



LUND UNIVERSITY

Characterization of endotoxin and 3-hydroxy fatty acid levels in air and settled dust from commercial aircraft cabins.

Hines, C J; Waters, M A; Larsson, Lennart; Petersen, M R; Saraf, A; Milton, D K

Published in:
Indoor Air

DOI:
[10.1034/j.1600-0668.2003.00175.x](https://doi.org/10.1034/j.1600-0668.2003.00175.x)

2003

[Link to publication](#)

Citation for published version (APA):

Hines, C. J., Waters, M. A., Larsson, L., Petersen, M. R., Saraf, A., & Milton, D. K. (2003). Characterization of endotoxin and 3-hydroxy fatty acid levels in air and settled dust from commercial aircraft cabins. *Indoor Air*, 13(2), 166-173. <https://doi.org/10.1034/j.1600-0668.2003.00175.x>

Total number of authors:
6

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Characterization of endotoxin and 3-hydroxy fatty acid levels in air and settled dust from commercial aircraft cabins

Abstract Endotoxin was measured in air and dust samples collected during four commercial aircraft flights. Samples were analyzed for endotoxin biological activity using the *Limulus* assay. 3-hydroxy fatty acids (3-OH FA) of carbon chain lengths C_{10:0}–C_{18:0} were determined in dust by gas chromatography–ion trap tandem mass spectrometry. The geometric mean (geometric standard deviation) endotoxin air level was 1.5 EU/m³ (1.9, *n* = 28); however, significant differences were found by flight within aircraft type. Mean endotoxin levels were significantly higher in carpet dust than in seat dust (140 ± 81 vs. 51 ± 25 EU/mg dust, *n* = 32 each, *P* < 0.001). Airborne endotoxin levels were not significantly related to either carpet or seat dust endotoxin levels. Mean 3-OH FA levels were significantly higher in carpet dust than in seat dust for C_{10:2}, C_{12:0}, and C_{14:0} (*P* < 0.001 for each), while the mean level of C_{16:0} was significantly higher in seat dust than in carpet dust (*P* < 0.01). Carpet dust endotoxin was significantly, but moderately, correlated with 3-OH–C_{12:0} and 3-OH–C_{14:0} (Pearson *r* = 0.52 and 0.48, respectively), while correlation of seat dust endotoxin with individual 3-OH FAs depended on the test statistic used. Mean endotoxin potency was significantly higher for carpet dust than for seat dust (6.3 ± 3.0 vs. 3.0 ± 1.4 EU/pmol LPS, *P* < 0.0001). Mean endotoxin levels in the air and dust of commercial aircraft cabins were generally higher than mean levels reported in homes and office buildings. These results suggest that exposure route and dust source are important considerations when relating endotoxin exposure to specific health outcomes.

C. J. Hines¹, M. A. Waters¹,
L. Larsson², M. R. Petersen¹,
A. Saraf², D. K. Milton³

¹National Institute for Occupational Safety and Health, Cincinnati, OH, USA, ²Department of Medical Microbiology, University of Lund, Lund, Sweden, ³Harvard School of Public Health, Boston, MA, USA

Key words: Endotoxin; 3-Hydroxy fatty acids; Aircraft; Exposure assessment; Air quality.

Cynthia J. Hines
National Institute for Occupational Safety and Health,
4676 Columbia Pkwy, R-14, Cincinnati, OH 45226, USA
Tel.: +1 513 841 4453
Fax: +1 513 841 4486
e-mail: chines@cdc.gov

Received for review 24 October 2001. Accepted for publication 8 May 2002.
© Indoor Air (2003)

Practical Implications

The commercial aircraft cabin is an often-overlooked indoor environment occupied by both flight crew and the general public. Endotoxin exposure in indoor environments has been linked to a variety of health conditions. In this study, endotoxin was readily detected in the air and dust of commercial aircraft during passenger flights, and mean air and dust endotoxin levels in the aircraft cabins were generally higher, and more potent, than those reported for homes and offices. Endotoxin levels also varied significantly by dust source (carpet or seat). Density of occupancy, duration of occupancy, and hygienic conditions may partly explain differences across studies.

Introduction

Endotoxin exposures and associated health effects have been studied in a wide range of agricultural, industrial, and indoor environments (Jacobs, 1997; Myatt and Milton, 2001). Endotoxins are biologically active lipopolysaccharide (LPS) molecules that form the outer membrane of Gram-negative bacteria. Endotoxin toxicity is related to the chemical structure of the lipid A portion of LPS, which varies among bacterial species. Hence, the bacterial source(s) of endotoxin may influence endotoxin-related health risks among individuals from different environments. Lipid A contains characteristic 3-hydroxy fatty acids (3-OH

FAs), primarily of carbon chain lengths C₁₀, C₁₂, C₁₄, C₁₆, and C₁₈ (Wilkinson, 1988). These 3-OH FAs can be used as chemical markers to estimate total LPS (Sonesson et al., 1994). Exposure to endotoxin in the air and dust of office buildings, homes, and other non-manufacturing indoor environments has been linked to a variety of health conditions and symptoms among occupants (Michel et al., 1991, 1996; Park et al., 2001; Reynolds et al., 2001; Rizzo et al., 1997; Rylander et al., 1989, 1992; Teeuw et al., 1994).

The commercial aircraft cabin is a non-manufacturing, non-agricultural indoor environment. Approximately 198 000 flight crew work on scheduled commercial aircraft in the United States (ATA, 2001).

A recent National Academy of Sciences report concluded that insufficient data on exposure to biological agents, including endotoxin, were available on commercial aircraft (NRC, 2001). To address this data gap, endotoxin levels in air and dust were measured on commercial aircraft flights as part of a larger National Institute for Occupational Safety and Health study of commercial aircraft cabin environmental quality (Waters and Bloom, 1997). The 3-OH FA content of the dust samples was also determined. These data were evaluated to determine if aircraft type, flight, source of dust (carpet or seat), and location within aircraft were significant predictors of endotoxin and 3-OH FA levels. The relationship, if any, between endotoxin levels in air and in dust was also investigated.

