Pilot Study of Residential Air Sampling Methods for Endotoxin

Lisa Pheatt

Abstract In light of research suggesting that endotoxin is an asthma 'trigger' and may play a role in childhood asthma development, this study was performed (1) to develop a sampling regime suitable for use in residential environments to measure airborne endotoxin concentrations and (2) to obtain information on the size of particles associated with airborne endotoxin. Indoor air was sampled in six Northern California homes for periods of 24 hours to 3 weeks using at least two particle-size-selective samplers. The samplers included a personal cascade impactor, a Harvard-type impactor, a cyclone/filter cassette, and a filter cassette alone. Each sampler was individually attached to a pump that was acoustically insulated to minimize noise. Phase 1 results suggested that samples of airborne dust of <0.25 mg may not be sufficient to measure endotoxin concentrations above the background of the analytical procedure. This was remedied in Phase 2 by collecting higher air sample volumes and baking the filters before use, a procedure that removes background endotoxin. The endotoxin content of the Phase 2 samples is not yet available, but the mass measurements indicated that cleaner environments may require collection of air sample volumes >3 x 10^4 m^3 to obtain a minimum sample mass of 0.25 mg. This was achieved most efficiently and conveniently with the Harvard-type impactor. Data from Phase 1 indicated that endotoxin concentrations were highest for particles 3.5 and 6.0 μm. This association with particles smaller than 10 μm is significant in assessing the related inhalation exposure to endotoxin as particles <5 μm can be deposited in the alveolar region of the lungs where the body responds differently than to dust deposited in the nose or throat. These findings differ from what has been seen in occupational environments, indicating that more research is needed in residential settings.

Introduction

Bioaerosols pose significant health risks to agricultural workers and their families in the state of California. Work in fields or processing plants exposes many workers to airborne contaminants that can cause chronic respiratory illnesses, among other health problems. As a result, studies on the environmental health risks for these workers and their families are extremely important and represent a growing field of research (Nieuwenhuijesen *et al*., 1998). One such bioaerosol is endotoxin, which is a lipopolysaccharide molecule that composes part of the outer membrane of Gram-negative bacteria (GNB). Common examples of GNB include species of *Aeromonas, Citrobacter, Enterbacter, Escherichia,* and *Pseudomonas*. GNB and endotoxin are commonly present in agricultural and textile manufacturing settings where they may become health hazards if they are aerosolized during the processing or decomposition of organic materials (Milton, 1999). GNB, which can be ingested via the gut and inhaled into the lungs, was associated with disease as early as 1942, when GNB first were studied as contaminants in drinking water. Subsequent studies suggest that endotoxin itself acts as an immunotoxicant, causing a variety of acute inflammatory responses in exposed persons including fever, increased asthma severity, mucous membrane irritation, chronic bronchitis, byssinosis, and toxic pneumonitis, and hypersensitivity pneumonitis (e.g., "humidifier fever" and "farmer's lung")(Milton 1996; Gyntelburg *et al*, 1994; Olenchock, 1994). On the other hand, some studies suggest that mild exposures to aerosolized endotoxin can actually be beneficial in some cases, as such exposures appear to stimulate the immune system.

Endotoxin's toxic properties have been the subject of numerous studies over the years, however, the connection between any particular endotoxin exposure and respiratory illness is still poorly understood. This problem is a result of many factors, including inconsistent associations between exposure and adverse health affects, limitations on the accuracy of current endotoxin analysis techniques, and only very limited information on the relationship between concentrations of endotoxin in air and settled dust (the latter being an easily obtained material often used for qualitative assessments of endotoxin exposure). Many of these unknowns for endotoxin persist because of underdeveloped sample collection techniques.

This study represents an attempt to expand our knowledge of endotoxin and its toxicity by developing and testing a new sampling method which will address two important properties of

this toxin which have yet to be established — (1) this toxin's particle size distribution and (2) its presence and behavior in residential settings.

