Indoor Air 2006; 16: 216–226
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INDOOR AIR
doi:10.1111/j.1600-0668.2005.00419.x

Particle size distributions and concentrations of airborne endotoxin using novel collection methods in homes during the winter and summer seasons

Abstract A comparison study of novel collection methods for airborne bacteria and endotoxin was performed in an environmentally controlled chamber and in pilot-field studies. Airborne particulate matter was collected in swirling liquid impingers, air-monitoring filter cassettes, and with a micro-orifice uniform deposit impactor (MOUDI) to evaluate aerodynamic particle size distributions. Environmentally controlled chamber studies showed that impingers and MOUDI recovered significantly more airborne bacteria than filter cassettes, whereas collection methods for airborne endotoxin were not significantly different. In addition, total airborne bacteria and endotoxin concentrations were measured indoors and outdoors at three homes in Boulder, CO during winter and summer seasons. Indoor concentrations collected with the three different samplers were significantly different for airborne endotoxin, but not for airborne bacteria. Total airborne bacteria indoors and outdoors significantly varied with seasons. Outdoor airborne endotoxin significantly varied with season; no seasonal variation was seen for indoor airborne endotoxin. Indoor and outdoor levels were not significantly different for both airborne bacteria and endotoxin. The largest proportion of endotoxin was associated with airborne particulate matter <1 μm.

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Key words: Airborne bacteria; Airborne endotoxin; Collection methods; Particle size distributions.

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Received for review 28 June 2005. Accepted for publication 2 December 2005.
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Practical Implications

This study compared sampling methods for airborne endotoxin, a potent and nonspecific immune system stimulant which can induce negative health responses. The data from this study showed that swirling liquid impingers and the micro-orifice uniform deposit impactor (MOUDI) recovered significantly more airborne endotoxin than the more widely adapted method of collecting airborne endotoxin on membrane filters, when collection methods were applied in realistic settings (homes). The MOUDI measured the particle size distribution of airborne endotoxin, which can be useful for determining endotoxin respiratory toxicity and its health effects.

Introduction

Endotoxin is a measure of the biologic activity of lipopolysaccharide, which is a component of the outer membrane of gram-negative bacteria. Endotoxin is increasingly recognized as a potent and nonspecific immune system stimulant, which can induce negative health responses (Martich et al., 1993; Ning et al., 2000; Olenchock, 1994). Low-level exposures, only slightly elevated above outdoor background levels, have been correlated to severe asthma attacks and sick building syndrome symptoms (Gyntelberg et al., 1994; Loppnow et al., 1990; Michel et al., 1991). As endotoxin recovery can be significantly influenced by collection method (Duchaine et al., 2001; Thorne et al., 1997; Walters et al., 1994; Zucker et al., 2000), extraction method, and is sensitive to analytical methods (yet to be standardized), care should be exercised when comparing the absolute levels reported in the literature in the context of exposure (Douwes et al., 1995; Reynolds et al., 2002; Thorne et al., 1997).

The main source of endotoxin is gram-negative bacteria. Several studies have reported total airborne bacteria concentrations in either indoor or outdoor environments (Bjornsson et al., 1995; Fabian et al.,

2005; Mochandreas et al., 2003; Toivola et al., 2002; Walters et al., 1994).

Endotoxin levels have been studied extensively as an occupational hazard in agriculture (Attwood et al., 1986; Donham et al., 1989; Duchaine et al., 2001; Zucker and Muller, 1998; Zucker et al., 2000), machining (Thorne et al., 1996), woodworking (Alwis et al., 1999; Dennekemp et al., 1999), and in office (Hines et al., 2000) buildings. Recent reports also document estimates of airborne endotoxin levels in non-occupational settings such as homes (Park et al., 2000, 2001), and in outdoor air (Heinrich et al., 2003). In general, occupational settings have much higher airborne endotoxin levels than homes, and in outdoor air, airborne endotoxin levels are typically lower than those reported indoors (Heinrich et al., 2003; Hines et al., 2000).

Few studies have attempted to optimize sampling methods for airborne endotoxin. In many studies related to health effects of endotoxin, samples are usually only collected from a reservoir such as settled house dust (Douwes et al., 1998; Long et al., 2001; Su et al., 2001). This method may inaccurately represent the quantity of allergens and pyrogens entering the lungs (Platts-Mills et al., 1995). In recent studies that have targeted airborne endotoxin, the most widely adapted practice is to collect known quantities of air through membrane filters, although standard AGI-30 liquid impingers (Ace Glass Inc., Vineland, NJ, USA) have been used and appear to be more efficient when compared with other particle samplers (Duchaine et al., 2001; Zucker et al., 2000).

Establishing the approximate aerodynamic particle size distribution for airborne endotoxin is an important element in determining endotoxin toxicity and its health effects. Previous studies on the particle size distribution of airborne endotoxin in agricultural environments (Attwood et al., 1986; Gordon et al., 1992; Olenchock et al., 1983) and outdoor air (Monn and Becker, 1999) showed that airborne endotoxin was associated with the airborne particulate matter $>1\ \mu$m.

This study was motivated by the need for a standard sampling method for airborne endotoxin. The objectives of this study were to demonstrate feasibility of liquid impingers and the micro-orifice uniform deposit impactor (MOUDI) for airborne endotoxin collection in an environmentally controlled environment, to compare these methods to the filter cassette sampling method, and then to validate if these sampling methods perform successfully when applied in more variable realistic conditions. An additional goal of the study was to show that the MOUDI was feasible for determining an endotoxin particle size distribution. In summary, this research evaluated the effects of sampler type, sampling in homes compared with an environmentally controlled chamber, seasonal variations, and indoor/outdoor sampling on airborne bacteria and endotoxin levels.

Materials and methods

Optimizing sampling methods for airborne bacteria and endotoxin in environmentally controlled chamber studies

During the first part of this study, gram-negative bacterial aerosol was generated in a test chamber to (i) compare recovery of collection methods; (ii) optimize an analytical protocol; and (iii) correlate total airborne bacteria concentration with airborne endotoxin levels.

