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Increased levels of bacterial markers and CO_2 in occupied school rooms

Alvin Fox,*[a] William Harley,[a] Charles Feigley,[b] Deborah Salzberg,[b] Aleksandra Sebastian[c] and Lennart Larsson[c]

[a]*Department of Pathology and Microbiology, University of South Carolina, School of Medicine, Columbia, SC 29208, USA. E-mail: afox@med.sc.edu; Fax: +803 733 3192; Tel: +803 733 3298*
[b]*Department of Environmental Health Sciences, University of South Carolina, School of Public Health, Columbia, SC 29208, USA*
[c]*Department of Medical Microbiology, Dermatology and Infection, University of Lund, Solvegatan 23, 223 62 Lund, Sweden*

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Our group previously demonstrated that carbon dioxide (CO_2) levels in heavily occupied schools correlate with the levels of airborne bacterial markers. Since CO_2 is derived from the room occupants, it was hypothesized that in schools, bacterial markers may be primarily increased in indoor air because of the presence of children; directly from skin microflora or indirectly, by stirring up dust from carpets and other sources. The purpose of this project was to test the hypothesis. Muramic acid (Mur) is found in almost all bacteria whereas 3-hydroxy fatty acids (3-OH FAs) are found only in Gram-negative bacteria. Thus Mur and 3-OH FA serve as markers to assess bacterial levels in indoor air (pmol m^{-3}). In our previous school studies, airborne dust was collected only from occupied rooms. However, in the present study, additional dust samples were collected from the same rooms each weekend when unoccupied. Samples were also collected from outside air. The levels of dust, Mur and $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ 3-OH FAs were each much higher (range 5–50 fold) in occupied rooms than in unoccupied school rooms. Levels in outdoor air were much lower than that of indoor air from occupied classrooms and higher than the levels in the same rooms when unoccupied. The mean CO_2 concentrations were around 420 parts per million (ppm) in unoccupied rooms and outside air; and they ranged from 1017 to 1736 ppm in occupied rooms, regularly exceeding 800–1000 ppm, which are the maximum levels indicative of adequate indoor ventilation. This indicates that the children were responsible for the increased levels of bacterial markers. However, the concentration of Mur in dust was also 6 fold higher in occupied rooms (115.5 *versus* 18.2 pmole mg^{-1}). This further suggests that airborne dust present in occupied and unoccupied rooms is quite distinct. In conclusion in unoccupied rooms, the dust was of environmental origin but the children were the primary source in occupied rooms.

1. Introduction

Microbial contamination of indoor air, from either environmental or human sources, may create health problems. These may be serious in schools and day-care centers where young children are present in crowded groups for 6–8 h per day.[1] There is no significant difference in the microbial content of outside air and in naturally ventilated homes. However, the total number of bacteria, and number of Gram positive cocci have been reported to be substantially higher in air-conditioned residences than in outside air.[2] Bacterial levels in a university office, auditorium, and apartments were found to be considerably elevated when people where present (925–1225 cfu m^{-3} occupied *versus* 92–182 cfu m^{-3} for unoccupied offices).[3] Endotoxin levels are higher in homes with animals.[4] However, recent studies of schools have not directly addressed the effect of occupancy on levels of bacteria or their constituents.[5–7]

Exposure to high levels of airborne organic dust in indoor environments, such as cotton mills, poultry houses and swine confinement buildings, can lead to pulmonary diseases including byssinosis and allergic hypersensitivity. Indeed, the respiratory problems associated with these situations are often related to bacterial contamination.[8,9] Although levels of biocontamination in schools are generally much lower, it may be premature to dismiss a relationship between indoor air quality in crowded schools and endotoxic and/or allergic reactions to bacterial antigens. Regardless of the mechanism, guidelines need to be established for acceptable levels of bacteria or their constituents present in indoor air.

Two major cell envelope components, derived from bacteria, have been demonstrated to be widely present in airborne dust: lipopolysaccharide (LPS), commonly referred to as endotoxin, and peptidoglycan (murein, PG). LPS is present only in Gram-negative bacteria, whilst PG is present in both Gram-negative and Gram-positive bacteria. Muramic (Mur) acid is a major constituent of the glycan backbone of PG; and $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ 3-hydroxy fatty acids (3-OH FAs) are constituents of the lipid A of LPS. Thus Mur and 3-OH FAs serve as markers for bacterial levels in indoor air.[10,11]

The research presented here builds on an earlier study in which significant correlations were found between air concentrations of dust, Mur, 3-OH FAs and CO_2 in two schools with differing air conditioning systems. Perhaps the most striking finding was that the amount of bacterial biomass (Mur, 3-OH FAs) positively correlated with CO_2, indicating that a considerable proportion of the air-borne bacteria emanated from the pupils themselves.[1] In the present study the levels of bacterial markers and environmental parameters, including CO_2 levels, were assessed in both occupied and unoccupied school rooms. If the children were responsible, then it was hypothesized that levels of bacterial markers in indoor air would be much higher in occupied rooms.

