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Characterization of an Aerosol Chamber for Human Exposures to Endotoxin

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The objective of this study was to develop and characterize an exposure chamber in which human subjects could be exposed to low dust concentrations carrying an endotoxin coating. An exposure chamber, dust dispersion method, and endotoxin characterization technique were developed for inhalation exposures. A 6.27 m^3 exposure chamber was designed and constructed from cinder block, glass windows, and Plexiglas. Using an acetone adhesion process, *Enterobacter agglomerans* were adsorbed onto respirable cellulose particles to create the endotoxin aerosol. The size distribution of the endotoxin-treated particles was verified using light microscopy and cascade impactors. A dry powder dust generator was refined to consistently disperse small quantities of the aerosol into the chamber to maintain dust concentrations at approximately 250 $\mu g/m^3$. Dust levels during the chamber exposures were monitored using a portable continuous aerosol monitor (PCAM). During initial exposure runs, PCAM monitoring stations were positioned at different locations within a 0.5-meter matrix to document mixing patterns. Total dust and cascade impactor samples were collected throughout each exposure period to characterize the chamber operating system and insure the mean airborne dust concentration fulfilled target levels. A one-factor analysis of variance at the 95 percent confidence interval illustrated that there was not a statistically significant difference in the mean dust concentration throughout the exposure runs compared to the individual runs. Together the consistency of the total dust filters, endotoxin concentrations, and aerosol-monitoring instrument were adequate to allow use of the chamber for experimental studies involving human volunteers.

Keywords Aerosol Chamber, Chamber Characterization, Endotoxin-Aerosol, Aerosol Dispersion

The inhalation hazards of endotoxin aerosols have been growing in importance with the recent attention given to indoor air quality and sick building syndrome. Researchers have also focused their attention on identifying endotoxin sources and the relationship between these sources and allergic disease.[1,2] The purpose of this study was to develop and characterize an exposure chamber in which human subjects could be exposed to low dust concentrations carrying an endotoxin coating. The chamber, dust dispersion method, and endotoxin characterization were necessary as preparation for a subsequent study that examined the bronchial reactivity of sensitive asthmatics following an inhalation exposure to endotoxin.

The goals of this investigation were threefold:

1. Construct an exposure chamber with ventilation characteristics which promote a well-mixed dust concentration inside the chamber;
2. Develop a dust generation system to disperse continuous quantities of the endotoxin aerosol and maintain uniform chamber dust concentrations at 250 $\mu g/m^3$;
3. Verify that the prepared endotoxin aerosol contains a respirable size distribution and a uniform endotoxin concentration of 325–425 ng endotoxin/m^3 or 1300–1700 ng endotoxin/mg dust.

Endotoxin, the lipopolysaccharide-protein complexes of the outer cell membrane of gram-negative bacteria, is common in agricultural, industrial, and office environments with low air exchange and an absolute humidity above 7 g/kg.[3,4] Exposure conditions were designed to simulate endotoxin exposures in a typical "sick" office environment as reported in a recent investigation.[5] Accordingly, the target endotoxin concentration inside the exposure chamber was established at 325–425 ng endotoxin/m^3 or, more descriptively, 1300–1700 ng endotoxin/mg dust at an air concentration of 250 $\mu g/m^3$. Since the Limulus Amebocyte Lysate (LAL) assay test has a 20-percent

coefficient of variation, a range of endotoxin concentration was acceptable.

METHODS AND MATERIALS

Exposure Chamber Design

The design of the exposure chamber was dependent upon the limited quantity of *Enterobacter agglomerans* available for the investigation. To maximize existing materials, a 6.27-cubic-meter chamber was partitioned from an existing dust room in the Aerosol Laboratory of the University of North Carolina at Chapel Hill (UNC-CH). The dust room was designed specifically for aerosol studies, with a floor drain and a separate ventilation system from the rest of the building to eliminate cross-contamination. Alternative chamber designs have been used to conduct similar aeroallergen exposure studies.[6–8] To reduce potential feelings of claustrophobia on the part of the human subjects, this exposure chamber was constructed of sealed cinder block, glass windows, and Plexiglas. The exposure chamber is illustrated in Figures 1 and 2.