An environmentally controlled 87-m^3 test chamber established at the University of Colorado at Boulder, as previously described (Kreider and Brandemuehl, 1991; Xu et al., 2003), was used for this part of the study. The test chamber has a natural infiltration rate of 0.1–0.3 air changes per hour (ACH or h^{-1}). All the experiments in the test chamber were performed at a temperature of 20–25°C and at a relative humidity of 20–30%.

Test bioaerosol Serratia marcescens (American Type Culture Collection 13880) and *Escherichia coli* (K-12) were aerosolized for the chamber experiments. *Serratia marcescens* is a gram-negative, non-motile rod-shaped bacterium with pigment that is influenced by culture conditions (Grimont and Grimont, 1984). *Escherichia coli* is a straight rod-shaped gram-negative bacterium that usually occurs in single or paired cells forming beige and opaque colonies on agar plates.

Both strains were grown on Soybean-Casein Digest Agar (Difco Laboratories, Detroit, MI, USA) at 37°C. Cultures were removed from the agar plate surface by scraping and suspending in pyrogen-free water (Cambrex Bio Science Inc., Walkersville, MD, USA). The extracellular polysaccharides of these pure cultures have been documented to have significant pyrogenic activity due to their endotoxin content (Fukushi et al., 1964). These microorganisms are environmentally sensitive and do not survive long upon becoming airborne regardless of relative humidity (Peccia, 2000). Two experiments with each test bioaerosol were performed, and denoted as test 1 and test 2 in this paper.

Bioaerosol generation The test bioaerosol was generated using a six-jet Collison nebulizer (CN 25; BGI Inc., Waltham, MA, USA). The nebulizer was operated at 138 kPa generated by a compressed air cylinder with an air supply system that includes a dehumidifier, a HEPA filter, and a regulator (Model 3074; TSI Inc., Shoreview, MN, USA). The volumetric flow rate of the suspension leaving the nebulizer was 0.30–0.42 ml/min. The test aerosol was released approximately 1.5 m above the floor. Bioaerosol was generated continuously for 60 min. No ventilation was provided during this period, and two box fans (48 cm diameter, Model 3723;

Lasko Inc., West Chester, PA, USA) were used to ensure complete mixing within the test room. Bioaerosol was sampled during the last 30 min of generation.

Bioaerosol collection The following sampling methods were employed for collecting airborne bacteria and endotoxin: (i) a new generation of swirling liquid impingers (BioSampler, SKC Inc., Eighty-Four, PA, USA), (ii) three-piece polystyrene 37-mm air-monitoring filter cassettes (Pall Corp., Ann Arbor, MI, USA), and (iii) a MOUDI (Model 110; MSP Co., Minneapolis, MN, USA).

The sampling protocol used in this study has been previously described (Hernandez et al., 1999; Kujundzic et al., 2005; Xu et al., 2003). Airborne bacteria and endotoxin were sampled at one room location. Previous research showed that under the current test chamber configuration with mixing fans operating, the room is well mixed and the bioaerosol concentration within the room air can be represented by a few samples (Xu and Miller, 1999). Liquid impingers were positioned in the breathing zone, 1.6 m above the floor. Two swirling liquid impingers were operated simultaneously to collect duplicate air samples. In addition, bioaerosol was sampled using four filter cassettes (two in open- and two in closed-face configuration) with 0.8 μm pore size Isopore™ membrane filters (Millipore, Bedford, MA, USA) at a flow rate of 12.5 l/min. Filter cassettes were operated at the higher flow rate of 12.5 l/min (Heikkinen et al., 2005) to avoid detection limit problems. The criteria for negligible sampling bias due to particle settling in still air was met by the selected flow rate. Filter cassettes were located next to liquid impingers.

A MOUDI was used to collect particles as a function of their aerodynamic diameter. The MOUDI was placed on the table with the liquid impingers and filter cassettes. The impactor has 10 stages that collect size ranges as defined by the stage's 50% cut-off of aerodynamic diameter, d_{50}: 10 μm (M1); 5.6 μm (M2); 3.2 μm (M3); 1.8 μm (M4); 1.0 μm (M5); 0.56 μm (M6); 0.32 μm (M7); 0.18 μm (M8); 0.1 μm (M9); 0.056 μm (M10) (Marple et al., 1991). Prior to sampling, each stage on the MOUDI was cleaned with a 70% ethanol solution and pyrogen-free water. The impactor collected particles at a flow rate of 30 l/min onto 47-mm diameter Isopore™ membrane filters with a 0.8-μm pore size (Millipore). The after-filter was a 37-mm diameter fiberglass filter with a 5-μm pore size (Whatman International Ltd, Kent, UK).

Before each experiment, liquid impingers were washed with endotoxin-free detergent (E-Toxa-Clean®; Sigma Chemical Co., St Louis, MO, USA) and pyrogen-free water, and then heated at 180°C for 4 h. Each liquid impinger was then filled with 20 ml of pyrogen-free water. Room air was sampled into liquid impingers and filter cassettes via high-flow sampling pumps (Model 1023-1 01 Q; Gast Manufacturing Inc., Benton Harbor, MI, USA) regulated with rotameters (Gillmont Instruments Div. of Barnant Co., Barrington, IL, USA) that were located outside the test chamber, and connected to the liquid impingers and filter cassettes with tubing. Liquid impingers, filter cassettes and MOUDI flow rates were calibrated using a primary flow meter (Dry Cal®; DC-Lite, BIOS International Co., Butler, NJ, USA). All three samplers were operated at the same time.