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2. Materials and methods

Monitoring site

A suburban elementary school in Columbia, SC, was identified by district personnel as a source of indoor air quality complaints. The school served approximately 600 students in pre-kindergarten through fifth grade, and had a total of 33 classrooms. Seven classrooms were selected for study from various parts of the school. Two of the seven were in the older wing of the building constructed *circa* 1935. These rooms (Rooms 6 and 7) were served by a central heating, ventilation, and air-conditioning (HVAC) system controlled by room thermostats. Air from this system was blown into each room only when that room was being heated or cooled. Each of the remaining five rooms (Rooms 1 through 5), located in the wing built in the 1970s, was heated and cooled by individual fan coil units (FCUs) in the exterior wall. Units were centrally controlled by district personnel and set to operate from 6:30 am to 3:45 pm each school day with fans running continuously during this period. The FCUs operated at other times if the temperature fell below 55 °F or rose above 85 °F. Teachers' practices regarding opening classroom doors and windows varied, but windows in most classrooms were kept closed during the sampling campaign. The samples were collected over a 21 day period from Friday, January 11, 2002, through Friday, February 1, 2002. All classrooms were unoccupied for the three weekends of the study and also on Monday, January 21, a school holiday.

Monitoring methods

Carbon dioxide, temperature, and humidity were monitored using an IAQ-Calc (Model 8760, TSI Inc., St. Paul, MN) instrument with a built-in data logger. Measurements were recorded at 10 min intervals. Suspended particulate matter was sampled using 3 piece air monitoring cassettes with Teflon filters (37 mm, 2 μm pore size, SKC Inc., Eighty Four, PA). Teflon filters were selected because of their inertness to chemicals used in the analytical procedure. All filters were pre-weighed using an electronic balance (Model GA200D, Ohaus). Each filter was attached to a rotary vane pump (VS-Series, 1/6-hp, Model 3032, Hi-Q, San Diego, CA) contained in a sound-deadening wooden box. An average air-flow rate of 37.5 l min^{-1} was used indoors and 44.5 l min^{-1} outdoors. Flow rates selected were slightly below the maximum capacity of each pump. This flow rate was needed to collect mg amounts needed for analysis in the time allotted (62.5 h per weekend [pumps continuously running] or 37.5 h per week, 7.5 h per school day). At higher flow rates pumps were too noisy for use. The collection efficiencies were not measured. There was some clogging, particularly with filters collected during the school day (since dust levels were higher). Thus flow rates were recorded at the beginning and end of each school day and the mean used for calculations.

Indoor and outdoor CO_2 data was used to calculate the effective air exchange rates for each room and each sampling period. For a classroom with sufficient mixing of air, the CO_2 concentration at the end of a short time period (C_t) may be expressed as a function of total CO_2 exhalation rate for all room occupants (G), the effective rate of air exchange between the classroom and outdoors (Q), the CO_2 concentration outdoors during the time period (C_0), and the classroom CO_2 concentration at the beginning of the time period (C_i):

$$C_t = \left(\frac{G}{Q} + C_0\right)\left(1 - e^{-Qt/V}\right) + C_i e^{-Qt/V} \qquad (1)$$

where t = duration of time period, and V = classroom volume.

To verify that classrooms were well mixed, CO_2 measurements were made at eight positions in a typical classroom. To estimate G and Q for each day's sampling interval, first eqn. (1) was applied to all 10 min monitoring time periods using initial approximations at the values of G and Q in an Excel® (Excel 97 SR-2) spreadsheet. The measured concentration was subtracted from the concentration estimated using eqn. (1) to obtain a residual value. The residuals were squared and added together to obtain the sum of squares of the residuals. The Excel Solver was used to minimize the sum of squares by varying G and Q by the Generalized Reduced Gradient nonlinear optimization method. The estimate of Q represents the effective outdoor air exchange rate, including exchanges through the HVAC system, doors, and windows, and by infiltration.