Endotoxin has been studied extensively as an occupational hazard. However, few of these studies have investigated the particle size range of this toxin and, of those that have, none agree. Furthermore, as sampling for airborne endotoxin in residential settings is extremely new, no attempt has ever been made to study endotoxin's particle size range in homes. Particle size is considered important information for any airborne toxin as the hazard associated with the inhalation of a particle is due in part to its size and mass, as this dictates the mechanism and location of its deposition in the human respiratory tract. In this way, establishing the approximate particle-size distribution for endotoxin is a critical element in determining its toxicity or health effects. Previous studies on this topic include Attwood *et al*. (1986), who reported that 40% of the total endotoxin they collected in swine confinement buildings was between 3.5 and 8.5 μm in diameter, Thorne *et al*. (1996) who reported a median diameter of 3.1 μm for grain dust and Monn and Becker (1999) who reported a higher concentration of endotoxin in the coarse (>10 μm) particle fraction of outdoor air. The differences in these studies' results may be due to actual differences in the airborne material in the different study environments or to the use of different collection techniques.

Past studies on airborne endotoxin primarily have been conducted in agricultural settings, as these environments tend to have abundant sources of GNB and thus are more likely to constitute a health risk from high endotoxin exposures for workers. However, the presence of airborne endotoxin in residential settings should not be overlooked, as there are many indications that endotoxin may play a part in the development of and possibly protection against pediatric respiratory disease. As a recent study suggests, infant exposure to increased house dust concentrations of endotoxin may actually protect against allergen sensitization by enhancing a certain type of immunity (Gereda *et al*, 2000). However, these data must be considered along with other information that support the notion that endotoxin exposure aggravates severe immune responses in certain people. The presence of ambient endotoxin in homes near agricultural areas is of particular interest because there exists a potential for high concentrations of such bioaerosols in these rural environments. Such exposures may present real health risks to residents and these remain largely undocumented. For studies on these risks to ensue, a suitable air sampling scheme must be developed.

Residential air sampling for endotoxin, as opposed to occupational sampling, presents new challenges, as the home environment requires accommodation for the practical needs of the occupants. These include a sample set-up that is considerate of space-, time-, and energy-conservation needs, as well as noise level. These logistical challenges are compounded with experimental challenges presented by the nature of endotoxin. As a substance with a considerable (and assay-specific) limit of detection, an effective sampling scheme may require that substantial sample volumes be collected in less contaminated environments.

To further the development of airborne endotoxin collection techniques, my study examined and tested options for measuring ambient endotoxin using a size-selective sampling system for use in residential settings. The air sampling methods that I tested will (1) provide an accurate measurement of the concentration of airborne endotoxin, (2) identify the distribution of endotoxin in different particle size fractions, and (3) be suitable for residential use. This study began with a review of past sampling methods. Several of these methods were modified for application in residential environments and tested at six sites in northern California.

Methods

Overview A review of past studies revealed that almost all airborne endotoxin measurements have been made in occupational environments. As the requirements of a sampling system in these environments are notably different from those in residential settings, past techniques need modification for residential applications. The first important difference between these two types of sampling environments is the relative abundance of ambient endotoxin. Endotoxin concentrations in many occupational environments, such as livestock houses and textile manufacturing plants, are quite high relative to typical concentrations found in residential settings. Thus, the minimum sample volume required to collect a detectable amount of endotoxin is smaller in highly contaminated (occupational) environments than in cleaner (residential) environments. That is, it is necessary to sample a greater volume of residential air, either via higher flow rates or longer sampling times, to collect a detectable amount of endotoxin.

$$\text{Endotoxin concentration (EU/m}^3\text{)} = \frac{\text{amount of endotoxin measured (endotoxin units or EU)}}{\text{volume of air sampled (cubic meters of air or m}^3\text{)}},$$

where

$$\text{air volume (m}^3\text{)} = \text{sampling flow rate (m}^3\text{/min)} \times \text{sampling time (min)}.$$