Ventilation measurements were collected with a Aridata Multimeter Series 4800 (Shortridge Instrument Inc, Scottsdale, AZ) to ensure a chamber air exchange rate of at least 10 air exchanges per hour as recommended in an indoor environment.[9] Air was exhausted from the chamber, pulled through a bag filter, and discharged into the atmosphere. A fan was used to induce turbulence and achieve uniform mixing throughout the exposure chamber.

Aerosol Characterization

Air particulate concentrations in an office environment typically range from 5 to 500 $\mu g/m^3$ depending upon office location, ventilation system, office equipment, and exterior dust concentration.[10,11] Therefore, a nominal concentration of 250 $\mu g/m^3$ of respirable dust containing 1300–1700 ng endotoxin/mg dust was targeted during exposures.

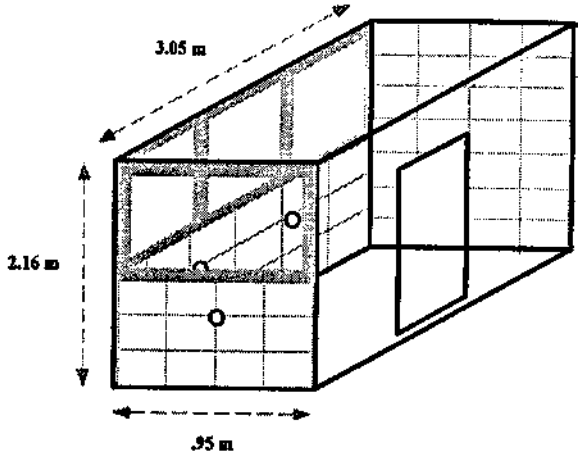


FIGURE 1
Schematic diagram of aerosol chamber.

Because exposure to cellulose causes minimal, if any, health effects at the low target concentrations, it was utilized as the carrier aerosol. After reviewing several samples of cellulose dust, a FMC microcrystalline lattice (LT-006) was selected due to its particle size distribution and content. Because the microcrystalline lattice could potentially contain background organic agents, the material was heated to 100°C for one hour to inactivate agents prior to endotoxin adhesion. Control, pre-heated, and heated cellulose samples were analyzed for endotoxin. Background endotoxin was minimal in these samples. Since the endotoxin analysis of the heated sample was below 5 ng endotoxin/m^3, the heated cellulose was accepted for use in the study.

The inert and heated base material was then coated with *E. agglomerans* using an acetone adsorption process. During this process, a preparation of *E. agglomerans*, disrupted by sonication to contain both whole and disrupted cells, was suspended in an acetone solution. Cellulose was added to the acetone preparation and then evaporated, while stirring at 60°C in a vented hood. The cellulose-*E. agglomerans* mixture was then "broken up" and analyzed by LAL for endotoxin in the bulk preparation. The preparation contained both extracted endotoxin (that extracted by the acetone and adsorbed on the cellulose) and cell wall fragments that may be adsorbed onto the cellulose or still associated with the cellulose after drying.

Light microscopy using a Porton graticule was performed on an aerosolized sample of endotoxin preparation to verify its respirable size distribution. This analysis resulted in a count median diameter (CMD) of 3.6 μm. Analysis of cascade impactor data (Graseby-Andersen, Smyrna, GA) indicated the endotoxin preparation had a mass median aerodynamic diameter (MMAD) of 1.6 μm and a geometric standard deviation (σ_g) of 2.06. These findings confirmed that the particles were respirable and could be inhaled into the alveolar region by the subjects.[12]

To verify endotoxin content, endotoxin preparations were aerosolized in the exposure chamber and collected on 37-mm glass fiber filters for total dust samples. At the conclusion of each exposure run, filter samples were weighted and placed in individual, sterile 50-mL centrifuge tubes. These tubes were then securely packed (no ice) and shipped for endotoxin analysis. If dust was lost from the filter during transit, it was captured in the centrifuge tube. Because the first step of the subsequent analysis was conducted in the centrifuge tube, there was no loss of sample. Media blank filters (at least 10 percent of total filter number) were also sent with the filters. These filters were then analyzed for endotoxin content using a Limulus Amebocyte Lysate (LAL) assay (Bio-Whittaker, Walkersville, MD). The LAL method was utilized in this study because it has been shown to quantify biologically active endotoxin from gram-negative bacteria and has been used in other investigations.[2,13,14]