Sampling plan

Monitoring of CO_2, temperature, humidity, and dust was performed at nine locations (7 classrooms and 2 outdoor points). At each of the nine locations, monitoring was performed over six time periods consisting of three weekends and three weeks. The weekend samples began on Friday afternoons after students were released and ended on Monday mornings before school began, except for the weekend that included Monday, January 21, a school holiday. For each week, composite dust samples were collected over the school day (not after school or at night) *i.e.* 7.5 h per day, 37.5 h per week. Also, composite dust samples were collected over the entire weekend periods (night and day) a total of 62 h. The pumps and IAQ-Calcs were manually turned on at the beginning of each school day and manually turned off at the end of each school day. In our previous school study, airborne dust samples were collected only during the school days.[1]

In classrooms, CO_2, temperature, and humidity instruments were placed on top of large cabinets, approximately 2.1 m above the floor. Filters for dust monitoring were placed close to the center of the rooms, about 2.1 m above the floor. Instruments and dust filters were placed to achieve samples representative of room contents. Outdoor air was monitored at two locations outside second floor windows, on different sides of the school buildings.

IAQ-Calcs were calibrated for CO_2 measurement at the beginning and end of the sampling period, and at least once each week using 1000 ppm manufacturer's calibration gas. Small adjustments to the instrument calibration settings were required on four occasions. Dust sampling flow rate readings were taken at least once every school day using a factory-calibrated Hi-Q flow meter (Cat. # v-flo-70l). Initial and final airflow readings were taken for each attached filter cartridge. On the weekends, airflow readings were taken at the beginning and end of the sampling period and once during the weekend, generally on Sunday mornings.

PG and LPS analysis

The gas chromatography-tandem mass spectrometry (GC-MS-MS) procedure for analysis of Mur (marker of PG) in airborne dust has been described elsewhere.[12,13] In brief, filter samples were hydrolyzed in 1.5 ml of 2 N sulfuric acid for 3 h at 100 °C. ^{13}C Mur (Isotec,Inc., Miamisburg, OH) was used as the internal standard. Samples were neutralized by mixing with 3.0 ml *N,N*-dioctylmethylamine : chloroform. The aqueous phase was passed through a C-18 column (J. T. Baker, Phillipsburg, VA) and reduced with sodium borohydride. To remove generated borate, evaporation was performed after addition of methanol–acetic acid (200 : 1 vol/vol). The alditols were acetylated at 100 °C overnight. Acetic anhydride was decomposed with water, chloroform added and after mixing the aqueous phase discarded. Ammonium hydroxide was added and the mixture passed through a Chem Elut column (Varian, Harbor City, CA) and the chloroform phase collected. Samples

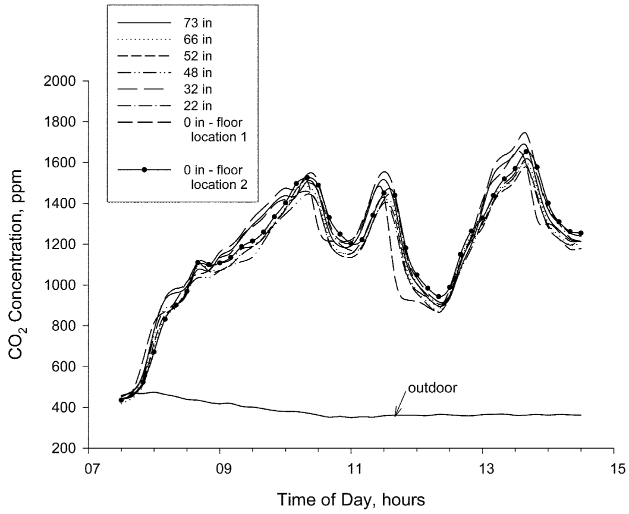

Fig. 1 Carbon dioxide concentrations outdoors and at various locations within a classroom.

were evaporated, then resuspended in chloroform for analysis. An ion trap tandem mass spectrometer (GCQ, Finnigan, Atlanta, GA) was employed. Ionization was performed in electron impact mode followed by collision induced dissociation and multiple reaction monitoring. The GC was equipped with a non-polar DB-5MS column (JW Scientific, Folsom, CA).