Secondly, occupational methods often utilize high flow-rate devices that are quite efficient for endotoxin collection but not suitable for use in residential settings because these systems emit a level of noise that can be disruptive to occupants. An effective sampling protocol for residential applications must be thoughtful of these differences. In addition to these considerations, the method developed in this study also was designed to examine the particle-size range of ambient residential endotoxin. Thus, the method developed in this study met several unique challenges. These were to (1) accurately and efficiently measure endotoxin concentrations in residential environments, (2) identify the particle-size distribution of residential endotoxin, and (3) accomplish these tasks via a method acceptable to residents. Qualitatively, development of this method involved the selection of a compact sampling train equipped with a medium to low flow-rate, particle-size separating sampler equipped with a quiet, AC adaptable pump, so as to minimize disturbance to residents over the course of the requisite sampling time.

Review of previous methods for endotoxin sampling There are a variety of sampling devices that can be used for endotoxin collection. Most of them utilize inertial impaction mechanisms or filtration. Impactor samplers include a family of liquid impingers and single-stage and multiple-stage ("cascade") impactors. Filtration samplers include a variety of open- and closed-face cassette filters with or without cyclone pre-separators, and the IOM (Institute of Occupational Medicine) sampler. There are also possible endotoxin applications for gravitational samplers, such as the passive aerosol sampler (Wagner and Leith, 2001). The passive sampler, which by definition requires no pump, is ideal for many residential applications as it is small and, more importantly, completely silent. However, passive samplers suffer from an inability to collect sufficient material for endotoxin analysis. In an effort to develop current sampling techniques, I reviewed environmental endotoxin studies dating from 1980 through 2000. A number of these impaction and filtration methods were used in these studies, however, no study I reviewed has ever been performed to compare a large number of these methods.

Among past studies, the most commonly used devices for endotoxin collection were filtration samplers. Liquid impingers, which collect airborne particles in a liquid medium as opposed to a filter, have been used as well but have been far less popular than filtration methods. This may be due in part to the relative ease of use and generally higher particle removal efficiency of a filter medium compared to a liquid medium. In a study that specifically compared AGI-30 impingers and a filtration sampling system, it was found that, in environments with high concentrations of endotoxin, the impinger was more efficient than the filtration set-up for endotoxin collection (Zucker *et al*., 2000). However, this study found this difference in efficiency in *occupational* environments with high levels of endotoxin –(calf and pig houses). Zucker *et al*. found no significant difference between the sampling systems in less-contaminated environments. As residential environments will have considerably less ambient endotoxin, this study suggests that a filtration system should perform as well as the impinger. Furthermore, there were other reasons to use a filtration or impaction sampling system for the purposes of my study.

Firstly, many impingers suffer from fluid loss (via evaporation) over the course of an extended sampling period that could contribute to a loss of collection efficiency. More importantly, single-stage impingers, of the sort tested in Zucker *et al*.'s study, are not particle-size separating samplers. For the purpose of examining the particle-size range of endotoxin, samplers with particle-size-specific collection stages or size-specific pre-separators are most suitable because they can separate airborne particles according to known size fractions. Furthermore, since high flow-rate samplers may be too noisy for use in a bedroom, samplers that require high flow rates, such as impingers, are not suitable for use in a residential study. Lower flow rate filtration and impactor samplers are a better option for this application.

The filtration and impactor samplers that have been used fall under the following general categories: cyclone pre-separators fitted with filter cassettes (cyclone/filter cassette), vertical elutriators, plastic filter cassettes, single-stage impactors, and cascade impactors. Each of these samplers has different merits for use in endotoxin or size-specific sampling. Unfortunately, no published study has been performed to compare such samplers' endotoxin collection efficiencies. Thus, I selected a group of suitable samplers of this kind for comparison in my study.

