) of 3-OH-9:0 and 3-OH-14:0, respectively.

As expected, the 3-O-PFBO-methyl esters all produced molecular radical ions as the base peak in negative ion chemical ionization MS (27), ions which were subsequently used in the selected-ion-monitoring analyses. The equation of the standard curve for E. coli LPS was y = 1.60 × 10⁻²x + 85.8 × 10⁻² (r² = 0.994) over the range 1 to 2,000 ng, and the detection limit was 1 ng/ml. The E. coli LPS standard was found to contain 50% (wt/wt) of 3-OH-14:0, which is in agreement with the results of previous studies (19).

The E. coli LPS standard was also analyzed with the LAL assay. The endotoxin standard of the Coatest (LAL) gave LPS values approximately half the 1,000- and 2,000-ng amounts, as compared with the GC-MS method (Table 1). For the freeze-dried preparations containing 1 to 50 ng of LPS, however, the LAL assay gave considerably lower values, probably due to ineffective extraction of the LPS adhered to the polypropylene tube wall (see Discussion).

LPS in E. coli samples. The LPS content of lyophilized preparations containing varying amounts of E. coli cells is presented in Table 2. Clearly, a decrease in LPS values follows a decrease in bacterial cell count. The quotients between the GC-MS values and the LAL values varied between 8 and 11 (except for the lowest value). The precision of the LAL assay (700 ng, coefficient of variation, 4.7%; n = 5) was similar to that of GC-MS (1,360 ng; coefficient of variation, 6.6%; n = 5) when aqueous solutions containing 10⁶ viable E. coli cells were analyzed. The higher values for LPS content/concentration of cells, obtained with the LAL assay in the precision study, may have been due to disruption of cells during pipetting steps in the preparation of the solutions, thus liberating LPS into the solution (see Discussion).

Dust samples. Results from the analyses of airborne dusts, collected in the three poultry slaughterhouses (S-H1, S-H2, and S-H3), are summarized in Table 3. The LPS content of dust, LPS, and DAP were obtained in samples from S-H1. The correlation between concentrations of dust and LPS content by the LAL assay was high in samples from S-H2 (r² = 0.92) and from S-H3 (r² = 0.90) but lower in those from S-H1 (r² = 0.20) (Fig. 1); with GC-MS, a low correlation was obtained for samples from all three plants studied (Fig. 1). The DAP values, indicating concentrations of gram-negative cell walls, were also poorly correlated with the dust levels (Fig. 2). Consequently, no good linear relationship was obtained between the GC-MS method and the LAL assay for measurement of LPS in the dust samples studied (Fig. 3). A good linear relationship was obtained, however, in samples from all three plants between LPS as measured by GC-MS and DAP values, whereas the relationship between LPS values obtained with LAL and DAP values was poorer (Fig. 4). The concentrations of airborne LPS were slightly higher when calculated from the DAP values than when calculated from the amounts of 3-hydroxy fatty acids (Table 3).

A variety of 3-hydroxy fatty acids were found in the dust samples analyzed by GC-MS. Most abundant were 3-OH-10:0 and 3-OH-12:0, which are major constituents of LPS, e.g., of Pseudomonas and Acinetobacter spp. (38). Other 3-hydroxy fatty acids found were 3-OH-14:0 (present in most LPS, especially of members of the family Enterobacteriaceae [38]) and 3-OH-16:0 and 3-OH-18:0 (e.g., found in LPS of Francisella and Agrobacterium spp. [38]), present in varying amounts but absent from some samples. No 3-hydroxy fatty acids or DAP was found in extracts of blank filters. Extracts of cellulose acetate filters have been shown to be inactive in the LAL test (5, 15). Culture studies of dust samples, described in Hagmar et al. (L. Hagmar, A. Schütz, T. Halberg, and A. Sjöholm, Int. Arch. Environ. Health, in
press) showed most of the viable airborne bacteria to belong to gram-positive species (mainly coagulase-negative staphylococci), which were 10- to 100-fold more abundant than gram-negative species found (Escherichia, Proteus, and Acinetobacter spp.). Total airborne bacteria content was \(4 \times 10^5\) to \(4 \times 10^6\) CFU/m\(^3\), whereas the number of gram-negative cells according to DAP values (Table 3), thus including viable and nonviable cells plus cellular debris, was \(10^6\) to \(10^7\) cells per m\(^3\). It is possible that the 3-OH-16:0 and 3-OH-18:0 detected derived from nonviable cells and debris. Additional microbial data as well as results from health studies of the slaughterhouse workers have been reported elsewhere by Hagmar et al. (in press).