Preparation of samples for bioaerosol quantification Liquid impinger contents were aseptically transferred to 50-ml polystyrene conical tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) and stored at 4°C until the next day, when they were analyzed for total bacteria by direct microscopy. Three milliliters of liquid impinger contents were transferred to cryogenic tubes (Nalge Nunc Brand Products, Rochester, NY, USA) and stored at −80°C until assayed for endotoxin content. Filter cassettes were stored at 4°C until the next day when airborne bacteria and endotoxin were eluted. In addition, filters from the MOUDI were kept in a storage container at 4°C until the next day when airborne bacteria and endotoxin were eluted. Filters from the MOUDI and filter cassettes were eluted with 10 ml of pyrogen-free water. In preliminary studies, filters samples were rotated for 1 h in a hybridization oven (Fisher Scientific, Hampton, NH, USA) at room temperature; the eluent was then subsequently centrifuged (GS-6; Beckman, Fullerton, CA, USA) for 10 min at 1100 g. The supernatant was then analyzed for its endotoxin content. As endotoxin recovery was lower when compared with those collected with liquid impingers, the experimental protocol was changed. Filters were eluted by sonication for 1 h in a sonication bath (Fisher Scientific) at room temperature, and then subsequently filtered through 25-mm syringe filter with 5 μm average pore size diameter (Pall Corp.). The samples were then stored in cryogenic tubes at −80°C until assayed for endotoxin content. Recovery was significantly higher using the sonication-based protocol; therefore, all endotoxin analysis employed this protocol.

Bioaerosol quantification Bioaerosol was quantified by direct microscopy and endotoxin content. Direct microscopy, using a sensitive biological stain 4′6-diamidino-2-phynyldole (DAPI) (Sigma Chemicals) in accordance with previously described methods (Hernandez et al., 1999), as modified by Peccia et al. (2001), was used to obtain total numbers of bacteria in the collected air samples. The collected air samples were not diluted before staining. Ten random microscopic fields were counted per slide, and only intact, brightly stained cells, with an obvious bacterial morphology, were counted. All counts were reported as the average of all microscopic fields counted.

Endotoxin potency is usually measured by determining the biological activity of a sample in a specified assay system. The widely accepted Limulus Amebocyte Lysate (LAL) assay was used in this study (Hines et al., 2000; Levin, 1987; Park et al., 2001; Reynolds et al., 2001). The enzyme signal was optimized by performing tests with different dilutions of the same sample to minimize inhibition. Endotoxin samples were vortexed for 3 min before being diluted, and again for 3 min before being dispersed in a 96-well assay plate (Corning Inc., Corning, NY, USA). The concentrations of endotoxin in samples were determined by standard reference (EC6, USP Reference Standard Endotoxin, 10 EU (endotoxin unit) = 1 ng) prepared for each assay in a linear range between 0.005 and 50 EU/ml. Endotoxin levels were analyzed using the Kinetic-QCL kit (Cambrex Bio Science Inc.) and El808 Ultra microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA) at 37°C, and color development was monitored every 40 s at the wavelength of 405 nm. Airborne endotoxin concentrations were reported in endotoxin units (EU) per cubic meter of air (EU/m^3).

Characterization of residential levels of total airborne bacteria, endotoxin and aerodynamic particle size distribution

Home selection Application of the previously tested samplers to home environments was conducted in three homes in Boulder, Colorado, USA. Air sampling was performed during the winter (February and March) and summer (July and August) of 2003. Seasonal variation of indoor total airborne bacteria and endotoxin levels were compared because during summer there are high pollen and allergens levels in outdoor air and in winter when residences have reduced air exchange with fresh outdoor air. All investigated homes had similar volumes (200–300 m^3), and similar home construction. The homes were unoccupied during the tests.

Questionnaires A questionnaire was given to one adult in each household. The purpose of the questionnaire was to characterize the home, including characteristics of the living space and building, evidence of smoking, presence of pet(s), house cleaning habits, the occupants' health, and surrounding environs.

Environmental conditions Temperature and relative humidity was monitored indoors every hour during sampling periods using an electronic monitor (Baxter Healthcare Co., Deerfield, IL, USA). Outdoor weather conditions during the sampling periods were provided by a local meteorological station.

Bioaerosol collection Bioaerosol was sampled in the main living area of the home, 1.3 m above the floor, using two swirling liquid impingers, two open-faced filter cassettes, and a MOUDI, according to the methods previously described in this paper. Two liquid impingers and two open-faced filter cassettes were stationed outdoors, near the home. The open-face filter cassettes were chosen for airborne bacteria and endotoxin collection as a series of experiments indicated that open-faced filter cassettes tended to collect up to three times more dust depending on the type of aerosol (Beaulieu et al., 1980).

The outdoor air samples provided direct information about background ambient airborne bacteria and endotoxin levels. Only one MOUDI was available for this study and was deployed inside the homes.

Experimental protocol Indoor and outdoor air was sampled between 10 AM and 2 PM in each home. During the first 2 h, air samples were collected indoors. Air was then sampled for 2 h outdoors.

Air exchange rate determination A tracer gas test was used to estimate the air exchange rate of the homes. Carbon dioxide (CO_2) was used as the tracer gas because it is a non-reactive gas, easy to monitor and does not pose a health threat at the concentrations used. Tracer gas was injected at a constant pressure into the main living area of the home from an 18 kg CO_2 cylinder; once CO_2 accumulated up to 5000 ppm, the cylinder discharge was ceased and CO_2 levels were then recorded every 1 min with a CO_2 monitor (Model L76; Langan Products Inc., San Francisco, CA, USA), until concentrations decayed to around 1000 ppm which is almost three times outdoor CO_2 levels (~350 ppm). The rate of change of the CO_2 concentration during the decay period was determined and described as the air exchange rate of the home.

Statistical analysis

Prior to statistical evaluation, a test for normality was applied to determine the appropriate statistical testing. In this study, the Anderson–Darling normality test was applied to airborne bacteria and endotoxin concentrations obtained from the environmentally controlled chamber and residential pilot-field studies. Concentrating on the tails of the distributions, this test measures the deviation of the data set from a prescribed statistical distribution, resulting in a *P*-value. Statistical standards in this study were established by choosing a 95% confidence level; *P*-values above $\alpha = 0.05$ were defined as a normally distributed data set. As judged by this test, airborne bacteria and endotoxin concentrations in this study could be fitted with a log-normal distribution. An analysis of variance (ANOVA) was performed on natural log-transformed airborne bacteria and endotoxin levels (Levine et al., 2001). A two-way ANOVA with replication was performed to test different effects in the environmentally controlled and

residential pilot-field studies. All significance testing was done at the $\alpha = 0.05$ level.