Choosing the sampling design

a) Samplers. One constraint on the design of this study was the limited pool of equipment available for testing. I had access to a variety of equipment owned by the California Department of Health Services Environmental Health Laboratory. Among those available and potentially useful for residential applications were plastic Millipore Filter Cassettes, a Passive Aerosol Sampler, IOM Inhalable Dust Samplers (SKC, Eighty Four, PA), a GS Cyclone/Filter Cassette sampler (SKC, Eighty Four, PA), a Marple 290 Personal Cascade Impactor (Andersen Instruments, Smyrna, GA), and a Harvard-type (PM10) Impactor (MS&T Area Sampler, Air Diagnostics and Engineering, Harrison, ME). Four of these samplers were tested: the cyclone/filter cassette sampler, the Harvard-type impactor, a filter cassette without a cyclone, and a cyclone/filter cassette sampler. Table 1 lists the flow rates and aerodynamic particle size ranges for the four samplers.

Sampler	**Manufacturer**	**Flow Rate (L/min)**	**# of Size Fractions**	**d_a Range (μm)**	**Filter Type**
GS Cyclone with filter cassette	SKC, Inc.	2.75	1	0– 4	Teflon
Harvard-type (MS&T) Impactor	Air Diagnostics and Engineering	10	1	0–10	Teflon
Filter Cassette (without cyclone)	Millipore Corp.	2	1	0–(>10)	Teflon
Marple Personal Cascade Impactor	Andersen Instruments, Inc.	2	5	0–(>10)	Phase 1: Mylar Phase 2: Teflon

Table 1. Samplers used

b) Filter type. Studies that compared filter types for particle recovery efficiency were used to determine which types are better for endotoxin applications. Douwes *et al*. (1995) found that the use of glass fiber, Teflon, or polycarbonate filters resulted in a two-fold increase in the level of detectable endotoxin over the use of cellulose-ester filters. In a more recent study, Taylor *et al*. (2000) assigned the best collection efficiency to glass fiber filters, noting that gelatin media had the highest recovery of endotoxin but is also much harder to manage than other filter media. As Taylor *et al*.'s study did not test Teflon filter types, these studies suggested that either glass fiber or Teflon filters would be reasonable options. As a matter of convenience, Teflon filters were used. The cascade impactor was used with mylar filter substrates in Phase 1 and Teflon substrates in Phase 2 with stages 3, 4, 5, and 6 in place (respective 50% cut points at 2 L/min of

9.8, 6.0, 3.5, and 1.55 μm) (Rubow *et al*., 1987). Teflon substrates were used in Phase 2 because it was determined that baking filters prior to use might reduce the background concentration of endotoxin. Teflon filters withstood baking better than Mylar.

c) Filter preparation. The Teflon filters used in all samplers for the first five trials were unbaked, while those for the sixth were baked as a measure to reduce the background levels of endotoxin that were observed in the preliminary results. Filters were placed in glass dishes in an oven and held at a temperature of 160–170°C for ≥2 hours.

All filters were conditioned in a weighing chamber for 24–48 hours before pre- and post-sample weighing. Temperature and relative humidity in the weighing chamber were ideally to be maintained at 22 ± 2°C and 44 ± 4%, respectively. Unfortunately, humidity conditions in the chamber were not constant and, due to time constraints, weighing was performed in some cases despite differences in the humidity level during pre- and post-weighing conditioning periods. Filters were weighed for total suspended particulate matter with a Cahn 26 Microbalance (Cerritos, CA) after passing each filter over a charge-neutralizing Po^{-210} strip (NRD Inc., Grand Island, NY). These procedures allowed masses to be measured to within 1 μg for all filters. All samples, including field blanks, were desiccated with Drierite™ once post-weighed to reduce the possibility of bacterial multiplication and change in endotoxin content. This step was eliminated in Phase 2 on notification from the analytical laboratory later that bacterial growth on filters at ambient indoor conditions was very unlikely.