Materials and methods

Air and dust samples were collected in the coach section of two types of commercial aircraft, an Airbus Industries A-320 and a Boeing 747-200, during four passenger flights on June 1, 2, 6, and 8 1998. A flight consisted of an entire trip between two cities, from take-off to landing. Different planes (i.e. with different tail or identification numbers) were flown on each flight; hence, flight and plane were confounded. The gate-to-gate times for the two A-320 flights (nos 30 and 33) were 292 and 209 min, respectively, while the gate-to-gate times for the two 747-200 flights (nos 31 and 32) were 618 and 519 min, respectively. The A-320 is a smaller aircraft (single aisle; 138 coach seats) than the 747-200 (double aisle; 296 coach seats). The coach section in each type of aircraft was divided into four groups of approximately the same number of continuous rows of seats. For the A-320, these groups or "zones" were rows 5–10 (zone A), rows 11–16 (zone B), rows 17–21 (zone C), and rows 22–27 (zone D); for the 747-200, these groups were rows 21–27 (zone A), rows 36–45 (zone B), rows 51–58 (zone C), and rows 59–66 (zone D). Two area air samples, two dust samples from different fabric-covered seats, and two dust samples from different sections of the aisle carpets were obtained in each zone.

Air samples were collected on 0.4-g polycarbonate filters in closed-face three-piece polystyrene, 37-mm filter cassettes at a nominal flow rate of 4 l/min using battery-operated personal sampling pumps (Model 224-PCXR8; SKC, Eighty Four, Pennsylvania). Pumps were turned on at the beginning of the flight (i.e. as flight crew and passengers were boarding) and off at the end of the flight (i.e. after the plane had landed). Filter cassettes were attached to seat backs. Personal air diffusers above the seats were turned off. Pumps were calibrated pre- and post-sampling on the ground at approximately NTP conditions (760 mmHg, 25°C) using a DryCal® DC-Lite flowmeter (BIOS International, Butler, New Jersey). A field blank was collected on each flight.

Settled dust samples were collected on glass-fiber filters in closed-face three-piece polystyrene, 37-mm filter cassettes. A stainless steel sampling probe, 38-mm long by 21-mm O.D. (outer diameter) crimped to a 3-mm slot was attached to the cassette inlet. The cassette outlet was attached by tubing to a personal sampling pump (described above) and the flow rate set to a nominal 4 l/min. A plastic sampling template (10 cm × 10 cm) was placed on the seat or carpet. Starting at one corner, the entire area within the template was swept back and forth in an "S" pattern with the sampling probe, first horizontally, then vertically. Horizontal and vertical swipes were repeated for 3 min. A new sampling probe was used for each dust sample.

A tube filled with the desiccant, Drierite™ (W.A. Hammond Drierite Co, Ltd, Xenia, OH, USA), was attached to the outlet of each air and dust cassette after sampling. Samples were refrigerated at 4°C and shipped within 1–2 days by overnight carrier to the laboratory. The mass of dust in the carpet and seat samples was determined gravimetrically. Endotoxin was extracted in both air and dust samples by sonication in buffer (0.05 M potassium phosphate, 0.01% triethylamine, pH 7.5) and quantified with the kinetic *Limulus* assay with resistant-parallel-line estimation (KLARE) method as previously described (Hines et al., 2000; Milton et al., 1992, 1997; Walters et al., 1994). *Limulus* amoebocyte lysate (LAL) was obtained from BioWhittaker, Inc. (Walkersville, Maryland), control standard endotoxin was obtained from Associates of Cape Cod (Woods Hole, Massachusetts), and reference standard endotoxin (EC6, Lot G) was obtained from the United States Pharmacopoeia (Rockville, Maryland). Samples with dose-responses that were not parallel to the standard curve were considered invalid and are not reported. Results were reported in endotoxin units (EU) with reference to EC6 (USP, Reference Standard Endotoxin Lot G, 1 ng = 10 EU). Potency of the control standard endotoxin was determined by direct assay with the reference standard, with both standards diluted in buffer. Endotoxin air levels in EU/m³ are reported at NTP conditions after correction for a 3.5% decrease in pump flow rate at typical in-flight cabin pressure conditions (582 mmHg or approximately 6000 ft) and for the time-weighted average pressure in the cabin while the sample was collected; i.e. $\text{EU/m}^3 = [\text{EU/filter} \times (760/P_{\text{aircraft-TWA}} \text{ in mm Hg})] / [(\text{average flow rate} \times 0.965 \text{ in m}^3/\text{min}) \times \text{sampling time in min}]$. In this paper, the term "endotoxin level" means the endotoxic biological activity of the sample in EU/m³ or EU/mg dust as measured by the *Limulus* assay, and the term "endotoxin potency" means the level of endotoxin biological activity in EU per picomole of total LPS as estimated by the total amount of 3-OH FAs.

Gas chromatography-ion trap tandem mass spectrometry (GC-MSMS) was used to determine 3-OH FAs

of 10–18 carbon chain lengths in 1-mg dust samples (Saraf et al., 1999). Dust samples were also analyzed by gas chromatography–mass spectrometry (GC–MS) using a quadrupole instrument in selected ion monitoring mode (SIM) (Saraf et al., 1997) to confirm the specificity of GCMSMS as compared to GCMS. LPS was quantified by assuming that 4 mol of 3-OH FA corresponded to 1 mol of LPS (Rietschel et al., 1984; Wilkinson, 1988).

Statistical analyses were done in SAS v. 6.12 (SAS Institute, Inc., Cary, North Carolina) and S-PLUS v. 4.5 (MathSoft, Seattle, Washington). All significance testing was done at the $\alpha = 0.05$ level. In order to reduce the probability of at least one Type 1 error, means of multi-level variables were compared only if the overall test was significant. Distributions of air and dust exposure variables were evaluated using graphical methods, the Shapiro–Wilk goodness-of-fit test, and by examining the residuals for each model.