Aerosol Dispersion Method

To inject the endotoxin-coated aerosol into the chamber, a method was developed for continuous and uniform aerosol dispersion. Several types of generators are currently available for aerosol distribution. As shown by other studies, it is difficult to

continually disperse a precise quantity of aerosol from a dust generator.[15] The predominant types disperse either a nebulized liquid or a dry aerosol. Nebulization techniques were not considered because gravimetric methods normally used to assess nebulizer output tend to overestimate aerosol production.[16] A dry aerosol generator was utilized, because it does not alter the original particle size or endotoxin content during dispersion.[17]

In other applications, turntable dust feeders have been used to consistently inject large quantities of dust into aerosol chambers.[18] This design worked with Arizona road dust, but had never been applied to an organic aerosol at the low dust concentrations in this study. To alleviate the powder flow problem in the hopper, a radical change in design was instituted in this dust feeder. Rather than use an inverted cone shape as found on most dust feeder units, a cylinder, open at both ends, was placed on the turntable, held in place by an idler wheel, and allowed to rotate freely as the turntable rotated. The powder to be dispersed was placed in the cylinder, which with its rotation, tended to wipe the powder across the turntable groove, filling the groove uniformly with powder. At the same time, the rotation of the cylinder kept the powder from clumping or bridging within the cylinder which otherwise could lead to intermittent dust loading of the groove. Dust was picked up from the turntable groove with an aspiration-type unit. By adjusting the internal nozzle position relative to the pick-up line the amount of suction on the aspirator could be controlled. This can also be controlled by adjusting the compressed air pressure applied to the unit, giving a wide range of flow rates through the aspirator. This adjustment is used to match dust pickup capability against those parameters which could degrade this capability such as turntable speed and/or dust characteristics. Figure 3 shows a photograph of the dust generator.

To combat particle charge, the dust generator was constructed of aluminum rather than plastic. The turntable diameter of the dust generator was 20.3 cm, and the supply cylinder diameter was 6 cm. To obtain low dust concentrations in the chamber, the turntable groove was a V-cut at a 60° angle. The turntable groove width was 0.021 cm and the groove depth at the vortex of the angle was 0.0381 cm. The dust feeder operates at an airflow of a minimum pressure drop of 2 cm Hg, but was normally operated at a pressure drop of 6 cm Hg.

The supply cylinder of the dust generator was filled with approximate 0.75 grams of the coated-endotoxin dust. This dust slowly filled the groove of the turnplate and was aspirated into the

This photo illustrates the exposure chamber and provides a sense of scale for future endotoxin exposure runs.

FIGURE 2
Exposure chamber.

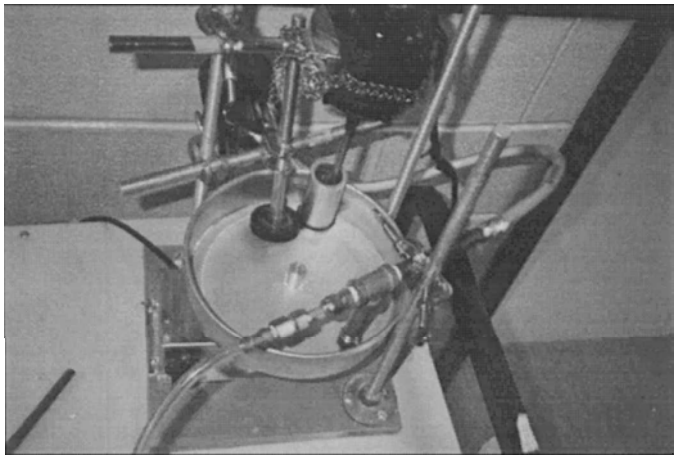


FIGURE 3
Dust generator.

chamber. The rotation speed of the turntable could be adjusted while the turntable was dispersing dust into the chamber. After the dust concentrations initially stabilized, the generator was fairly self-sufficient.