d) Sampler Operation. In air sampling, the sampler-type in conjunction with the estimated concentration of the material being sampled dictate the flow rate and thus the pump type used for a sampling event. Low flow-rate samplers, such as the personal cascade impactor, the cyclone/filter cassette, and filter cassette, are commonly used with small, personal sampling pumps. Flow rates for these samplers vary from 1.5 to 2.75 liters per minute (L/min) (Table 1). The Harvard-type (PM10) impactor is designed to be operated at a substantially higher flow rate of 10 L/min. Operation of samplers at this high a flow rate can be noisy because larger air movers are needed. However, this can be remedied to a certain extent if the pump is well insulated acoustically. One such device is the SP-280 series pump (Air Diagnostics Inc., Harrison, Maine). This pump was selected for my study as it is ideal for such applications. It is AC adaptable, contained in an acoustically insulated case, capable of supporting operation of multiple samplers, and self adjusts for pressure changes over the course of extended sampling.

The pressure drop of a sampler changes as material is collected. Without compensation, flow rate through a sampler would decrease with time. The SP-280 pump also is conveniently equipped with a self-timer, which allows for precise start and stop times and automatic (unattended) operation. Separate flow rates for the samplers (Table 1) were maintained with a series of valves (Figure 1). Individual flow rates were measured with in-line rotameters. All samplers, rotameters, and valves were supported by a single ring stand, which was placed in each sampling location on a level surface at a height of 1.2 m (4 ft) above the floor and 0.3 m (1 ft) from the nearest wall (Park *et al*., 2000) (Fig. 2).

Data Collection

a) Sampling overview. A study lead by Jeff Wagner, Ph.D., conducted at the California Department of Health Services' Environmental Health Laboratory, constituted Phase 1 of this study. Dr. Wagner's specific interest was to compare the sampling efficiencies (in terms of recovered mass) of four different samplers, a cyclone/filter cassette, the IOM, a Harvard-type impactor, and a personal cascade impactor, with a passive aerosol sampler he designed (Wagner and Leith, 2001). Dr. Wagner treated the samples appropriately for later endotoxin analysis and, subsequent to the completion of his project, donated them to my study. Endotoxin results from these samples, which were taken at residences in Oakland (Site 1), Soledad (Site 2), and Richmond (Site 3), were instrumental in recommending a sample design. Phase 2 was composed of 3 additional sampling events at residences in Berkeley (Site 4), Albany (Site 5), and Sonoma (Site 6). At Sites 4 and 5, the cyclone/filter cassette, the Harvard-type impactor, and a close-faced filter cassette were used. At Site 6, the cyclone/filter cassette and filter cassette were removed from the set up and the cascade impactor was used along side of the Harvard-type impactor. Figure 1 and Tables 2 and 3 describe the sampling set-up at each of the six sites.

Site #	Residence Location	Sampling Time per Sample	Sample #	Activity in Room	Outdoor Surroundings
1	Oakland, CA	24 hours	1	yes	Urban, residential (mixed housing, near highways)
2	Soledad, CA	1 week	7	yes	Rural, residential and agricultural (single-family homes)
3	Richmond, CA	2 weeks	1	no	Urban, residential and commercial (mixed housing, near construction)
4	Berkeley, CA	24 hours	3	yes	Urban, residential (mixed housing, near highways)
5	Albany, CA	24 hours	7	yes	Urban, residential (single-family homes with yards)
6	Sonoma, CA	48 hours 14 days	7 1	yes	Rural, residential and agricultural (single-family homes)