Endotoxin air data appeared to fit an approximately lognormal distribution and a natural log transformation was applied. An analysis of variance (ANOVA) was performed to test the effect of aircraft type (two levels), flight (two levels in each aircraft type, treated as random), zone (four levels) and the interaction of aircraft by zone on airborne endotoxin. Each combination of aircraft, flight, and zone had two observations, except four observations were missing due to assay interferences. As the data were not balanced, interactions were generally tested first, and the one with the largest *P*-value was removed from the model if that *P*-value was greater than 0.05.

After examining residuals, an approximately normal distribution was assumed for the endotoxin dust data. An ANOVA was performed to test the effect of aircraft type, flight within aircraft type (treated as random), zone, source of dust (carpet or seat), and the interactions aircraft type by source, aircraft type by zone, source by zone, and aircraft type by source by zone on dust endotoxin levels. Each combination of aircraft type, flight, source, and zone had two observations. Again, after examining residuals, an approximately normal distribution was assumed for the concentration of 3-OH FAs in the dust samples (expressed as pmol LPS/mg dust). An ANOVA was performed separately for carpet and seat data to test the effect of aircraft type, flight within aircraft type (treated as random), zone, replicates within each combination of aircraft type, flight, and zone (treated as random), carbon chain length, and the interactions for aircraft type by zone, aircraft type by chain length, chain length by zone, aircraft type by chain length by zone, and chain length by flight within aircraft type on 3-OH FA concentrations in the dust samples. A least-squares means analysis was done to examine significant interactions. A more complex analysis was also performed on the combined carpet and seat dust data

to specifically test the effect of source of dust (carpet or seat) on 3-OH FA concentrations.

An ANOVA was also performed to test the effect of aircraft type, flight within aircraft type (treated as random), source, zone, and the interactions for aircraft type by source, aircraft type by zone, source by zone, and aircraft type by source by zone on estimates of endotoxin potency for the carpet and seat dust samples. After examining residuals, the potency variable was assumed to be approximately normally distributed. A one-way ANOVA was used to test separately, the effect of zone and the effect of source, on the mass of dust collected in the carpet and seat samples, which was assumed to have an approximately normal distribution.

The relationship between endotoxin levels in air and endotoxin levels in carpet and seat dust was examined separately using regression analysis. The mean endotoxin level in the air and the mean endotoxin level in carpet or seat dust for each zone on each flight ($n = 15$) was used as independent and dependent variables, respectively. Six regression models were fit for each dust source. In three models, the mean of the natural log-transformed endotoxin air levels was used as the dependent variable and either a linear or quadratic model was fit to the independent variable, or a linear model was fit to a natural log-transformation of the independent variable. In the other three models, conditions were the same as described, except the dependent variable was not log-transformed. The correlation between endotoxin levels and 3-OH FA concentrations in seat and in carpet dust was evaluated for each carbon chain length using Pearson's and Spearman's correlation coefficients.

Results

Thirty-two air samples were collected on four flights. A valid estimate of the endotoxin level could not be obtained for four samples due to possible assay interferences. Endotoxin levels for the remaining 28 samples were above the limit of detection (LOD = 0.19 EU/filter or 0.12 EU/m³ for a mean flight time of 473 min, mean flight pressure of 656 mmHg, and a mean sampling rate of 4 l/min). The geometric mean (GM) and geometric standard deviation (GSD) were 1.5 EU/m³ and 1.9, respectively ($n = 28$, range 0.53–5.3 EU/m³). All field blanks were below the LOD. Endotoxin levels on flight 33 were significantly higher than on flight 30, and endotoxin levels on flight 32 were significantly higher than on flight 31 (Table 1). Type of aircraft, zone, and the aircraft by zone interaction were not significant ($P = 0.93, 0.35, 0.70$, respectively).

Sixty-four dust samples were collected on four flights ($n = 32$ each for carpets and seats). Total dust mass harvested was obtained for 58 samples (29 each for

Table 1 Arithmetic and geometric means and standard deviations for endotoxin levels in air by flight within aircraft type

Aircraft	Flight	<i>n</i>	AM (EU/m ³)	SD (EU/m ³)	GM (EU/m ³)	GSD	Range (EU/m ³)
A-320	30	8	1.3	0.43	1.2 ^a	1.4	0.91–2.1
	33	5	2.3	0.98	2.1 ^a	1.6	0.97–3.7
747-200	31	8	0.91	0.32	0.86 ^b	1.5	0.53–1.3
	32	7	3.6	31.4	3.3 ^b	1.6	1.6–5.3

^a $P < 0.05$; ^b $P < 0.001$. Values with the same letter are significantly different. AM, arithmetic mean; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

carpets and seats). The mean (\pm SD) amount of dust harvested from carpets and seats was 447 ± 93.7 (range 281–624) and 423 ± 140 (range 132–662) mg/sample, respectively. These means did not vary significantly by zone within the aircraft ($P > 0.8$) or by dust source, i.e. carpet or seat ($P > 0.4$).

All 64 dust samples had detectable levels of endotoxin (LOD = 0.01 EU/mg dust). Mean (\pm SD) endotoxin levels in carpet and in chair dust samples were 140 ± 81 (range 3.8–420, $n = 32$) and 51 ± 25 (range 2.3–142, $n = 32$) EU/mg dust, respectively; the GMs were 120 (GSD = 2.3) and 44 (GSD = 1.9) EU/mg dust, respectively. The mean endotoxin level for carpet dust was significantly higher than for seat dust ($P < 0.001$). Aircraft type, flight within aircraft type, and zone were not significant ($P = 0.93, 0.46,$ and 0.39 , respectively). A statistically significant linear, quadratic, or exponential relationship between airborne endotoxin levels and endotoxin levels in either carpet or seat dust was not found in any of the regression models (data not shown).