In the environmentally controlled chamber study, the impact of fixed effects was tested: sampler type (three levels) and bacteria type (two levels) on airborne bacteria and endotoxin concentrations. In addition, the interaction of sampler and bacteria type on airborne bacteria and endotoxin levels was tested. Total airborne bacteria and endotoxin concentrations from two impingers and four filter cassettes were averaged, and then the ANOVA was performed on the data set.

In the pilot-field study, the ANOVA was conducted to evaluate a number of effects and interactions. The impact of fixed effects – sampler type (three levels) and season (two levels) – was tested, as well as interaction of these two parameters, on indoor airborne bacteria and endotoxin levels. The effect of the home within Boulder (treated as random effect; three levels) and season was tested, and interaction between home and season, on indoor airborne bacteria and endotoxin levels. And finally, the effect of the home and season was tested, and interaction between home and season, on outdoor airborne bacteria and endotoxin levels. Before performing the ANOVA, the concentrations of total airborne bacteria and endotoxin were averaged from the same sampler.

Total airborne bacteria concentrations recovered from liquid impingers and filter cassettes were calculated as the arithmetic mean (AM) and geometric mean (GM) and corresponding standard deviations (s.d. and GSD) of 10 microscopic fields from each sampler. Airborne bacteria levels recovered from the MOUDI were calculated by first counting 10 microscopic fields for each MOUDI stage and averaging, then summing all stage averages (denoted as AM); the s.d. was calculated by propagating the standard deviation of the 10 microscopic fields. Airborne endotoxin concentrations recovered from liquid impingers and filter cassettes were calculated as the AM, GM, s.d., and GSD of mean endotoxin content from each sampler. Airborne endotoxin levels recovered from the MOUDI were calculated from mean endotoxin content on all stages and then summed; the s.d. was calculated by propagating the range of endotoxin content on each stage.

Results

Validating sampling methods for airborne bacteria and endotoxin in environmentally controlled chamber studies

The results of experiments conducted in the test chamber using two different gram-negative bacteria (*S. marcescens* and *E. coli*) and three different bioaerosol samplers (liquid impingers, filter cassettes, and MOUDI) are shown in Tables 1 and 2. In test 1 with *S. marcescens*, endotoxin concentrations in the nebulizer were an order of magnitude higher compared with test 2 while total airborne bacteria levels were similar. An elevated endotoxin concentration in the nebulizer resulted in correspondingly elevated endotoxin concentrations recovered from the air. In tests 1 and 2 with *E. coli*, the airborne endotoxin levels and total airborne bacteria levels in the chamber were comparable, and airborne endotoxin concentrations in the nebulizer were similar. As judged by the ANOVA, a significant difference was found in total airborne bacteria

Table 1 Total airborne bacteria concentrations recovered by filter cassettes, liquid impingers, and MOUDI in environmentally controlled chamber studies

Nebulizer concentration	Filter ($n = 4$)	Impinger ($n = 2$)	MOUDI
Serratia marcescens			
Test 1: 376,000/ml			
AM (m^{-3})	15,700,000	59,400,000	10,600,000
s.d. (m^{-3})	3,040,000	12,000,000	7,340,000
GM (m^{-3})	15,433,000	58,300,000	
GSD	1.23	1.22	
Test 2: 221,000/ml			
AM (m^{-3})	20,200,000	76,700,000	24,400,000
s.d. (m^{-3})	6,550,000	11,600,000	3,600,000
GM (m^{-3})	18,800,000	75,800,000	
GSD	1.50	1.80	
Escherichia coli			
Test 1: 371,000/ml			
AM (m^{-3})	4,730,000	18,800,000	4,730,000
s.d. (m^{-3})	1,520,000	5,530,000	986,000
GM (m^{-3})	4,410,000	17,800,000	
GSD	1.51	1.45	
Test 2: 390,000/ml			
AM (m^{-3})	13,100,000	44,000,000	20,500,000
s.d. (m^{-3})	5,880,000	6,760,000	2,350,000
GM (m^{-3})	11,700,000	43,400,000	
GSD	1.66	1.17	

Table 2 Airborne endotoxin concentrations recovered by filter cassettes, liquid impingers, and MOUDI in environmentally controlled chamber studies

Nebulizer concentration	Filter ($n = 4$)	Impinger ($n = 2$)	MOUDI
Serratia marcescens			
Test 1: 10,700 EU/ml			
AM (EU/m^3)	349	635	427
s.d. (EU/m^3)	123	75	14
GM (EU/m^3)	331	634	
GSD	1.04	1.11	
Test 2: 1760 EU/ml			
AM (EU/m^3)	23	54	73
s.d. (EU/m^3)	5	1	3
GM (EU/m^3)	22	54	
GSD	1.39	1.00	
Escherichia coli			
Test 1: 6390 EU/ml			
AM (EU/m^3)	75	193	134
s.d. (EU/m^3)	54	164	6
GM (EU/m^3)	60	193	
GSD	1.45	1.00	
Test 2: 8270 EU/ml			
AM (EU/m^3)	106	270	446
s.d. (EU/m^3)	84	33	12
GM (EU/m^3)	91	33	
GSD	2.95	1.90	

concentrations recovered by swirling liquid impingers, filter cassettes, and MOUDI ($P = 0.04$). No significant difference was found in airborne endotoxin concentrations recovered by these samplers ($P = 0.57$). There was no significant difference in recovered total airborne bacteria ($P = 0.09$) and endotoxin ($P = 0.81$) between the two tested bacteria types. No interaction between the tested parameters for the chamber study was found.