Table 2. Sampling environments

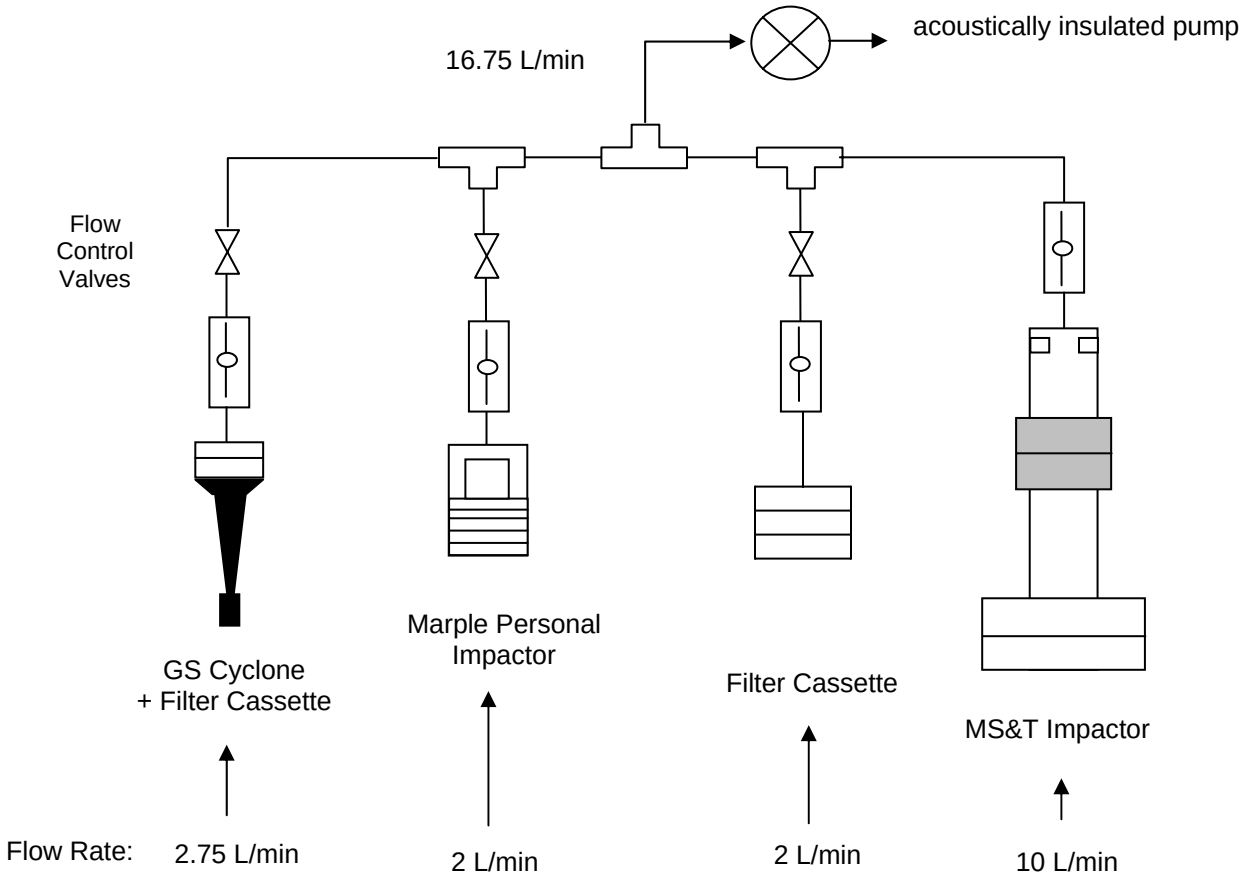

Figure 1. Sampling setup. Cassette filter samples were not used at Sites 1–3. Personal impactor samples were not taken from Sites 4 and 5. Cyclone and filter cassette samples were not taken from Site 6. (Modified from Wagner and Macher, 2001) See Table 4 for details on which samplers were used at each site.

Site #	Harvard-type (MS&T) Impactor	GS Cyclone with Filter Cassette	Filter Cassette without Cyclone	Marple Personal Cascade Impactor
1	●	●	-	●
2	●	●	-	●
3	●	●	-	●
4	●	●	●	-
5	●	●	●	-
6	●	-	-	●

Table 3. Samplers used at each site

b) Sampling location and participant involvement. Because my study was a component of a longitudinal exposure study being conducted by the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), the sampling locations were chosen with respect to the goals of the CHAMACOS project. Hence, at each residence, the sampling train was to be assembled and left undisturbed as close as possible to the place where a child sleeps, assuming there was a child in residence at that site (Fig.2). Otherwise, the sampling train was placed in another occupied bedroom. Residents of each sample environment participated in data collection in several capacities. They were asked to (1) check and adjust sampler flow rates, if necessary, (2) record household activities on a sample tracking form, and (3) load and unload samplers at specified time intervals. Participants were provided with written instructions as well as special training on sampler operation.