Detectable levels of 3-OH FAs of each carbon chain length were also found in all 64 dust samples (LOD = ~ 10 ng LPS/sample). The source (carpet or seat) by carbon chain length interaction was significant ($P < 0.001$) (Table 2). Mean 3-OH FA levels were significantly higher in carpet dust than in seat dust for 3-OH-C_{10:0}, 3-OH-C_{12:0}, and 3-OH-C_{14:0} ($P < 0.001$ for each). The level of 3-OH-C_{16:0}, however, was significantly higher in seat than in carpet dust

Table 2 Arithmetic means and standard deviations for 3-OH FA concentrations^a in dust by source and carbon chain length ($n = 32$)

Chain length	Dust source					
	Carpet (pmol LPS/mg dust)			Seat (pmol LPS/mg dust)		
	AM	SD	Range	AM	SD	Range
C _{10:0}	2.0 ^b	0.91	0.62–3.9	0.80 ^b	0.47	0.075–1.7
C _{12:0}	4.8 ^c	1.4	2.1–8.9	2.2 ^c	0.45	1.1–3.4
C _{14:0}	6.5 ^d	2.0	3.0–13	3.2 ^d	0.71	2.2–5.0
C _{16:0}	6.1 ^e	1.2	2.8–9.0	6.6 ^e	1.4	4.5–11
C _{18:0}	3.7	0.89	2.1–6.0	4.0	1.2	1.4–7.8

^a 3-OH FA concentrations in pmol/mg dust divided by 4 to give moles of LPS. ^{b–d} $P < 0.001$; ^e $P < 0.01$. Values with the same letter are significantly different. AM, arithmetic mean; SD, standard deviation.

($P < 0.01$). When carpet and seat samples were analyzed separately, two interaction terms were significant in each model; in the carpet dust model, the chain length by flight within aircraft interaction ($P < 0.001$) and the aircraft by chain length by zone ($P = 0.034$) interaction were significant (Tables 3 and 4); in the seat dust model, the chain length by flight within aircraft interaction ($P = 0.032$) and the chain length by zone interaction ($P = 0.032$) were significant (Tables 4 and 5).

Endotoxin potency estimates of carpet and seat dust in EU/pmol LPS were determined by dividing the endotoxin level in EU/mg dust by the total estimated LPS concentration in pmol/mg dust (sum of 3-OH-C_{10:0} through 3-OH-C_{18:0}) for each sample. Carpet and seat dust mean (\pm SD) endotoxin potency estimates were 6.3 ± 3.0 ($n = 32$, range 0.18–15) and 3.0 ± 1.4 ($n = 32$, range 0.16–7.3) EU/pmol LPS, respectively. The mean potency estimate for carpet dust was significantly higher than for seat dust ($P < 0.0001$).

Carpet dust endotoxin levels were significantly correlated with 3-OH-C_{12:0} and 3-OH-C_{14:0} (Pearson $r = 0.52$ and 0.48 , respectively; $P < 0.01$ and $n = 32$ for both). Seat dust endotoxin levels were significantly correlated with 3-OH-C_{14:0} and 3-OH-C_{16:0} (Pearson $r = 0.42$ and 0.46 , respectively; $P < 0.05$ and $P < 0.01$, respectively; $n = 32$ for both). Spearman correlations gave similar results for carpet dust; however, all correlations for seat dust were not significant when the Spearman statistic was used, most likely because the Pearson statistic was strongly influenced by an observation that had the highest value for endotoxin, 3-OH-C_{14:0} and 3-OH-C_{16:0}.

GC-MSMS offered considerably improved detection specificity in comparison with GC-MS used in the SIM mode (Figure 1). The two lowest tracings show in detail the time interval around 3-OH_{16:0} using SIM; ions of mass-to-charge ratio (m/z) 343 and m/z 175 are monitored. It is not possible to accurately quantify this acid (using m/z 175) due to the presence of a partly co-eluting compound that forms ions of the same mass-to-charge ratio, i.e. m/z 175. By contrast, this disturbing compound was not registered when using GC-MSMS, which is, therefore, preferred for determination of LPS 3-OH FA in environmental samples.

Discussion

The air and dust of commercial aircraft, like that of other indoor environments such as homes, offices, and schools, contains readily detectable levels of endotoxin. Endotoxin results can vary across laboratories depending on extraction protocol, source of sample, filter media, and LAL lot (Chun et al., 2000). Therefore, comparisons across laboratories should be interpreted with caution. In the comparisons below, we have noted

Table 3 Arithmetic means and standard deviations for 3-OH FA concentrations in carpet dust by aircraft, zone, and carbon chain length ($n = 4$), units = pmol LPS/mg dust

Chain length	Aircraft 747-200; zone, AM (SD)				Aircraft A-320; zone, AM (SD)			
	A	B	C	D	A	B	C	D
C _{10:0}	2.4 (0.80)	1.9 (0.94)	2.8 (1.3)	1.9 (0.80)	1.7 (0.93)	1.4 (0.82)	2.0 (1.0)	1.7 (0.55)
C _{12:0}	5.5 (1.6)	5.4 ^a (0.66)	6.6 ^{a,b} (1.1)	4.5 ^b (1.1)	4.0 (1.6)	3.7 (0.81)	4.6 (1.5)	4.2 (0.69)
C _{14:0}	6.2 ^c (1.6)	6.7 (1.4)	9.4 ^{c,d,e} (2.9)	6.0 ^e (1.2)	5.9 (2.4)	5.1 ^{f,g} (1.4)	6.5 ^f (1.5)	6.4 ^g (1.0)
C _{16:0}	6.8 (2.0)	6.9 (1.0)	6.4 (0.47)	6.3 (1.1)	5.3 (1.3)	5.0 ^h (1.5)	6.2 ^h (0.79)	5.7 (0.63)
C _{18:0}	3.9 (1.7)	3.6 (0.55)	3.3 (0.68)	3.6 (0.85)	3.5 (1.0)	3.5 (1.0)	3.9 (0.90)	3.8 (0.59)

a,f-h $P < 0.05$; b $P < 0.001$; c-e $P < 0.0001$. Values with the same letter are significantly different. AM, arithmetic mean; SD, standard deviation.