For analysis of 3-OH FAs, filter samples were heated in 2 M methanolic HCl overnight at 85 °C after which the internal standard (methanolysate of ^{13}C-labelled cyanobacterial cells) was added. The methyl esters formed were extracted twice with n-heptane (1 ml). The combined heptane layers were evaporated under a stream of nitrogen, redissolved in 1 ml of a hepane : dichloromethane mixture (1 : 1 v/v), and applied onto a disposable silica gel column (1 ml, Bond-Elut, Analytichem, Harbour City, CA) to separate the methyl esters of hydroxylated acids from those of non-hydroxylated acids. The hydroxy fatty acid methyl esters were eluted with 2 ml of diethyl ether, evaporated, and converted to trimethylsilyl (TMS) derivatives by heating in 50 : 1 *N*,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and 5 : 1 of pyridine at 80 °C for 20 min. After evaporation of the pyridine, n-hepane was added to a final volume of 100 µl; 1 µl of which was used for GC-MS-MS. Samples were ionized in electron impact mode and analyzed using a Saturn 2000 ion-trap GC-MS instrument (Varian, Palo Alto, CA, USA) equipped with Combi Pal auto-sampler (CTC Analytics AG, Zwingen, Switzerland) and a fused-silica capillary column (CP-Sil 8 CB low bleed, 0.25 µm film thickness, 30 m × 0.25 mm id) (Chrompack, Middelburg, The Netherlands).[14–18]

Table 1 Average CO_2 concentrations measured in a school

Average CO_2 (ppm)								
Time Period	Outside	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]
Weekend 1	449	411	450	443	407	415	405	391
Weekdays 1	415	1155	1485	1017	1451	1585	1561	1359
Weekend 2	428	386	450	436	425	423	398	385
Weekdays 2	399	1295	1622	1030	1416	1529	1541	1327
Weekend 3	441	411	481	426	424	424	399	389
Weekdays 3	371	1304	1609	1102	1391	1110	1736	1511
Weekend 4	446	414	483	377	383	394	401	383

[a]School room.

Statistical analysis

In addition to being presented in graphs and tables, the data were analyzed using correlation and linear regression (Minitab 13.1, Minitab Inc., State College, PA and SAS System Software Statistical Analysis System Inc., Cary, NC). With SAS, regressions were performed using the GLM procedure with Bonferroni *t*-tests to assess the effects of categorical variables. The classroom data used in these analyses consisted of independent variables (room, type of HVAC system, occupancy status, and effective air exchange rate) and monitoring results (CO_2, temperature, absolute humidity; concentrations of dust, Mur and LPS in air; and concentrations of Mur and LPS in airborne dust). Statistical significance was indicated by a p value of 0.05 or less.

3. Results

To explore the uniformity of a contaminant emanating from room occupants, CO_2 was monitored at eight locations in a typical classroom. Pearson correlation coefficients of CO_2 concentrations for the eight monitoring locations ranged from 0.96 to 0.99, suggesting that a single monitoring location was appropriate for characterizing contaminant levels within the rooms (Fig. 1). The CO_2 concentration, averaged over dust sampling periods, was about 437 ppm in unoccupied rooms (weekend averages) and outside air and ranged from 1017 to 1736 ppm in occupied rooms (weekly averages during school hours, Table 1).

The average levels of dust, Mur, LPS, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ 3-OH FAs, were each much higher in the occupied rooms than in the unoccupied rooms (Table 2 and Figs. 2 and 3). Moles of LPS were calculated by summing the individual 3-OH FAs and dividing by four.[14] For example, the mean dust levels (± standard deviation) in unoccupied rooms were: weekend one 7.6 ± 1.2; weekend two 6.5 ± 1.2; and weekend three 8.5 ± 1.4 µg m^{-3}; whereas dust levels in occupied rooms were: week one 65 ± 36; week two 60 ± 39; and week three 49 ± 23 µg m^{-3}. The levels of Mur in unoccupied rooms were: weekend one 0.09 ± 0.01; weekend two 0.16 ± 0.05; and weekend three 0.16 ± 0.16 pmol m^{-3}; whereas, for occupied rooms: week one 8.5 ± 6.2; week two 6.5 ± 5.0; and week three 5.9 ± 3.1 pmol m^{-3}. The levels of these markers in outdoor air were found to be slightly higher than those of unoccupied classrooms, but much lower than those of occupied classrooms.