Figure 2. Sample location at Site 5, Albany, CA.

c) Sample number and sampling period. The sampling schedule varied because the goals of Phases 1 and 2 differed and the scheme was revised as information became available (Tables 3 and 4). A constant goal was to account for potential within-house variability in endotoxin concentration over time. Therefore, sampling times were ≥24 hours as a provision to collect a detectable amount of endotoxin from residential environments. Phase 1 samples were collected continuously, while Phase 2 samples were collected over 24 or 48-hour periods separated by 24-hour breaks to allow for the filters to be changed.

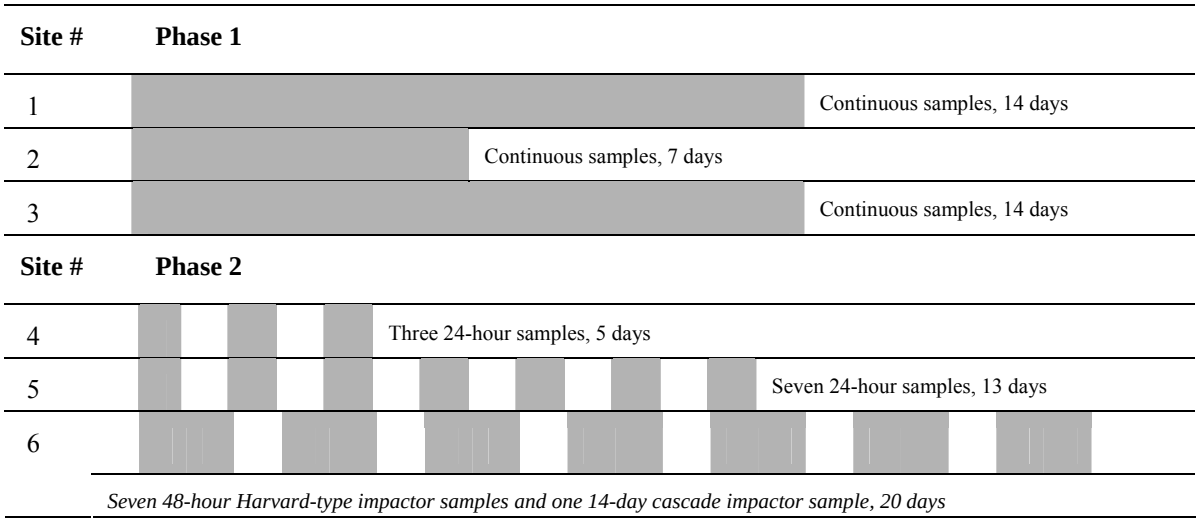

Table 4. Numbers of samples and sampling periods by site (See Table 3 for samplers used in each event.)

Endotoxin results from Phase 1 samples were received from the analytical laboratory just prior to the start of sampling at Site 6. Consequently, Phase 1 endotoxin data were guided for changes in sample design for the trial at Site 6 only. Phase 1 data indicated that 24-hour samples may not collect a detectable amount of endotoxin and that reliable information from the cascade impactor was obtained only at Site 1, so several measures were taken to remedy these problems. First, the lower flow rate samplers, the filter cassette and cyclone/filter cassette, were eliminated from the set-up and the personal cascade impactor was added. Seven samples again were collected with the Harvard-type sampler, to estimate day-to-day variability, but sampling time was increased from 24 to 48 hours. A single sample was collected with the cascade impactor to gather more information on particle size distribution, because this sampler operates at a lower flow rate than the Harvard-type sampler. Both samplers were operated on the same days, but the

collection substrates in the cascade impactor were not changed during the 20-day study period. Finally, filters for this trial were baked prior to use to reduce background endotoxin.