Aerodynamic particle size distribution of airborne bacteria and endotoxin in environmentally controlled chamber studies

Figure 1 summarizes the aerodynamic particle size distribution in the range 0.32–3.2 μm for airborne bacteria collected with the MOUDI during experiments conducted in the environmentally controlled chamber. The errors reported for each MOUDI stage were derived using the standard deviation in 10 microscopic fields used to obtain the average number of total bacteria in an air sample. During preliminary tests, all 10 MOUDI stages were used for sampling, but analyses showed that airborne endotoxin was only associated with smaller particle sizes, so the first three stages (M1, M2, and M3) were excluded from later observations. In addition, for direct microscopy counting of bacteria only MOUDI stages in the size range 0.32 and 3.2 μm were used as the resolution of epifluorescent microscope was calculated to be > 0.2 μm (Davidson, 2004).

Figure 2 illustrates the aerodynamic particle size distribution in the range 0.056–3.2 μm for airborne endotoxin associated with *S. marcescens* and *E. coli* during experiments conducted in the environmentally controlled chamber. The errors reported for each MOUDI stage were derived from the difference in duplicate samples analyzed by the LAL assay.

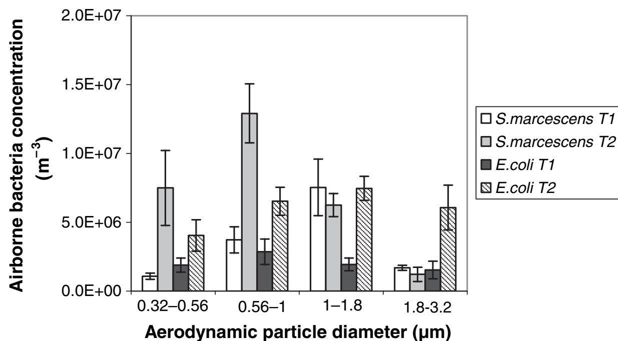

Fig. 1 Aerodynamic particle diameter size distribution of total gram-negative bacteria in the range 0.32–3.2 μm in environmentally controlled chamber studies. Experiments were repeated twice, and T1 indicates test 1, T2 indicates test 2. The errors reported were derived using the standard deviation of total bacteria observed in eluents from each MOUDI stage. Count median aerodynamic diameter 1.0 μm

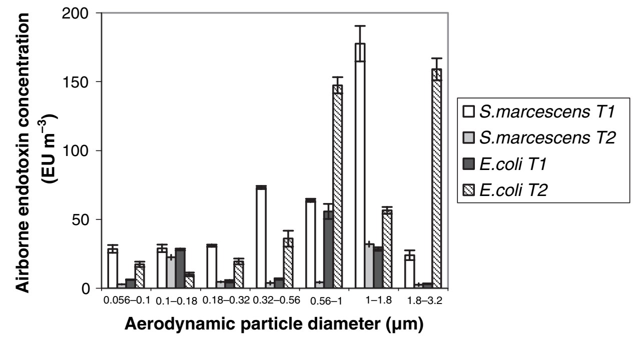

Fig. 2 Aerodynamic particle diameter size distribution of airborne endotoxin in the range 0.056–3.2 μm, in environmentally controlled chamber studies. Experiments were repeated twice, and T1 indicates test 1, T2 indicates test 2. The errors reported for each MOUDI stage were derived using the range of endotoxin content observed in eluents from each MOUDI stage. Mass median aerodynamic diameter 0.84 μm

Characteristics of the homes and environs

The mean air exchange rate was 0.32 h^{-1} ($n = 3$; s.d. = 0.25 h^{-1}) during winter. The mean air exchange rate in the summer was higher: 0.75 h^{-1} ($n = 3$; s.d. = 0.18 h^{-1}). These data span the mean infiltration rate of 43 southern California homes of 0.63 $\pm$ 0.33 h^{-1} (Lee et al., 1999), and agree with the air exchange rates of 0.2–0.6 h^{-1} measured in three other residences in Boulder, Colorado (Dutton et al., 2001).

In the winter, the mean outdoor temperature was 4°C ($n = 25$; s.d. = 12°C), and the mean relative humidity was 42% ($n = 21$; s.d. = 33%). During the summer, the mean outdoor temperature was 30°C ($n = 25$; s.d. = 4°C), and the mean relative humidity was 24% ($n = 24$; s.d. = 10%). The mean indoor temperature in the winter was 18°C ($n = 21$; s.d. = 1°C), and mean relative humidity was 33% ($n = 21$; s.d. = 18%). The mean indoor temperature in the summer was 26°C ($n = 21$; s.d. = 2°C), and the mean relative humidity was 32% ($n = 21$; s.d. = 4%).

Residential levels of total airborne bacteria and endotoxin

The total indoor airborne bacteria and endotoxin levels measured in three homes during the winter and summer seasons are presented in Tables 3 and 4. Statistical analysis showed no significant difference in total indoor airborne bacteria concentrations recovered with swirling liquid impingers, filter cassettes, and all the MOUDI stages (excluding M1, M2, and M3) added together ($P = 0.14$) during winter and summer seasons. Statistical analysis showed a significant difference in indoor airborne endotoxin concentrations recovered with swirling liquid impingers, filter cassettes, and MOUDI ($P = 0.001$) during winter and summer seasons.

Statistical analysis showed no significant difference in total indoor airborne bacteria concentrations between

Table 3 Total indoor airborne bacteria concentration recovered by filter cassettes, liquid impingers, and MOUDI in three Boulder homes during the winter and summer

	Filter ($n = 2$)		Impinger ($n = 2$)		MOUDI	
Home	Winter	Summer	Winter	Summer	Winter	Summer
Home 1						
AM (m^{-3})	1,030,000	3,640,000	825,000	5,570,000	1,830,000	2,380,000
s.d. (m^{-3})	445,000	1,790,000	364,000	1,350,000	651,000	441,000
GM (m^{-3})	956,000	3,230,000	739,000	5,430,000		
GSD	1.46	1.69	1.67	1.28		
Home 2						
AM (m^{-3})	353,000	4,100,000	310,000	15,500,000	2,290,000	15,300,000
s.d. (m^{-3})	248,000	546,000	149,000	4,440,000	441,000	1,340,000
GM (m^{-3})	276,000	4,070,000	287,000	14,900,000		
GSD	2.10	1.14	1.61	1.34		
Home 3						
AM (m^{-3})	1,050,000	2,430,000	1,650,000	5,340,000	993,000	2,230,000
s.d. (m^{-3})	419,000	692,000	352,000	997,000	475,000	375,000
GM (m^{-3})	978,000	2,340,000	1,610,000	5,240,000		
GSD	1.50	1.33	1.25	1.22		