The concentration of Mur in airborne dust in occupied rooms was 6 fold higher than the concentration in unoccupied rooms (115.5 *versus* 18.2 pmole mg^{-1}) (Table 3). This suggests that airborne dust sampled from occupied rooms was distinct from airborne dust when rooms were unoccupied and may be from different sources. By contrast, dust concentrations of the 3-OH FAs were not obviously affected by occupancy

Table 2 Average levels of dust and bacterial markers in school air

	Dust/µg m^{-3}	Muramic acid/ pmole m^{-3}	$C_{10:0}$ 3-OH FA/pmole m^{-3}	$C_{12:0}$ 3-OH FA/pmole m^{-3}	$C_{14:0}$ 3-OH FA/pmole m^{-3}	$C_{16:0}$ 3-OH FA/pmole m^{-3}	LPS/ pmole m^{-3}
Unoccupied classrooms	7.5 ± 1.5	0.14 ± 0.10	0.11 ± 0.06	0.16 ± 0.08	0.31 ± 0.06	0.67 ± 0.12	0.31 ± 0.048
Occupied classrooms	57.9 ± 32.4	6.97 ± 4.82	0.93 ± 1.21	2.192 ± 1.4	3.09 ± 1.87	3.51 ± 1.59	2.43 ± 1.464
Outdoor air	21.6 ± 7.6	0.82 ± 0.52	0.31 ± 0.25	0.803 ± 0.71	0.68 ± 0.23	1.03 ± 0.26	0.70 ± 0.316

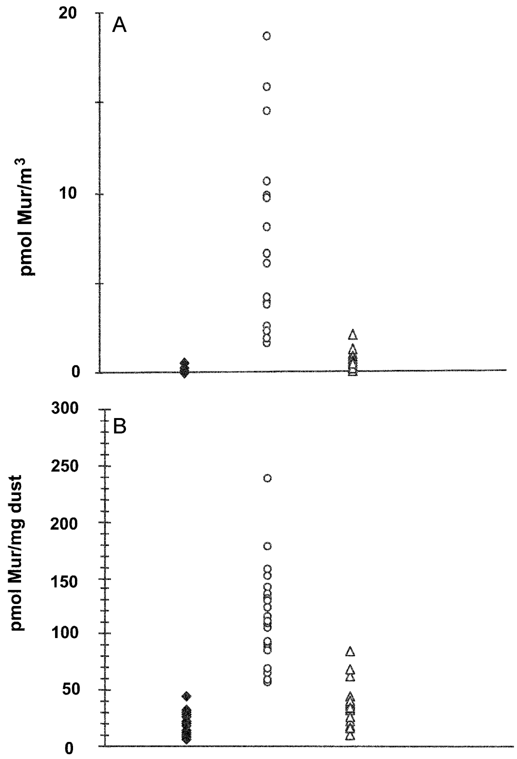

Fig. 2 (A) Levels of muramic acid in indoor air and (B) concentration of muramic acid in airborne dust in a school. Unoccupied rooms (bold diamonds), occupied rooms (circles) and outdoor air (triangles).

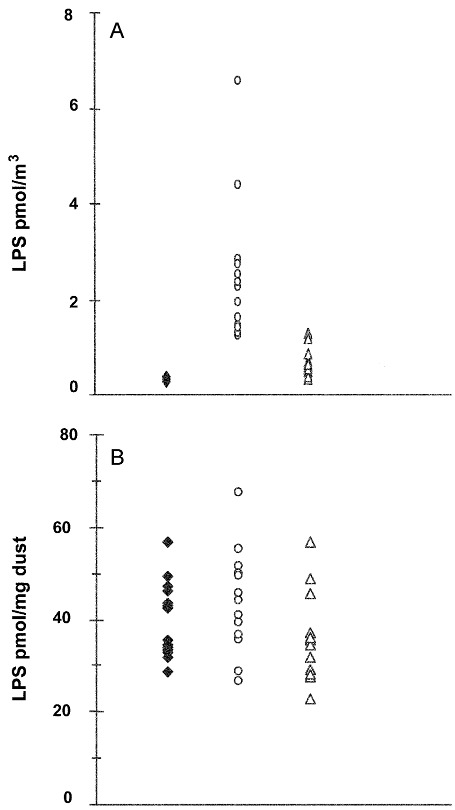

Fig. 3 (A) Levels of LPS in indoor air and (B) concentration of LPS in airborne dust in a school. Unoccupied rooms (bold diamonds), occupied rooms (circles) and outdoor air (triangles).

status. Regression analyses were performed to examine these relationships further.