Chamber Characterization

To determine the reproducibility and accuracy of the chamber operating system, a series of exposure trial runs were completed. Prior to each run, chamber walls were coated with a thin layer of dust to insure an equilibrium dust concentration. Without wall conditioning, the injected aerosol particles were attracted to the inside surfaces of the chamber, and dust concentrations would not stabilize. This practice of wall conditioning is widely accepted by aerosol scientists and is attributed to a particle adhesion phenomenon.[19]

This coating process was conducted by running the dust generator at a pressure drop of 8 cm Hg and dispersing dust into the clean chamber for approximately two hours. After two hours, the airflow to the dust generator was turned down to a pressure drop of 6 cm Hg and the turntable speed was reduced to normal levels. If the dust chamber concentration would stabilize, the coating process was complete. If the chamber concentration was still highly variable, the coating process was continued in 15-minute intervals. The chamber walls were washed after each exposure run and re-coated for each subsequent run.

During each four-hour exposure, three total dust filters, and one cascade impactor sample with nine filters were collected. To collect the maximum quantity of endotoxin material, total dust samples were collected at a flow rate of 10.5 liters/min using a modified National Institute for Occupational Safety and Health (NIOSH) 0500 Method.[20] The cascade impactor sampled at a flow rate of 6.8 liters/min to collect size specific filter samples.

Endotoxin recovery efficiency is dependent upon filter media and the specific aerosol material being collected.[21,22] In some cases, the endotoxin aerosol binds tightly to the filter media and cannot be recovered during endotoxin analysis. Therefore, two separate, comparison runs of four hours were completed to determine which filter type was most efficient in collecting the cellulose-endotoxin aerosol.

During each comparison run, samples were collected on five filter types: mixed cellulose ester, polyvinylchloride, gelatin, zeta, and glass fiber. Sampling conditions were identical to a typical exposure, except filters were sampled in the center of the chamber at a height of three feet and approximately six inches apart. Immediately following both runs, all filter samples

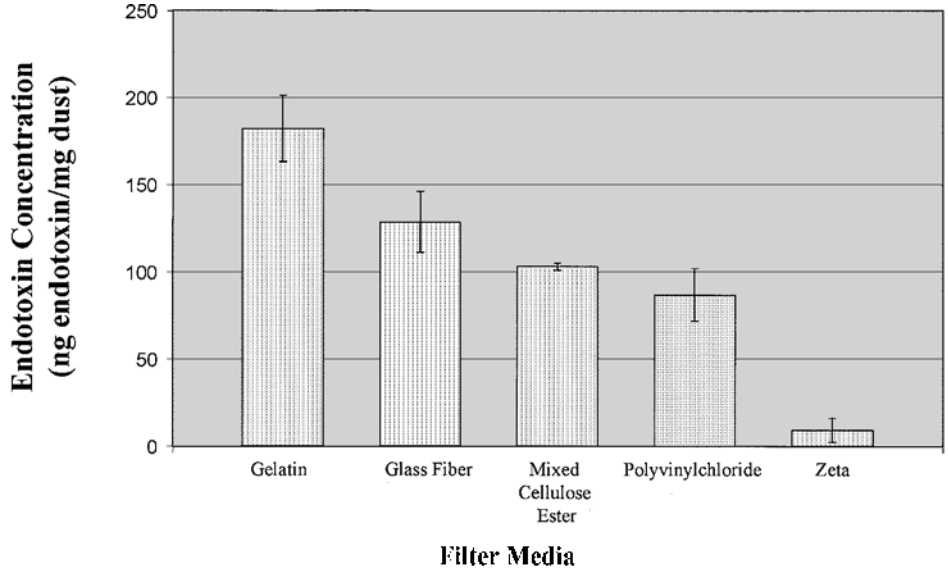


FIGURE 4
Evaluation of filter media and endotoxin aerosol on endotoxin recovery.

were shipped for endotoxin analysis. Two blank filters from the same manufacturing lot of each filter type were analyzed for background endotoxin contamination. These filters were handled identically to determine if potential endotoxin contamination resulted from either the sampling procedure or background endotoxin. In all cases, the blank filters contained no endotoxin contamination.