Table 4 Arithmetic means and standard deviations for 3-OH FA concentrations in carpet and seat dust by carbon chain length and flight within aircraft ($n = 8$), units = pmol LPS/mg dust

Chain length	Aircraft 747-200; flight, AM (SD)		Aircraft 747-200; flight, AM (SD)	
	31	32	30	33
Source: carpet				
C _{10:0}	1.9 (1.1)	2.6 (0.77)	1.6 (0.68)	1.8 (0.89)
C _{12:0}	5.2 (1.3)	5.8 (1.3)	4.3 (0.72)	3.9 (1.5)
C _{14:0}	7.7 ^a (2.7)	6.4 ^a (1.5)	6.8 ^b (0.87)	5.1 ^b (1.7)
C _{16:0}	6.6 (1.5)	6.7 (0.83)	5.8 (0.64)	5.3 (1.4)
C _{18:0}	3.6 (1.3)	3.7 (0.61)	4.2 ^c (0.45)	3.3 ^c (0.91)
Source: seat				
C _{10:0}	0.68 (0.46)	0.81 (0.54)	0.59 (0.29)	1.1 (0.46)
C _{12:0}	2.3 (0.45)	2.3 (0.66)	1.9 (0.16)	2.2 (0.32)
C _{14:0}	3.0 (0.69)	3.3 (0.79)	3.0 (0.70)	3.4 (0.75)
C _{16:0}	6.0 ^d (1.0)	7.1 ^d (1.4)	6.1 ^e (0.69)	7.4 ^e (1.9)
C _{18:0}	13 ^f (1.1)	4.6 ^f (1.5)	3.6 ^g (0.56)	4.4 ^g (0.81)

a,d $P < 0.01$; b $P < 0.0001$; c,g $P < 0.05$; e-f $P < 0.001$. Values with the same letter are significantly different. AM, arithmetic mean; SD, standard deviation.

Table 5 Arithmetic means and standard deviations for 3-OH FA concentrations in seat dust by zone and carbon length ($n = 4$), units = pmol LPS/mg dust

Chain length	Zone, AM (SD)			
	A	B	C	D
C _{10:0}	1.0 (0.50)	0.70 (0.40)	0.77 (0.63)	0.71 (0.32)
C _{12:0}	2.4 (0.46)	2.1 (0.27)	2.0 (0.49)	2.3 (0.52)
C _{14:0}	3.8 ^{a,b} (0.81)	2.8 ^a (0.39)	2.9 ^b (0.42)	3.3 (0.75)
C _{16:0}	7.2 ^{c,d} (1.9)	6.3 ^{c,e} (0.99)	6.0 ^{d,f} (1.1)	7.1 ^{e,f} (1.3)
C _{18:0}	3.8 ^g (0.65)	4.0 ^h (0.91)	3.4 ⁱ (1.2)	4.7 ^{g,h,i} (1.5)

a-c,g $P < 0.01$; e,h $P < 0.05$; d,f $P < 0.001$; i $P < 0.0001$. Values with the same letter are significantly different. AM, arithmetic mean; SD, standard deviation.

where laboratories were the same and where they were different.

The GM endotoxin air level in the aircraft cabins was approximately six-fold higher than that reported in a single office building (Hines et al., 2000) and approximately two-fold higher than the GM level reported in homes (Park et al., 2000). The foregoing studies can be directly compared as they used the same laboratory

and method, and adjusted for the effects of variation in LAL lot sensitivity to environmental endotoxin in house dust. The GM endotoxin air level in the aircraft was also higher (1.1–3-fold) than in five of six office buildings studied by Reynolds et al. (2001) (different laboratory).

The mean endotoxin level in aircraft cabin carpet dust was approximately two-fold higher than the mean level found in carpet dust from a single office building (same laboratory) (Hines et al., 2000) and three orders of magnitude higher than levels found in carpet or floor dust from office buildings in two other studies (different laboratories) (Gyntelberg et al., 1994; Mølhav et al., 2000). By contrast, the mean endotoxin level in aircraft cabin seat dust was only slightly higher than the mean level reported for office building chair dust (same laboratory) (Hines et al., 2000). When compared to dust from homes, the mean endotoxin level in aircraft carpet dust was slightly higher (less than two-fold) than levels in bedroom and kitchen floor dust, and the mean endotoxin level in aircraft seat dust was comparable to levels in family room and bed dust (Park et al., 2000, 2001) (same laboratory). Compared to studies using different laboratories, aircraft carpet dust endotoxin levels were usually higher (2–16-fold) (Bischof et al., 2002; Douwes et al., 1998; Michel, 1996; von Mutius et al., 2000; Rizzo et al., 1997; Thorne et al., 2000) and sometimes lower (Gereda et al., 2000; von Mutius et al., 2000) than levels in floor or carpet dust from homes. Endotoxin levels in aircraft seat dust were higher (2–16-fold) than reported endotoxin levels in home mattress dust (Douwes et al., 1998; Michel et al., 1996).

In previous studies reporting endotoxin results separately for floors and furnishings, mean endotoxin levels in floor or carpet dust were higher than mean endotoxin level in beds (Park et al., 2000, 2001), office chairs (Hines et al., 2000), and mattresses (Douwes et al., 1998), except in Michel et al. (1996) where no significant difference was found between mean endotoxin levels in mattress and in bedroom floor dust. We similarly found a significantly higher mean endotoxin level in aircraft carpet dust than in seat dust.

As in this study, Park et al. (2000) did not find a significant association between endotoxin levels in air