Stepwise multiple regression was utilized with the following continuous dependent variables: total airborne dust, Mur in air (pmol m^{-3}), Mur in airborne dust (pmol mg $dust^{-1}$), LPS in air (pmol m^{-3}), LPS in airborne dust (pmol mg $dust^{-1}$), $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ 3-OH FAs in airborne dust (pmol mg $dust^{-1}$), and $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, and $C_{16:0}$ 3-OH FAs in air (pmol m^{-3}). Independent variables in the regressions were occupancy status, HVAC system type (FCU or central), and room number. Carbon dioxide, temperature, and humidity were all confounded with occupancy status for a combination of reasons: for instance, occupants were the source for CO_2 and humidity, and ventilation systems were only operated during school hours. Thus, a categorical variable for occupancy status was included in the stepwise regressions, whereas CO_2, temperature and humidity were not. Table 4 shows the stepwise regression results including all statistically significant ($p < 0.05$) independent variables for the first five dependent variables (Dust, Mur, Mur/dust, LPS, and LPS/dust); Table 5

Table 3 Average concentration of bacterial markers in dust (pmole mg^{-1})

	Muramic acid	$C_{10:0}$ 3-OH FA	$C_{12:0}$ 3-OH FA	$C_{14:0}$ 3-OH FA	$C_{16:0}$ 3-OH FA	LPS
Unoccupied classrooms	18.2 ± 9.6	13.9 ± 8.9	21.1 ± 11.1	39.4 ± 7.2	85.1 ± 16.1	39.87 ± 8.13
Occupied classrooms	115.5 ± 43.6	14.6 ± 10.8	39.1 ± 10.9	55.4 ± 15.8	65.0 ± 15.0	43.54 ± 10.75
Outdoor air	38.3 ± 21.5	15.6 ± 10.8	39.3 ± 29.1	35.1 ± 7.2	54.5 ± 10.6	35.17 ± 10.29

Table 4 Stepwise regression results for dust and chemical markers over all classrooms and sampling periods with all statistically significant independent variables

	Factor coefficient and p-value					
Dependent variables	Constant	Occupied	FCU	Room 1	Room 3	R^2
Dust[a]/μg m^{-3}	22.82	50.4 <0.001	−26.1 0.001		23.2 0.013	0.696
Mur[a]/pmol m^{-3}	1.99	6.84 <0.001	−3.5 0.002		4.4 0.002	0.670
Mur/dust[a]/pmol mg^{-1}	23.44	97.3 <0.001		−36.0 0.007		0.762
LPS[b]/pmol m^{-3}	1.16	2.12 <0.001	−1.19 0.004			0.670
LPS/dust[b]/pmol mg^{-1}	50.06		−13.4 <0.001	8.6 0.054		0.414

[a] $n = 42$ Observations. [b] $n = 28$ Observations.

Table 5 Stepwise regression results for individual 3-OH FAs in dust over all classrooms and sampling periods with all statistically significant independent variables

	Factor coefficient and p-value							
Dependent variables	Constant	Occupied	FCU	Room 1	Room 3	Room 5	Room 6	R^2
$C_{10:0}$/pmol mg dust^{-1}	11.08			11.1 0.027			11.2 0.026	0.276
$C_{12:0}$/pmol mg dust^{-1}	19.17	18.0 <0.001					13.6 0.019	0.536
$C_{14:0}$/pmol mg dust^{-1}	39.79	16.0 <0.001			−10.6 0.029	−11.1 0.023	19.1 <0.001	0.734
$C_{16:0}$/pmol mg dust^{-1}	96.60	−20.1 <0.001	−16.2 0.010					0.474

n = 28 observations.

shows the stepwise regression results for the four individual 3-OH FAs in dust. Each row of the tables represents an equation for predicting the dependent variable in the first column. For example, the first row of Table 4 is equivalent to the equation: Dust = 22.82 + 50.4 × Occupied − 26.1 × FCU + 23.2 × Room 3, where each of the variables "Occupied," "FCU," and "Room 3" takes on the value 1 for true and 0 for false. For instance, on a day when Room 3 (which has an FCU) is unoccupied, the airborne dust level predicted by the regression equation: Dust = 22.82 + 50.4 × 0 − 26.1 × 1 + 23.2 × 1 = 19.9 µg m^{-3}; on a day when Room 6 (no FCU) is occupied, the predicted airborne dust level is: Dust = 22.82 + 50.4 × 1 − 26.1 × 0 + 23.2 × 0 = 73.2 µg m^{-3}.