Mean endotoxin concentrations from each media are presented in Figure 4. Within the two runs, gelatin media provided the greatest reclamation of endotoxin. However, significant attention was necessary to sample with this filter media. Specifically, the gelatin filters required 10-mm filter cassettes, and special temperature treatment before and after the exposure run. The research team determined that the gelatin filters introduced additional technical difficulties due to the temperature requirements and a potential source of experimental error. Concurrently, research at the University of Alabama concluded that glass fiber filters have marginal increased endotoxin extraction efficiency compared to other filter types.[23] Therefore, because glass fiber filters allow maximum reclamation with minimal attention, they were selected for use during collection.

One of the limitations for gravimetric filters is the potential to absorb moisture. To control for this source of error, all filters were kept in a filter desiccator and were weighed with a sensitive microbalance to detect any changes in filter mass.

During each exposure run, a PCAM (Model 151, PPM Inc, Knoxville, TN) measured dust concentrations inside the chamber. The PCAM has a detection limit of 2000 $\mu g/m^3$ and was originally calibrated for cotton dust. The PCAM was re-calibrated for cellulose during this study. The dust concentration is determined by measuring the amount of incident light scattered by particles in a specified volume into a sensitive receptor. The quantity of light scattered from a particle into the detector is used to determine the diameter of a particle and the frequency of light-scattering pulses indicates particle concentration. Theoretical response settings for the PCAM can be determined using Mie theory and illustrate that the mass response of the scattered power is a function of the mass mean diameter.

The PCAM was used to provide a real-time assessment of dust concentrations inside the chamber during an exposure. If dust concentrations varied by more than ± 20 $\mu g/m^3$ from the target concentration of 250 $\mu g/m^3$, the dust generator rotation speed was increased or decreased accordingly. For these studies, the dust generation process was extremely consistent and did not require significant attention.

The PCAM was also used during preliminary runs to document uniform mixing inside the chamber. During initial exposure runs, PCAM monitoring stations were positioned at different locations within a 0.5 m matrix to document mixing patterns. Other investigators have utilized a similar sampling protocol to verify uniform chamber concentrations.[24] Because concentration fluctuations were not evident, the investigators were satisfied that chamber mixing was relatively consistent.

A lengthy exposure trial testing period was completed to accurately assess dust and endotoxin concentrations in the chamber. The results from the total dust samples, impactor samples, and monitoring data from the trial runs were analyzed to optimize the chamber operating parameters.

RESULTS

Exposure Chamber

The three initial research objectives were achieved for this chamber characterization. An aerosol chamber was constructed from Plexiglas, cinderblock, and glass. The chamber flow rate was originally measured at 585 cubic feet per minute. After the exhaust rate was damped, the flow rate was reduced to 50 cubic feet per minute. This ventilation configuration resulted in an air exchange rate of approximately 11.5 air exchanges per hour in the chamber. These ventilation measurements provided evidence that the chamber simulated an indoor environment for a whole body exposure.

Secondly, the chamber design was cost-effective because it was built maximizing existing materials. As shown in Figure 2, the Plexiglas wall exaggerated the perceived chamber volume to increase subject comfort. Finally, the chamber construction withstood excessive washing between exposure runs allowing investigators to test multiple conditions quickly. In summary, the constructed exposure chamber proved to be an excellent facility to host the endotoxin exposures.

Uniformity of Dust Concentrations

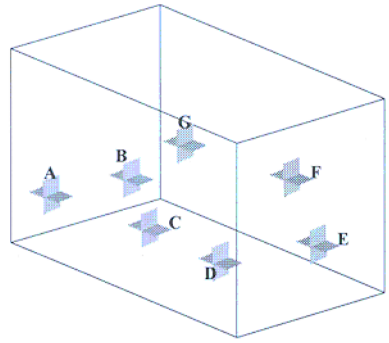
Uniform dust concentrations were documented inside the chamber using total dust filters and PCAM measurements during every exposure. After each exposure, the mass of each total dust sample was divided by the sample air volume to obtain the airborne dust concentration. A mean dust concentration was calculated from the three samples for each run.

During preliminary runs, sample locations were changed periodically to verify chamber mixing. Figure 5 illustrates the sampling locations and the resulting dust concentrations from some preliminary chamber runs. The variation between dust concentration on the different total dust filters within a given chamber run is quite low. After chamber mixing was achieved, sample locations were moved to monitor the air directly surrounding the area where the human subject would reside during future human exposures.