Table 4 Airborne indoor endotoxin concentration recovered by filter cassettes, liquid impingers, and MOUDI in three Boulder homes during the winter and summer

	Filter ($n = 2$)		Impinger ($n = 2$)		MOUDI	
Home	Winter	Summer	Winter	Summer	Winter	Summer
Home 1						
AM (EU/m^3)	0.87	0.71	1.5	1.8	11	5.1
s.d. (EU/m^3)	0.31	0.14	1.1	0.2	4	3.4
GM (EU/m^3)	0.83	0.69	1.2	1.8		
GSD	1.5	1.2	2.3	1.1		
Home 2						
AM (EU/m^3)	0.56	0.84	2.7	ND[a]	7.3	0.86
s.d. (EU/m^3)	0.34	0.78	1.9	–	2.1	0.97
GM (EU/m^3)	0.47	0.49	2.1	–		
GSD	2.0	1.5	2.3	–		
Home 3						
AM (EU/m^3)	0.91	2.6	2.5	2.0	7.0	7.4
s.d. (EU/m^3)	0.44	0.3	2.0	1.8	0.6	2.6
GM (EU/m^3)	0.61	2.5	2.4	1.2		
GSD	2.8	1.1	2.6	1.2		

[a]ND, not detected.

homes ($P = 0.53$) during two seasons. ANOVA showed that there was a significant difference in total indoor airborne bacteria levels between two seasons ($P = 0.0007$). There was no significant difference in total indoor airborne endotoxin levels between three homes ($P = 0.57$) and for two seasons ($P = 0.64$).

Total indoor and outdoor bacteria and endotoxin concentrations are detailed in Table 5; the results are presented for the three sampler types and the two seasons, and were averaged across the three homes. Compared with concentrations measured by filter cassettes, liquid impingers and MOUDI sampled up to three times more airborne bacteria and up to 10 times more airborne endotoxin. Statistical analysis showed no significant difference between total indoor and outdoor airborne bacteria concentrations ($P =$

Table 5 Indoor and outdoor airborne bacteria and endotoxin concentrations during winter and summer recovered by filter cassettes, liquid impingers, and MOUDI averaged across three Boulder homes

	Filter ($n = 6$)		Impinger ($n = 6$)		MOUDI ($n = 3$)	
	Winter	Summer	Winter	Summer	Winter	Summer
Indoor: bacteria						
AM (m^{-3})	502,000	3,390,000	928,000	8,803,000	1,704,000	6,637,000
s.d. (m^{-3})	491,000	863,000	676,000	5,801,000	658,000	7,503,000
GM (m^{-3})	337,000	3,100,000	750,000	7,725,000	161,000	4,330,000
GSD	2.14	1.72	1.78	1.59	1.50	2.09
Outdoor: bacteria						
AM (m^{-3})	609,000	3,680,000	608,000	5,560,000		
s.d. (m^{-3})	517,000	491,700	710,000	3,282,000		
GM (m^{-3})	447,500	3,660,000	343,000	4,896,000		
GSD	2.79	1.93	2.92	1.89		
Indoor: endotoxin						
AM (EU/m^3)	0.78	1.38	2.25	1.86	8.43	4.45
s.d. (EU/m^3)	0.19	1.06	0.65	0.10	2.23	3.32
GM (EU/m^3)	0.76	1.16	2.18	1.86	8.25	3.19
GSD	1.31	2.03	1.38	1.06	1.28	3.16
Outdoor: endotoxin						
AM (EU/m^3)	1.25	3.26	2.39	8.30		
s.d. (EU/m^3)	1.29	2.31	3.02	9.05		
GM (EU/m^3)	0.74	2.78	1.35	5.28		
GSD	4.01	1.97	3.57	4.25		

0.38) and between indoor and outdoor airborne endotoxin concentrations ($P = 0.45$).

Outdoor total bacteria concentrations were up to nine times higher during the summer compared with the winter, and statistically different between three homes ($P = 0.03$) and for two seasons ($P = 0.0002$). Statistical analysis showed significant difference in outdoor airborne endotoxin concentrations between homes ($P = 0.004$) and during winter and summer ($P = 0.009$). No interaction between all tested parameters for the pilot-field study was found.

Association of airborne endotoxin with aerodynamic particle size distribution in residential environments

Figures 3 and 4 present the total indoor airborne bacterial aerodynamic particle size distribution in the range 0.32–3.2 μm in study homes, during winter and summer seasons. Airborne bacteria were found on all four MOUDI stages investigated in this part of study.

Figures 5 and 6 present the total indoor airborne endotoxin aerodynamic particle size distribution in the range 0.056–3.2 μm during winter and summer in study homes. Airborne endotoxin was detected on all seven stages investigated during this part of study.