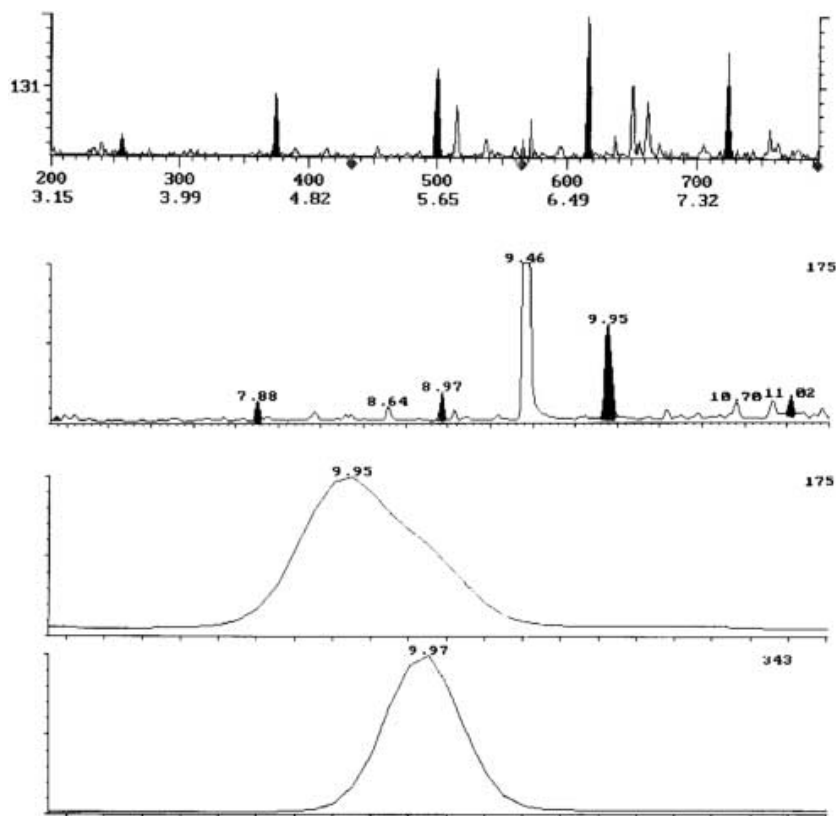


Fig. 1 Analysis of 3-hydroxy fatty acids of 10, 12, 14, 16, and 18 carbon chain lengths (blackened) in a dust sample by using GC-MSMS focusing at m/z 131 and GC-MS focusing at m/z 175 (upper two tracings); detail of the region of 3-hydroxyhexadecanoic acid in the same sample preparation achieved by using GC-MS focusing at m/z 175 and m/z 343 (lower two tracings). Abscissa: minutes; ordinate: relative ion intensity

and endotoxin levels in bed, bedroom floor, or kitchen floor dust. Similarly, air and dust levels of muramic acid, a marker for bacterial peptidoglycan, were not correlated in schools (Liu et al., 2000). Thus, settled dust levels of bacterial endotoxin and peptidoglycan may not be good predictors of airborne exposure, although they may be reasonable exposure markers if brief exposure to puffs of material pumped out of seats or resuspended from carpets is important.

Mean endotoxin potency estimates for aircraft cabin seat and carpet dust (3.0 and 6.3 EU/pmol LPS, respectively) were approximately 6–7-fold higher than mean endotoxin potency estimates for office building chair and carpet dust (0.50 and 0.88 EU/pmol LPS, respectively) (Hines et al., 2000), and 2–4-fold higher than for house dust (~1.4 EU/pmol LPS) (Saraf et al., 1997). Moreover, the mean carpet dust endotoxin potency estimate was approximately two-fold higher than the potency estimate for seat dust in both the aircraft cabins and in the office building (Hines et al., 2000). Higher endotoxin levels and potency estimates for carpet dust as compared to seat dust in both the aircraft cabins and in the office building suggests that these two dust reservoirs have different bacterial populations.

The explanation for the relatively high endotoxin potency estimates in the aircraft cabins as compared to other indoor environments becomes clearer by examining differences in 3-OH FA levels in these environments. In general, mean levels of individual 3-OH FAs in both aircraft carpet and seat dust were several folds lower than mean levels reported for office building carpet and chair dust (Hines et al., 2000). In addition, the range of total estimated LPS in the aircraft carpet and seat dust, computed as the sum of the LPS for C_{10} – C_{18} , (12–33 and 11–25 pmol LPS/mg dust, respectively) was much lower than for house dust (7.4–175 pmol LPS/mg dust) (Saraf et al., 1997) and for office building carpet and seat dust (53–81 and 68–92 pmol LPS/mg dust, respectively) (Hines, unpublished data). The combination of relatively high endotoxin levels and relatively low total LPS produced higher endotoxin potency estimates for aircraft dust as compared to the office building or house dust.

The 3-OH FA profile for aircraft cabin carpet and seat dust was different than that found in an office building study (Hines et al., 2000). Office building carpet and chair dust had significantly different levels of $C_{10:0}$, $C_{12:0}$, $C_{16:0}$, and $C_{18:0}$ fatty acids, while the aircraft cabin carpet and seat dust had significantly

different levels of C_{10:0}, C_{12:0}, C_{14:0}, and C_{16:0}. The predominant 3-OH FAs in the office building carpet and chair dust were C_{16:0} and C_{18:0} (62 and 78% of total, respectively) (Hines, unpublished data), whereas in aircraft carpet dust, C_{14:0} and C_{16:0} comprised 55% of the mean total 3-OH FAs (75% if C_{12:0} included), and C_{16:0} and C_{18:0} predominated in aircraft seat dust (63% of total). In air samples collected from schools (Liu et al., 2000), C_{16:0} and C_{18:0} were the predominating 3-OH FAs (73% of total). These studies suggest that C_{16:0} and C_{18:0} may be the predominating 3-OH FAs in some, but not all, indoor reservoirs.

The correlation between endotoxin level and 3-OH FA concentrations varies by type of indoor environment. Endotoxin in house dust correlated best with carbon chain lengths C_{10:0}, C_{12:0}, and C_{14:0} (Spearman $r = 0.65$, $r = 0.56$, $r = 0.65$, Saraf et al., 1999; Pearson $r = 0.89$, $r = 0.90$, $r = 0.77$, Saraf et al., 1997), while endotoxin in carpet and seat dust from an office building was most positively correlated with C_{12:0} (Pearson $r = 0.61$) and most negatively correlated with C_{16:0} (Pearson $r = 0.67$) (Hines et al., 2000). In the current study, endotoxin in aircraft carpet dust correlated best with C_{12:0} followed by C_{14:0} (Pearson $r = 0.52$ and 0.48 , respectively); and endotoxin in aircraft seat dust correlated best with C_{16:0} followed by C_{14:0} (Pearson $r = 0.46$ and 0.42 , respectively). In summary, endotoxin in dust collected from homes, an office building, and aircraft cabins seems to correlate most consistently with 3-OH FA C_{12:0} and 3-OH FA C_{14:0}.