To determine whether the differences in mean dust concentrations were statistically significant throughout the exposure runs compared to the individual exposure runs, a one-factor analysis of variance (ANOVA) statistical test was performed using SAS 6.12 (SAS Institute, Inc. Cary, NC). This analysis illustrated that the difference between the means was not statistically significant at the 95 percent confidence interval.

The average dust concentration logged by the PCAM was compared to the mean dust concentration obtained from the total dust samples. An R^2 value of 0.825, $n = 10$, and a slope of 1.2 were obtained from data using linear regression.

The logged PCAM measurements were also used to monitor the consistency of the dust generator throughout exposure runs.



Total dust samples were collected at three locations in each chamber run. Sampling locations are indicated on the chamber diagram and the bar graph below.

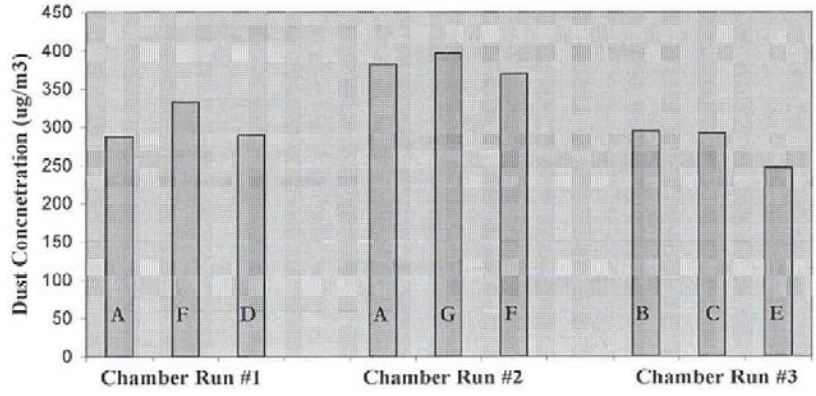


FIGURE 5
Total dust concentrations in each chamber run (by location).

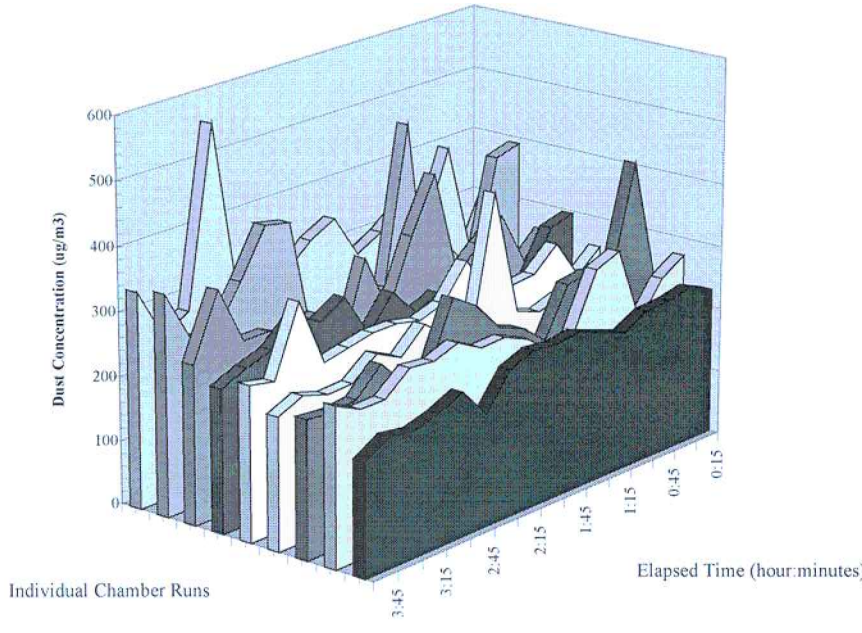


FIGURE 6
Dust concentrations throughout individual chamber runs.