Discussion

Environmentally controlled chamber studies comparing methods for airborne bacteria and endotoxin collection showed that total airborne bacteria concentrations recovered from swirling liquid impingers and

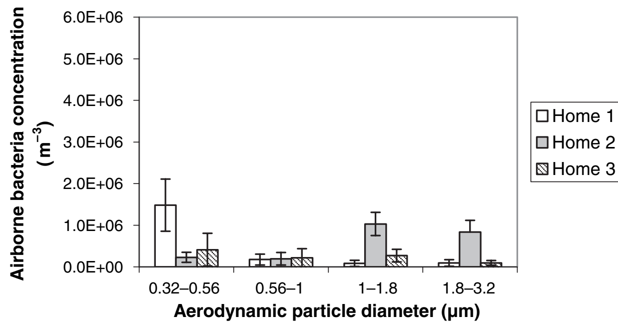

Fig. 3 Aerodynamic particle diameter size distribution in the range 0.32–3.2 μm of total indoor airborne bacteria concentrations in three homes during winter. The errors reported were derived using the standard deviation of total bacteria observed in eluents from each MOUDI stage. Count median aerodynamic diameter: home 1, <0.32 μm; home 2, 1.51 μm; home 3, 0.70 μm

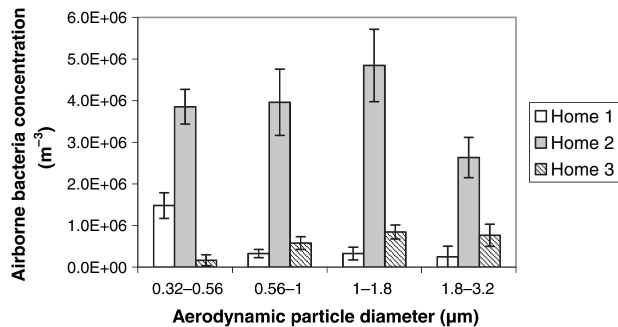

Fig. 4 Aerodynamic particle diameter size distribution in the range 0.32–3.2 μm of total indoor airborne bacteria concentrations in three homes during summer. The errors reported were derived using the standard deviation of total bacteria observed in eluents from each MOUDI stage. Count median aerodynamic diameter: home 1, <0.32 μm; home 2, 0.98 μm; home 3, 1.35 μm

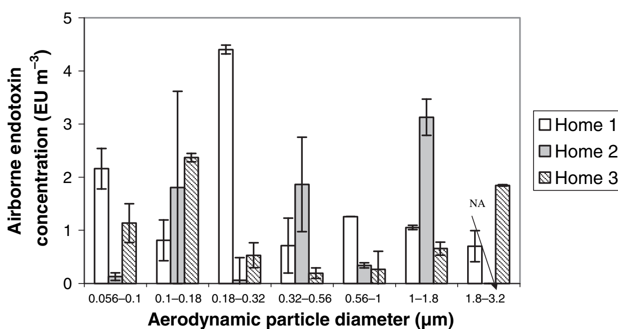

Fig. 5 Aerodynamic particle diameter size distribution in the range 0.056–3.2 μm of total indoor airborne endotoxin concentrations in three homes during winter. The errors reported were derived using the range of endotoxin content observed in eluents from each MOUDI stage. Mass median aerodynamic diameter: home 1, 0.25 μm; home 2, not available; home 3, 0.18 μm

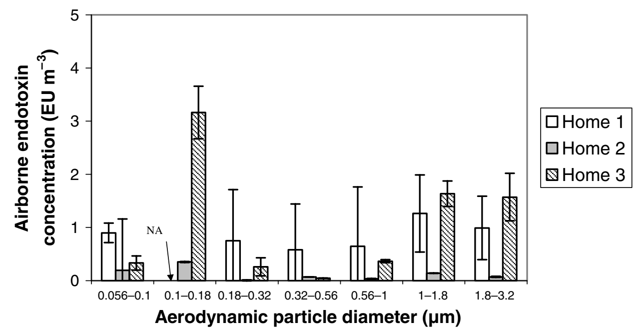

Fig. 6 Aerodynamic particle diameter size distribution in the range 0.056–3.2 μm of total indoor airborne endotoxin concentrations in investigated homes during summer. The errors reported were derived using the range of endotoxin content observed in eluents from each MOUDI stage. Mass median aerodynamic diameter: home 1, not available; home 2, 0.15 μm; home 3, 0.28 μm

MOUDI were higher than those recovered from filter cassettes. Liquid impingers recovered on average three times more airborne bacteria compared with MOUDI under identical conditions, and on average up to four times more airborne bacteria than filter cassettes using the elution protocols outlined (Table 1). Airborne endotoxin concentrations obtained by liquid impingers and the MOUDI were higher than airborne endotoxin concentrations obtained by filter cassettes but not significantly different. Liquid impingers and MOUDI recovered on average up to two times more airborne endotoxin compared with filter cassettes under identical conditions (Table 2).

During the pilot-field study, the liquid impingers and MOUDI collected more airborne bacteria (not significantly different) and endotoxin (significantly different) than filter cassettes. Liquid impingers and MOUDI recovered on average two times more airborne bacteria, and two to six times more airborne endotoxin, than filter cassettes (Tables 3 and 4). MOUDI recovered the most airborne endotoxin in pilot-field studies.

Findings on differences in sampler type in the environmentally controlled chamber study do not agree with the less controlled pilot-field study. The differences in these results may be due to variance in sampled bioaerosol and/or also due to differences in the age of sampled bioaerosol. In addition, the mass median aerodynamic diameter (MMAD, Figures 2, 5 and 6) for airborne endotoxin was much higher for the aerosols used in the chamber study compared with the pilot-field study. The difference in recovery of these three different sampling methods is an indication that other whole biological particles, or fractions of particles, were recovered from the liquid impinger or MOUDI, but not by filter cassettes. The advantage of sampling by liquid impingers is that no elution process is needed and therefore recovery artifacts are minimized. The swirling liquid impinger is nearly 100%

efficient for particles >1 μm, and the collection efficiency decreases to approximately 89% at 0.5 μm (Willeke et al., 1998). The MOUDI has been designed to collect particles as small as 0.056 μm and as large as 10 μm (Marple et al., 1991). The 37-mm-diameter filter cassettes can collect 70% of particles <10 μm and collects a few particles ($<10\%$) above 25 μm (Buchan et al., 1986).

Indoor air concentrations of biocontaminants generally vary greatly over time depending on the activity of occupants, number of pets, relative humidity, etc. Therefore many samples need to be collected within homes to reliably assess exposure and make comparisons between homes and seasons. This study of three homes was designed to validate novel sampling methods for airborne endotoxin and bacteria in realistic conditions. A larger study should be conducted in more homes to support further the conclusions found here.