The reasons for the generally higher endotoxin levels in the air and dust of aircraft cabins as compared to homes and offices are not entirely clear. Aircraft cabins have a higher density of human occupancy per unit volume and per unit area than either office buildings or homes. Daily duration of occupancy in aircraft cabins (i.e. 16–18 h/day) is usually greater than most office buildings (i.e. 8–10 h/day), yet comparable to homes that are occupied most of the day (i.e. 16–18 h/day). Carpets and seats in aircraft cabins are not routinely vacuum cleaned. Duration of occupancy in apartments by the same individual and infrequent vacuum cleaning have been shown to be significant predictors of endotoxin in living room floor/carpet dust (Bischof et al., 2002). Absolute humidity in homes has been significantly associated with increased airborne endotoxin levels (Park et al., 2000); however, neither absolute humidity nor relative humidity has been associated with endotoxin levels in settled dust (Bischof et al., 2002; Douwes et al., 1998; Park et al., 2000). In our study, relative humidity (which averaged 12–20% on the four flights) was confounded with aircraft type and its effect could not be independently evaluated. Temperature in homes has not been significantly associated with either airborne or dust endotoxin levels (Bischof et al., 2002; Douwes et al., 1998; Park et al., 2000). Differences in hygienic conditions among

aircraft cabins, homes, and offices may partly explain differences in endotoxin levels across studies.

Another possible explanation for higher endotoxin levels in aircraft cabins as compared to homes and offices is that the relative proportions of C_{12:0} and C_{14:0} 3-OH FAs were greater in aircraft cabin dust than in home or office dust (Saraf et al., 1997; Hines, unpublished data). Previous investigation of the endotoxin potencies of several bacterial species (Saraf et al., 1997) suggests that higher endotoxin potencies are associated with the presence of C_{12:0} or C_{14:0} 3-OH FAs. Thus, the types of bacteria and, therefore, the type of LPS present, may simply be different and more potent in the aircraft cabins.

Except for certain chain lengths of 3-OH FA in carpet dust, differences in endotoxin and 3-OH FA levels between the two aircraft types were not found. The limited number of flights sampled and treating flight as a random effect reduced the power to detect differences. Significant spatial variability, i.e. differences between zones, was found only for some 3-OH FAs in carpet and seat dust, but not for endotoxin in either air or dust. The lack of spatial variability in airborne endotoxin levels suggests that the air was well mixed and that highly localized sources of airborne endotoxin were not present. The absence of spatial variability for endotoxin in either seat or carpet dust, and the limited spatial variability observed for certain 3-OH FAs, suggests that the influence of any exposure-modifying factors was similar throughout the coach section.

In summary, differences in endotoxin characteristics by dust source indicate the importance of considering possible exposure pathways when designing sampling strategies. The absence of a correlation between endotoxin in air and endotoxin in indoor surface dust, both in this study and in Park et al. (2000), suggests that different factors may contribute to endotoxin levels in air than in dust. Epidemiological studies that include both air- and source-specific dust measures of exposure are needed to understand the mechanisms of endotoxin exposure in indoor environments as they relate to health outcomes.

Acknowledgments

We gratefully acknowledge the assistance of Thomas F. Bloom, Donald E. Booher, Kevin H. Dunn, Timothy E. Jiggins, Daryl Johnson, Jun Ju, Xiudong Lei, and Mimi Nguyen during this study. We especially thank Drs James Whinnery, Noal May, and Jerry Hordinsky of the Civil Aeromedical Institute, US Federal Aviation Administration (CAMI-FAA), the airline companies, the flight crews, and the Swedish Council for Building Research for their support and assistance. This study was funded in part by the CAMI-FAA, Interagency Agreement DTI7A0197 F80353.