TABLE I
Early exposure runs illustrating endotoxin aggregates

Media description	Chamber run (8196)[A] Endotoxin concentration (ng/mg)	Chamber run (10296)[A] Endotoxin concentration (ng/mg)	Chamber run (82196)[B] Endotoxin concentration (ng/mg)
Impactor stage 5 (1.7 um)	164	387	3614
Impactor stage 4 (2.8 um)	186	765	2646
Impactor stage 3 (4.2 um)	315	1266	2779
Impactor stage 2 (10.2 um)	213	4637	1186
Impactor stage 1 (11.7 um)	5200	10770	2600
Impinger sample (mean n = 2)	330	N/A	N/A
Total dust filter (mean n = 2)	N/A	1012	2558

[A]Original acetone preparation, without additional sonication.
[B]Modified acetone preparation, with additional sonication.
N/A not applicable during this chamber run.

If the dust generator dispersed an inconsistent aerosol concentration, then the logged dust concentrations from the PCAM would show a high coefficient of variation. The coefficient of variation throughout the nine dust runs (n = 8) was 19 percent. Figure 6 depicts the chamber dust concentration in 15-minute intervals throughout the 4-hour exposures.

Endotoxin Concentrations

The endotoxin preparation was adsorbed onto the cellulose particles using an acetone extraction process. The size distribution of the endotoxin aerosol was verified by light microscopy using a porton graticle and cascade impactor filters. LAL analysis was conducted on every collected sample to verify a uniform endotoxin concentration in each size distribution of the dust.

During early exposures, the endotoxin content of total dust filters did not reflect the expected endotoxin concentrations (Table I). Several runs were repeated with a preparation of the endotoxin to determine whether sampling conditions during the dust dispersion were causing a reduction in endotoxin concentrations. In each run, cascade impactor samples were compared to total dust filters or impinger samples. However, the LAL analysis of cascade impactor filters indicated that high concentrations of endotoxin were occasionally collecting on upper stages of the cascade impactor (Table I, column 1–2). This data suggested that there were aggregates of endotoxin in the bulk dust that would rapidly settle after dispersion and occasionally collect on the upper stages of the cascade impactor. In response to these findings, the acetone preparation process was refined to include an additional step of sonication. After these new steps were introduced, the endotoxin content in the aerosolized total dust samples and the cascade impactor filters correlated closely (Table I, column 3). Subsequent cascade impactor sample data from other exposure runs confirmed that the endotoxin concentration was representative throughout different size distributions and on total dust filters (Table II).

TABLE II
Endotoxin concentrations on select impactor stages

Media description	Exposure run (82196) Endotoxin concentration (ng/mg)	Exposure run (09496) Endotoxin concentration (ng/mg)	Exposure run (102396) Endotoxin concentration (ng/mg)	Exposure run (101196) Endotoxin concentration (ng/mg)
Impactor stage 5 (1.7 um)	1930	1778	2420	2356
Impactor stage 4 (2.8 um)	1680	1785	1384	1774
Impactor stage 3 (4.2 um)	3807	2085	1540	1366
Total dust filters	1507[A]	1351[B]	1766[B]	1706[B]

[A](mean, n = 2).
[B](mean, n = 3).

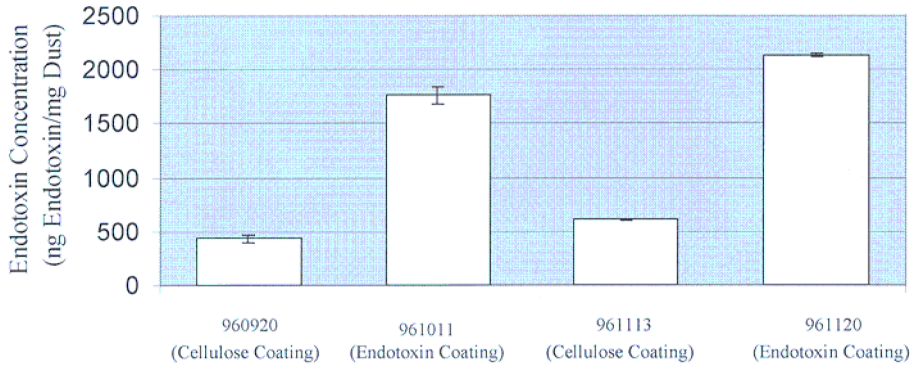


FIGURE 7
Wall coating effect on endotoxin concentration.