Total airborne bacteria indoors were in the range 5.02×10^5 to 8.80×10^6 m^{-3} (Table 5). The levels of airborne bacteria, reported here were consistent with total indoor airborne bacteria collected with liquid impingers, reported in homes enriched with bioaerosol due to water damage (Fabian et al., 2005), and slightly higher than those concentrations recovered by membrane filters in Scandinavian homes (Bjornsson et al., 1995; Toivola et al., 2002). Outdoor total airborne bacteria concentrations were in the range from 6.08×10^5 and 5.56×10^6 m^{-3}, and were consistent with outdoor total airborne bacteria levels previously reported and collected with a high-volume wet cyclone sampler (Tong and Lighthart, 1999), and liquid impingers (Fabian et al., 2005).

Indoor airborne endotoxin concentrations were in the range from 0.78 to 8.43 EU/m^3 (Table 5). As a basis for comparison, in 15 Boston homes (Park et al., 2000), airborne endotoxin was in the range from 0.02 to 19.82 EU/m^3. Outdoor airborne endotoxin levels were in the range from 1.25 to 8.30 EU/m^3, and were higher than outdoor airborne endotoxin levels reported elsewhere (Heinrich et al., 2003; Hines et al., 2000; Park et al., 2000; Rose et al., 1998). The differences in these results may be due to actual variance in the sampled airborne material, and/or also due to different sampling protocols (all other studies mentioned above sampled with filter cassettes). In addition, a sample processing method may have played a role in differences in airborne bacteria/endotoxin concentrations. Membrane filters from filter cassettes and MOUDI were sonicated and filtered whereas swirling liquid impinger air samples were not.

The aerodynamic particle size distribution obtained by the MOUDI in the environmentally controlled chamber study showed that on average 85% of total gram-negative bacteria concentration were <1.8 μm aerodynamic diameter and the count median aerodynamic diameter (CMAD) was 1 μm. The particle size distribution of total gram-negative bacteria in the environmentally controlled study may have been influenced by the operation of the Collison nebulizer, which was used to generate the test bioaerosol. The aerodynamic size distribution of bacteria generated with a Collison nebulizer typically peaks at around 0.75 μm, but the size distribution is wide due to the generation of large numbers of particles with both larger and smaller aerodynamic diameters (0.3–0.6 μm) (Reponen et al., 1997).

Total indoor airborne bacteria aerodynamic particle size distribution in the range between 0.32 and 3.2 μm varied between the homes, and between seasons. The CMAD for home 1 was markedly smaller, <0.32 μm, compared with the CMADs for homes 2 and 3, which ranged from 0.7 to 1.5 μm.

The aerodynamic particle size distribution obtained by the MOUDI in the environmentally controlled chamber study showed that on average 66% of airborne endotoxin was found in 0.56–3.2 μm size range. The MMAD was 0.84 μm. The size distribution of airborne endotoxin recovered from homes 1 and 3 suggested that airborne endotoxin was associated with the 0.18–0.32 μm size range in home 1 and with the 0.1–0.18 μm size range in home 3 during winter season. The highest proportion of airborne endotoxin (on average 42%) in homes 2 and 3 was found in the 0.1–0.18 μm size range during summer season. The MMAD ranged from 0.15 to 0.28 μm. These results, which agree with the environmentally controlled chamber results, showed that airborne endotoxin is associated with particulate matter <1 μm, and differ from other findings on airborne endotoxin distributions in other environments (Attwood et al., 1986; Gordon et al., 1992; Monn and Becker, 1999; Olenchock et al., 1983). The different size fractions may be indicative of different sources of airborne endotoxin in homes. It could also be the case that endotoxin exists as cell fragments, or associated with particulate matter in the air, and also suggests that whole bacteria cells can retain significant endotoxin activity.

Few studies have been carried out on seasonal variation of endotoxin in homes. A study on endotoxin levels in house dust from homes in Sao Paulo, Brazil, showed that the highest endotoxin levels were detected during months with higher outdoor temperatures, whereas the lowest were detected during lower outdoor temperatures (Rizzo et al., 1997). Season had a high correlation with domestic endotoxin levels in house dust in investigated homes in Taiwan (Su et al., 2001). A similar study on airborne endotoxin showed a significant seasonal pattern in outdoor airborne endotoxin levels; mean outdoor endotoxin levels varied by more than a factor of four across seasons (Park et al., 2000). Increase in vegetation cover and plant material might contribute to higher airborne endotoxin levels in

spring/early summer (Rylander, 2002). It has been suggested that outdoor gram-negative bacteria are shed from leaves of growing plants (Milton, 1999). Also the surface of pollen grains can serve as an additional source of growth of gram-negative bacteria (Spiewak et al., 1996). However, some studies have not detected seasonal variation in endotoxin levels in homes (Michel et al., 1990; Park et al., 2001).

Data from this pilot-field study show that outdoor airborne endotoxin concentrations vary with season in Colorado. Indoor airborne endotoxin levels, however, did not vary with season. When compared with total airborne bacteria, which varied with season in both indoor and outdoor air samples, it may be that the sources of indoor airborne endotoxin are different than indoor total airborne bacteria sources in Colorado. Noteably, indoor and outdoor total airborne bacteria and endotoxin levels were not significantly different. Further, it maybe that outdoor sources dominate indoor total airborne bacteria and endotoxin levels, however, indoor sources of airborne endotoxin are important. Interestingly indoor total airborne bacteria and endotoxin were also not significantly different between homes, suggesting a similarity in sources in this small sample size.

Acknowledgements

Authors are grateful to the families that provided access and all necessary help during air sampling in their homes. The authors thank Damir Kujundzic, Cody Howard, Mari Rodriguez and Joanne Hayes, University of Colorado at Boulder, for their assistance in collecting and analyzing samples. Authors are grateful to Prof. Jana Milford and Prof. Michael Hannigan, Mechanical Engineering Department, University of Colorado at Boulder for their assistance. We would like to acknowledge the Joint Center for Energy Management personnel for providing essential equipment and operating the test facility.

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