From the regression equations in Table 4 it may be seen that total dust, Mur in air, and LPS in air were much higher in occupied rooms than on weekends and much higher in rooms served by a central HVAC system rather than by an individual FCU. Dust concentrations of LPS, but not of Mur, were significantly lower in rooms with FCUs. Room 3 was much larger and had more occupants than the other rooms, and had significantly higher levels of dust and Mur than the other rooms with FCUs. Air exchange rates were not significantly different in rooms with FCUs *versus* rooms connected to a central HVAC system (p = 0.62) (data not shown). Thus, the impact of HVAC seems to be independent of air exchange differences and could result from different filtration characteristics.

LPS levels in airborne dust, unlike Mur levels, were related to occupancy in a complex fashion. Although it initially appeared that LPS in dust was not related to occupancy (Table 4), when the individual 3-OH FAs were used as dependent variables (Table 5), it was clear that occupancy was an important factor in those levels. Stepwise regression revealed that the concentrations of C_{12} and C_{14} 3-OH FAs in dust were significantly higher in occupied classrooms; the concentration of C_{16} 3-OH FA was significantly lower in occupied classrooms; and the concentration of C_{10} 3-OH FA in dust was unaffected by occupancy status. Hence, the effect of occupancy on the concentrations of individual 3-OH FAs in dust was variable suggesting different sources of the individual 3-OH FAs, *i.e.* a change in the Gram negative bacterial population. It was also noted that the average levels in air (pmol m^{-3}) of the individual 3-OH FAs were higher when the rooms were occupied and lower in rooms with FCUs (data not shown).

4. Discussion

According to the American Society of Heating, Refrigeration, and Air Conditioning Engineers, CO_2 concentrations in indoor air should be no greater than 700 ppm above that of outdoor air,[19] which translates to approximately 1100 ppm in this school. However, that standard was exceeded for 19 of the 21 weekly CO_2 concentration averages during school hours (3 weeks × 7 rooms). The National Institute of Occupational Safety and Health recommends that indoor CO_2 concentrations of greater than 800 ppm should trigger examination of the building's HVAC system.[20] Carbon dioxide maxima in all classrooms frequently exceeded 1000 ppm, and, toward the end of the school day, often exceeded 2500 ppm in three of the seven classrooms tested.

Levels of airborne dust, bacterial markers and CO_2 were found to be much higher in occupied schoolrooms than in unoccupied rooms and in outdoor air. In addition, the concentration of Mur in dust from occupied rooms was six-fold higher than in unoccupied rooms. This suggests that dust from occupied and unoccupied rooms is distinct and from different sources. The effect of occupancy on the dust concentrations of the individual 3-OH FAs was inconsistent, suggesting different sources of these markers. Mur is present in both Gram-positive and Gram-negative bacteria but at much higher levels in the former. 3-OH FAs are present in Gram-negative but not in Gram-positive bacteria. Thus, it is suggested that the concentrations of Gram-positive bacteria in the airborne dust from occupied rooms may be higher than those from unoccupied rooms. Alternatively, there may be a change in the Gram positive population from those with a low Mur content to those with a high Mur content. For example, bacilli pre-dominate in environmental samples, whereas staphylococci are of human origin (*Bacillus cereus* cells are around 0.3% Mur, whereas *Staphylococcus aureus* are 2.6% Mur on a dry weight basis.[11] The differences in these chemical marker concentrations in dust may also have been due to their presence in particles of different characteristics. For instance, Mur may have been carried by smaller particles with longer settling times than those containing LPS such that these carriers were still present in room air on weekends. When the rooms were occupied and the HVAC systems were operating, larger particles could have been more efficiently removed by filtration. It is also possible that occupation will disturb and make airborne, heavier particles which have settled into dust deposits during unoccupied times. It will be important in future work, to identify the microbial population of occupied and unoccupied rooms.

From the above, it is strongly suggested that room occupants were responsible for the increased levels of airborne dust and chemical markers; however, this study does not show how room occupation increases the levels. These initial findings do suggest, however, that surveys of classrooms when the children are not present will grossly underestimate airborne exposures.

In our previous school study, Mur was well correlated with CO_2, but only occupied classrooms were studied.[1] In the present study, when the data from occupied rooms were analyzed separately by stepwise regression, the best predictor of airborne Mur concentration was found to be air exchange rate (results not shown). This is not surprising because CO_2 concentrations and air exchange rates are confounded in occupied room data. Also, in the previous study, air exchange rates were determined in the evenings with all windows closed, using a tracer gas dilution method; whereas CO_2 monitoring and dust sampling for Mur was conducted during the school day, when one of the schools had windows open in most classrooms. Thus, the measured air exchange rate in that study may have underestimated actual air exchange during the sampling periods.