After numerous chamber endotoxin trials, it also became evident that the aerosol material used to coat the chamber walls affected the endotoxin levels in the total dust filters. Prior to this discovery, chamber walls were coated with inert cellulose in an effort to conserve endotoxin material. As shown by Figure 7, when inert cellulose was used to condition the walls, the subsequent exposure run results yielded total dust samples with approximately half the expected endotoxin concentrations. However, after the endotoxin aerosol was dispersed to coat the walls, the endotoxin results were within the desired range. Prior to all subsequent runs, chamber walls were conditioned with endotoxin treated cellulose.

After learning of this phenomenon and adjusting the experiments accordingly, eight exposure chamber runs were conducted. Figure 8 illustrates the mean endotoxin concentration from the total dust filters during several exposure runs. Given the 20-percent coefficient of variation of the LAL analysis in the laboratory, the consistency of the endotoxin concentrations is impressive.

DISCUSSION

The extensive preparation process has shown that there are complex aerosol interactions occurring inside the exposure chamber. The seemingly simple task of dispersing an organic dust into a chamber and controlling it at low concentrations is quite complicated. However, the final result of the evaluative process is a well-characterized chamber operating system with consistent endotoxin concentrations.

Although not an original goal, the lessons learned throughout the chamber characterization may be the most significant aspect of this research. For instance, the need to coat chamber walls with the specific dust to be dispersed in the chamber exposure was an unexpected necessity. The investigators noted that the cellulose dust exhibited a strong static charge during preliminary runs with the dust generator. The "lost" endotoxin concentration is attributed to the electrostatic properties of both the inert cellulose particles and the endotoxin-coated particles. Amid other aerosol investigators, it appears that the knowledge to coat chamber walls to stabilize dust concentrations is common, but

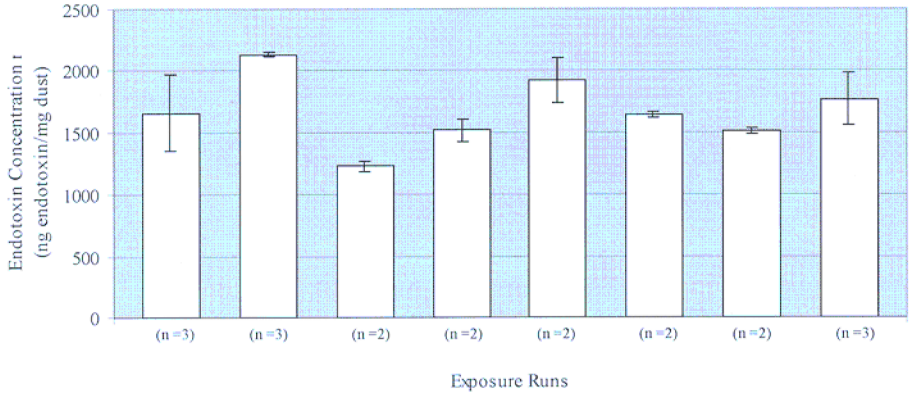


FIGURE 8
Observed endotoxin concentrations throughout exposures.

the exact theory behind the practice is uncertain. Recent research has investigated the relationship of particle deposition to air velocities in an indoor environment.[25] The behavior of an aerosol to adhere or deposit on a chamber wall may be related to deposition problems during the production of wafers in many "clean" industries. Given the diversity of applications, the specific aerosol behavior behind the wall phenomena offers a unique opportunity for research.

CONCLUSIONS

The results of this study confirm the chamber operating system achieves the three primary goals of this study. Given the importance of endotoxin in several occupational settings, the value of a successful chamber operating system is significant. With some modifications in dust generator rotation speed, dust type, and ventilation configuration, this chamber could be used for other investigations such as inhalation studies, respirator fit testing, and even controlled exposures to radioactive particles. Additional investigators could also utilize a similar characterization process in other aerosol exposure chambers.

However, this study also emphasizes the need for a lengthy evaluation period before an aerosol chamber can be used for human exposures. Exposing human subjects to low concentrations of an organic dust is extremely difficult and should be approached as a formidable task. Study limitations include the inherent variability of the LAL analysis techniques and complicating aerosol factors such as dust dispersion and aerosol electrostatic properties. Researchers should recognize the challenges associated with endotoxin aerosols and the complexity of interacting factors in a chamber operating system. Only after researchers fully evaluate chamber mechanisms can confidence in a chamber characterization be obtained.

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