References

- ATA (2001) Annual report of the Air Transport Association. Washington, DC.
- Bischof, W., Koch, A., Gehring, U., Falilbusch, B., Wichmann, H.E. and Heinrich, J. (2002) Predictors of high endotoxin concentrations in the settled dust of German homes. *Indoor Air*, **12**, 2–9.
- Chun, D.T.W., Chew, V., Bartlett, K., Gordon, T., Jacobs, R.R., Larsson, B., Lewis, D.M., Liesivuori, J., Michel, O., Milton, D.X., Rylander, R., Thorne, P.S., White, E.M. and Brown, M.E. (2000) Preliminary report on the results of the second phase of a round-robin endotoxin assay study using cotton dust. *Appl. Occup. Environ. Hyg.*, **15**, 152–157.
- Douwes, J., Doekes, G., Heinrich, J., Koch, A., Bischof, W. and Brunekreef, B. (1998) Endotoxin and $\beta(1-3)$ -glucan in house dust and the relation with home characteristics: a pilot study in 25 German houses. *Indoor Air*, **8**, 255–263.
- Gereda, J.E., Leung, D.Y.M., Thatayatikom, A., Streib, J.E., Price, M.R., Klinnert, M.D. and Liu, A.H. (2000) Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet*, **355**, 1680–1683.
- Gyntelberg, F., Suadicani, P., Wohlfahrt, N.J., Skov, P., Valbjørn, O., Nielsen, P.A., Schneider, T., Jørgensen, O., Wolkoff, P., Wilkins, C.K., Gravesen, S. and Norn, S. (1994) Dust and the sick building syndrome. *Indoor Air*, **4**, 223–238.
- Hines, C.J., Milton, D.K., Larsson, L., Petersen, M.R., Fisk, W.J. and Mendell, M.J. (2000) Characterization and variability of endotoxin and 3-hydroxy fatty acids in an office building during a particle intervention study. *Indoor Air*, **10**, 2–12.
- Jacobs, R.R. (1997) Endotoxins in the environment: a criteria document. *Int. J. Occup. Environ. Health*, **3** (Suppl. 1), S3–S5.
- Liu, L.J.S., Krahmer, M., Fox, A., Feigley, C.E., Featherstone, A., Saraf, A. and Larsson, L. (2000) Investigation of the concentration of bacteria and their cell envelope components in indoor air in two elementary schools. *J. Air Waste Manage. Assoc.*, **50**, 1957–1967.
- Michel, O., Ginanni, R., Duchateau, J., Vertongen, F., LeBon, B. and Sergysels, R. (1991) Domestic endotoxin exposure and clinical severity of asthma. *Clin. Exp. Allergy*, **21**, 441–448.
- Michel, O., Kips, J., Duchateau, J., Vertongen, F., Robert, L., Collet, H., Pauwels, R. and Sergysels, R. (1996) Severity of asthma is related to endotoxin in house dust. *Am. J. Respir. Crit. Care Med.*, **154**, 1641–1646.
- Milton, D.K., Feldman, H.A., Neuberg, D.S., Bruckner, R.J. and Greaves, I.A. (1992) Environmental endotoxin measurement: the kinetic *Limulus* assay with resistant-parallel-line estimation. *Environ. Res.*, **57**, 212–230.
- Milton, D.K., Johnson, D.K. and Park, J.-H. (1997) Environmental endotoxin measurement: interference and sources of variation in the *Limulus* assay of house dust. *Am. Ind. Hyg. Assoc. J.*, **58**, 861–867.
- Mølhave, L., Schneider, T., Kjægaard, S.K., Larsen, L., Norn, S. and Jørgensen, O. (2000) House dust in seven Danish offices. *Atmos. Environ.*, **34**, 4767–4779.
- von Mutinus E., Braun-Fahrlander, C., Schierl, R., Riedler, J., Ehlerman, S., Maisch, S., Waser, M. and Nowak, D. (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin. Exp. Allergy*, **30**, 1230–1234.
- Myatt, T.A. and Milton, D.K. (2001) Endotoxins. In: Spengler, J.D., Samet, J.M. and McCarthy, J.F. (eds) *Indoor Air Quality Handbook*. McGraw-Hill, New York, pp. 42.1–42.14.
- NRC (National Research Council) (2001) Biological agents. In: *The Airliner Cabin Environment and Health of Passengers and Crew*. National Academy Press, Washington DC, pp. 131–181.
- Park, J.-H., Spiegelman, D.L., Burge, H.A., Gold, D.R., Chew, G.L. and Milton, D.K. (2000) Longitudinal study of dust and airborne endotoxin in the home. *Environ. Health Perspect.*, **108**, 1023–1028.
- Park, J.-H., Gold, D.R., Spiegelman, D.L., Burge, H.A. and Milton, D.K. (2001) House dust endotoxin and wheeze in the first year of life. *Am. J. Respir. Crit. Care Med.*, **163**, 322–328.
- Reynolds, S.J., Black, D.W., Borin, S.S., Breuer, G., Burmeister, L.F., Fuortes, L.J., Smith, T.F., Stein, M.A., Subramanian, P., Thorne, P.S. and Whitten, P. (2001) Indoor environmental quality in six commercial office buildings in the Midwest United States. *Appl. Occup. Environ. Hygiene*, **16**, 1065–1077.
- Rietschel, E.T., Wollenweber, H.W., Brade, H., Zähringer, U., Lindner, B., Seydel, U., Bradaczek, H., Barnickel, G., Labischinski, H. and Giesbrecht, P. (1984) Structure and conformation of the lipid A component of lipopolysaccharides. In: Rietschel, E.T. (ed.) *Handbook of Endotoxin*, Vol. 1. Elsevier Science, Amsterdam, pp. 187–220.
- Rizzo, M.C., Naspitz, C.K., Fernández-Caldas, E., Lockey, R.F., Mimica, I. and Solé, D. (1997) Endotoxin exposure and symptoms in asthmatic children. *Pediatr. Allergy Immunol.*, **8**, 121–126.
- Rylander, R., Sörensen, S., Goto, H., Kazumi, Y. and Tanaka, S. (1989) The importance of endotoxin and glucan for symptoms in sick buildings. In: Bieva, C.J., Coutois, M. and Govaerts, M. (eds) *The Present and Future of Indoor Air Quality*. Elsevier Science, Amsterdam, pp. 219–226.
- Rylander, R., Persson, K., Goto, H., Yuasa, K. and Tanaka, S. (1992) Airborne beta-1,3-glucan may be related to symptoms in sick buildings. *Indoor Environ.*, **1**, 263–267.
- Saraf, A., Larsson, L., Burge, H. and Milton, D. (1997) Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography–mass spectrometry: comparison with fungal culture and determination of endotoxin by a *Limulus* amoebocyte lysate assay. *Appl. Environ. Microbiol.*, **63**, 2554–2559.
- Saraf, A., Park, J.-H., Milton, D.K. and Larsson, L. (1999) Use of quadrupole GC–MS and ion trap GC–MS–MS for determining 3-hydroxy fatty acids in settled dust: relation to endotoxin activity. *J. Environ. Monit.*, **1**, 163–168.
- Sonesson, H.R.A., Zähringer, U., Grimmecke, H.D., Westphal, O. and Rietschel, E.T. (1994) Bacterial endotoxin: chemical structure and biological activity. In: Brigham, K. (ed.) *Endotoxin and the Lungs*. Marcel Dekker, New York, pp. 1–20.
- Teeuw, K.B., Vandenbroucke-Grauls, C.M.J.E. and Verhoef, J. (1994) Airborne gram-negative bacteria and endotoxin in sick building syndrome. *Arch. Inter. Med.*, **154**, 2339–2345.
- Thorne, P.S., Kulhankova, K., O'Neill, M.E., Vojta, P.J., Friedman, W. and Zeldin, D.C. (2000) A national survey of endotoxin in house dust. *Am. J. Respir. Crit. Care Med.*, **160** (Suppl. A), 908.
- Waters, M. and Bloom, T. (1997) Evaluation of cosmic radiation and cabin environmental exposures of flight attendants: study protocol. Division of Surveillance, Hazard Evaluation, and Field Studies, NIOSH, Cincinnati, OH, USA.
- Walters, M., Milton, D., Larsson, L. and Ford, T. (1994) Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques. *Appl. Environ. Microbiol.*, **60**, 996–1005.
- Wilkinson, S.G. (1988) Gram-negative bacteria. In: Ratledge, C. and Wilkinson, S.G. (eds) *Microbial Lipids*, Vol. 1. Academic Press, New York, pp. 299–488.