The weekly average total airborne dust level in occupied rooms found at this elementary school, 58 μg m^{-3}, was similar to the levels found in the two elementary schools studied previously, 39 and 54 μg m^{-3}.[1] However, the average weekend levels in unoccupied schoolrooms, 7.5 μg m^{-3}, were much lower and similar to that seen in lightly occupied laboratories, 4 μg m^{-3}.[21] Exposure to particulate matter has been reported to be higher in schools than in outdoor air.[22] The levels in occupied school rooms are still lower than those found in industrial arts classrooms, which ranged between 120 and 1118 μg m^{-3} and those typically found in agricultural and industrial settings.[23] Dust levels in cotton mills, at the lower end of agricultural exposures, can range from 70 to 270 μg m^{-3}.[24] Examples of intermediate exposure include swine confinement buildings with dust levels ranging between 5.6 and 24 mg m^{-3},[25] and the paper industry with levels exceeding 5 mg m^{-3}.[26] One of the highest exposure groups were in the potato processing industry with dust levels reaching 100 mg m^{-3}.[27]

Spirometric studies of human volunteers exposed to cotton dust in an experimental card room provided the first direct evidence for the toxicity of airborne LPS.[8] On acute inhalation exposure of guinea pigs in an environmental chamber, only high doses of purified LPS of 9.6 and 50 μg m^{-3} produced significant decreases in specific airway conductance.[28] However, doses as low as 0.05 μg m^{-3} substantially increased total leukocyte counts (primarily neutrophils) in lavage fluids. In agreement with these animal studies, on low-level human exposures, bronchoalveolar lavage fluid exhibited a 100 fold increase in neutrophils.[9]

Peptidoglycan displays toxicity for the hamster tracheal epithelium *in vitro*.[29] In one recent study in swine houses, there was a correlation between airborne PG, but not LPS levels, with blood granulocyte levels and body temperature.[30] In a later study, upon exposure to swine dust, granulocyte levels increased dramatically in bronchoalveolar lavage and nasal lavage fluid. IL-6 and TNF levels also increased significantly. LPS levels, but not PG levels, were correlated with IL-6 levels.[31] Compost workers also display increased IL-8 levels on exposure to bio-aerosols.[32] Exposure to dust in swine confinement buildings, which contains high concentrations of bacteria, causes intense inflammation in healthy people.[25]

The significance of exploring exposure to PG and LPS and the factors associated with this exposure may be seen by consideration of the distribution and trends of respiratory illnesses, particularly pediatric asthma. Asthma is recognized as an important public health problem.[33–36] The prevalence of asthma in children has more than doubled in the last 20–30 years[37] and hospitalization rates for asthma have increased while other types of pediatric admissions have decreased dramatically.[38] In persons with a genetic predisposition, asthma development and attack occurrence have been found to be associated with environmental factors, such as volatile organic compounds in commercial products (*e.g.*, paints, adhesives, perfumes), components of building materials, outdoor air pollutants, animal and insect allergens, molds, and environmental tobacco smoke.[39,40] Because PG and LPS are inflammatory agents, even at relatively low exposure levels, they may be responsible in some children for asthma development; and they may also trigger asthma attacks. However, exposure of children to PG and LPS has not been well characterized.

PG and LPS exposures in schools may play some role in diminished student performance as well as having direct student health impacts. Improved air handling systems, including better air distribution, better temperature control systems, and greater air exchange were found to reduce students' symptoms, significantly reduce student reaction times in neuro-behavior tests, and reduce indoor air quality and comfort-related complaints.[41] The physical condition of school buildings in Washington, DC, was found to account for a statistically significant portion of achievement test scores, after removing the effects of community income and racial composition, parent involvement in schools, school age, and school enrolment.[42] Average achievement scores in schools with poor physical conditions were 6% below schools in fair condition and 11% below schools in excellent condition. Studies in Virginia and North Dakota obtained very similar results[43–45] The North Dakota studies are important because they are less likely to be confounded by racial and socio-economic factors. Other studies concluded that HVAC systems and indoor air quality were important determinants of student achievement test results[46] and their ability to concentrate.[47] Based on a national survey of 10,000 facilities in the US, energy efficiency and ventilation are the building characteristics most often mentioned as deficient US General Accounting Office.[48] An estimated 11,545,000 students are taught in schools with poor ventilation in the US. Inadequate ventilation may result in poor indoor air quality and have a negative impact on building occupants' health and performance. In this case, building occupants are mostly children whose learning and health may be adversely affected.

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