In studies in which polyvinyl chloride filters were used, LPS values by GC-MS were \(10^5\) to \(10^7\) times those obtained with LAL (Table 4), indicating that LPS collected on the filters were not quantitatively released into the water extract used in the LAL assay.

**DISCUSSION**

The presence of LPS-containing airborne dust in the working environment has been associated with a variety of health hazards and lung diseases. Byssinosis among cotton workers has been related to the presence of gram-negative bacteria and LPS in cotton dust (3, 21, 23, 24). In several studies, high levels of LPS have been reported in poultry and hog confinement buildings, as well as in poultry processing plants, and constitute potential health hazards (5, 8, 15, 16). Thus, reliable and selective methods of measuring airborne LPS are needed to be able to relate LPS concentrations to occupational health effects.

Hitherto, the LPS content in airborne dust has generally been measured with the LAL test, an assay in which lipid A activates the clotting cascade in lysate from amoebocytes of *Limulus polyphemus* (30). The molecular environment of lipid A is critical for the activation of this biological effect (12). Most of the cell wall LPS is inactive in the test since lipid A is not exposed to the *Limulus* enzymes at the surface of the outer membrane (14), and thus it is predominantly cell-wall-dissociated LPS that is measured. As the activity of the lysate may vary both between different commercial preparations and between different batches, standardization is a crucial feature of analysis (18). The GC-MS method, on the other hand, measures specific structural components of lipid A. Hence, the total amount of LPS in a sample is determined equally efficiently regardless of whether the LPS is present in a cell-wall-dissociated or associated state or attached to other organic molecules. This was reflected in our analyses of the *E. coli* suspensions: we obtained larger values for LPS with GC-MS than with LAL (Table 2). Similar findings have been reported for meningococcal strains without excessive release of LPS (2). Munford and Hall (13) measured LPS-containing fragments of outer membranes by both the LAL assay and a radioimmunoassay (measuring the amount of the LPS polyasaccharide part) and found the radioimmunoassay values to be as much as 10 times greater than those obtained with LAL.

The airborne dust collected on the filters in this study was probably of heterogenic origin, containing particles of skin, feathers, feces, various fibers, etc. The correlation between the amounts of dust and LPS as determined by the LAL assay was high in the two plants with lower dust levels (mean values, 3.4 and 1.9 mg/m\(^3\)) but lower at S-H1, where the concentration of airborne dust was higher (mean value, 7.9

**TABLE 3. LPS and gram-negative cells present in airborne dust sampled from three poultry slaughterhouses (S-H)**

<table>
<thead>
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<tr>
<td></td>
<td>GM(^a)</td>
<td>Range</td>
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<tr>
<td>Total dust</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg</td>
<td>0.55</td>
<td>0.06-9.50</td>
<td>1.19</td>
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<tr>
<td>mg/m(^3)</td>
<td>0.20-11.8</td>
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<tr>
<td>LPS (LAL)(^b)</td>
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<tr>
<td>ng/mg(^d)</td>
<td>120</td>
<td>22-270</td>
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<tr>
<td>ng/m(^3)</td>
<td>770</td>
<td>20-2760</td>
<td>51</td>
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<tr>
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<td>7060</td>
<td>20-64400</td>
<td>10900</td>
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<tr>
<td>DAP(^f)</td>
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<tr>
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<td>190</td>
<td>13-370</td>
<td>180</td>
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<tr>
<td>ng/m(^3)</td>
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<td>7-8430</td>
<td>320</td>
</tr>
<tr>
<td>Cells (DAP)(^g)</td>
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<tr>
<td>(10^9/mg(^d)</td>
<td>5.5</td>
<td>0.4-11</td>
<td>5.2</td>
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<tr>
<td>(10^9/m(^3)</td>
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<td>0.2-140</td>
<td>9.3</td>
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<tr>
<td>LPS (DAP)(^h)</td>
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<td>1,650</td>
<td>110-320</td>
<td>1,570</td>
</tr>
<tr>
<td>ng/m(^3)</td>
<td>11,800</td>
<td>61-42,000</td>
<td>2,780</td>
</tr>
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</table>

\(^a\) Number in parentheses is number of samples.

\(^b\) GM, Geometric mean.

\(^c\) LPS determined with the LAL assay.

\(^d\) Present in total dust.

\(^e\) LPS determined with GC-MS selected-ion monitoring.

\(^f\) Gram-negative cells calculated from the concentration of DAP assuming that 1 ng of DAP corresponds to \(2.9 \times 10^6\) cells (33).

\(^g\) Cells calculated from the amount of DAP, assuming that 1 ng of DAP corresponds to \(8.7 \times 10^6\) cells (33).

\(^h\) LPS calculated from the amount of DAP, assuming that 1 ng of DAP corresponds to \(8.7 \times 10^6\) LPS (28).
mg/m$^3$). Thelin et al. (32) also reported a good correlation between dust and LPS (LAL) in samples from a poultry farm. In the present study, the correlation was poor between dust and LPS (GC-MS), as it was between dust and DAP, although the mean values (nanograms per milligrams of total dust) at the different plants were similar (Table 3). That LPS values obtained with GC-MS were higher than those obtained with LAL reflects the different LPS states being analyzed with the two methods.

The LPS values obtained with GC-MS were calculated assuming that the value of 3-OH-14:0, the sum of 3-OH-10:0 and 3-OH-12:0 values, and the sum of 3-OH-16:0 and 3-OH-18:0 values each constituted 20% (wt/wt) of LPS. Usually 4 mol of 3-hydroxy fatty acids is present in lipid A, corresponding to approximately 15 to 30% (wt/wt) of LPS (7), and the 3-OH-14:0 in the E. coli LPS standard was in that range in our study (i.e., 20%, wt/wt). That these approximations were applicable was supported by the good correlation between LPS (GC-MS) and DAP (Fig. 4). Calculation of LPS values from the DAP value gave amounts which were approximately twice the LPS values obtained with GC-MS,

FIG. 1. Correlation between total airborne dust and LPS as measured by GC-MS and LAL in (a) SH-1, (b) SH-2, and (c) SH-3.

FIG. 2. Correlation between total airborne dust and DAP measured by GC-ECD in (a) SH-1, (b) SH-2, and (c) SH-3.
though the differences were not statistically significant (Student's t test; \( P > 0.01 \)). The GC-MS method has earlier been used in a study of a few dust samples from poultry confinement buildings (27); in that study, no 3-hydroxy fatty acids were found, although high levels of DAP were detected (thus suggesting the possible presence of DAP-containing gram-positive bacteria [25]).

The method used here for determining the content of LPS in airborne dust is based on the assumption that the LPS collected on the filters is released into the water quantitatively. This assumption is doubtful, however, as it is well known that LPS may adhere very strongly to both polar and nonpolar surfaces (36). This may explain the low recovery in the present study when samples containing small amounts of LPS (<50 ng) and bacterial cells (<10^6 CFU) were extracted from polypropylene tubes for analysis with LAL (Tables 1 and 2). Morris et al. (11) reported large differences in reproducibility between the use of cold and warm water when extracting LPS from cotton. An approach suitable for the GC-MS method might be direct hydrolysis of the filter. Cellulose acetate filters are not suitable, however, as the
filter is degraded by the acid solution. The polyvinyl chloride filters used withstand the acidic conditions during hydrolysis, and the results indicate that only low proportions of the LPS collected on the filters are extracted into the water. By using direct hydrolysis of polyvinyl chloride filters followed by GC-MS, the total amount of material collected on the filter is available for study.

Studies have shown LPS values obtained with the LAL assay also correlate with the pyrogenticity of its lipid A, but not with other toxic (i.e., endotoxic) effects of lipid A. Several substances of microbial origin have been shown to activate the LAL test nonspecifically (17, 37), and other substances have been found to inactivate the assay (35). The GC-MS method measures the total amount of LPS in a sample, even when LPS is present in a biologically inactive form. However, it may be possible that LAL-inactive LPS, e.g., present in cellular debris, becomes biologically active when inhaled into the lung. It is known also that peptidoglycans may cause certain endotoxic effects (4, 34), and several studies have reported the majority of airborne viable bacterial cells in poultry plants to be gram positive (1, 5, 8; Hagmar et al., in press). GC-MS analysis of such biomarkers as d-alanine, DAP, and 3-hydroxy fatty acids provides useful information of the amounts of gram-positive and gram-negative cells and LPS in airborne dusts for evaluating the potential risk of endotoxic effects of the working environment.

ACKNOWLEDGMENTS

We are indebted to Ragnar Rylander and colleagues, University of Gothenburg, Gothenburg, Sweden, for analyses of the filter samples with the LAL test, and to Göran Ohdam and Anders Valeur, Laboratory of Ecological Chemistry, Lund University, for providing a GC-MS and for assistance with mass spectrometry. This work was supported by the Swedish Work Environment Fund.

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