d) Endotoxin analysis. All samples and lab blanks were analyzed using a method known as the *Limulus* amebocyte lysate (LAL) assay. This is a comparative method that measures the biological reactivity of samples in a colorimetricassay system. The LAL method estimates the *relative toxicity* of a sample rather than providing a quantitative estimate of endotoxin concentration (Milton, 2000). Values were reported in endotoxin units per cubic meter of air (EU/m^3). Phase 2 samples were not analyzed for endotoxin concentrations prior to the completion of this project, thus, only mass data were available for analysis at these sites. Insight gained from comparison of collected dust mass and endotoxin concentration for the Phase 1 samples was used to evaluate the success of the methods used in Phase 2.

Results

Mean particle mass concentrations and particle size distributions varied by site.

As detailed in Figure 3, mean mass concentrations ($\mu g/m^3$) for particles sampled by the Harvard-type impactor were comparable for Sites 2, 3, 5, and 6 while Sites 1 and 4 had roughly twice the mass concentrations of these sites.

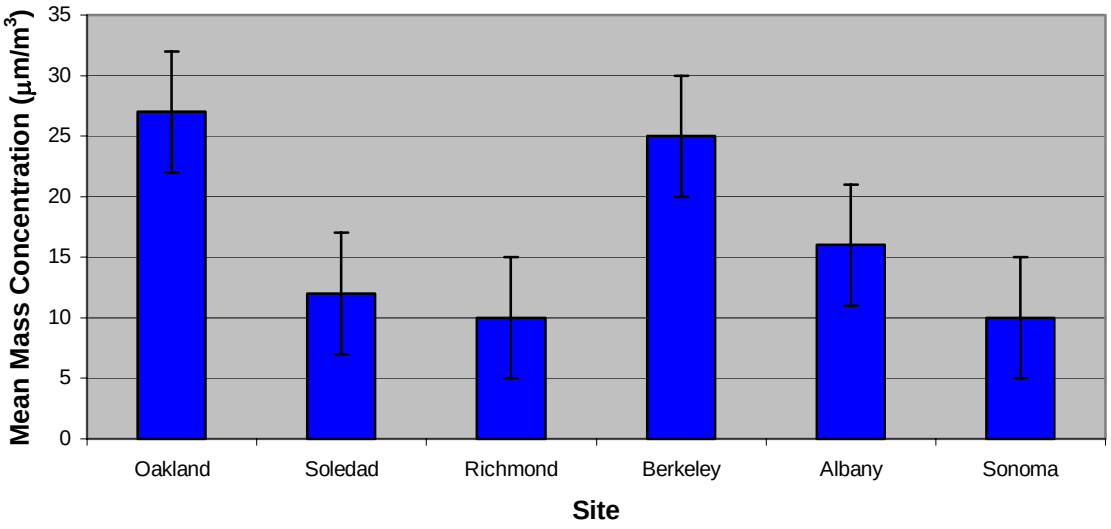

Figure 3. Mean mass concentration as measured by the Harvard-type sampler.

Figure 4 shows particle-size distributions determined by cascade impactor measurements for Sites 1–3 and 6. (Cascade impactor samples were not taken at Sites 4 and 5.) Site 1 was characterized by a much higher percentage of fine particles than the other 3 sites. This is quantitatively represented by the mass median aerodynamic diameter (d_{50}) for each site. The d_{50} value is the particle size associated with the median mass on a particle size distribution plot, as in Figure 4. D_{50} values are helpful in characterizing the composition of airborne particulate in each sampling environment. D_{50} values for Sites 2, 3, and 6, were 5.8 µm, 6.8 µm, and 7.2 µm respectively, while the d_{50} for Site 1 was1 µm. These values demonstrate that airborne particles of the inspirable (inhalable) mass fraction at Sites 2, 3, and 6 have similar size distributions and contain fewer fine (<2 µm) particles than Site 1, where approximately 50% of the inspirable mass fraction was <1 µm in size.

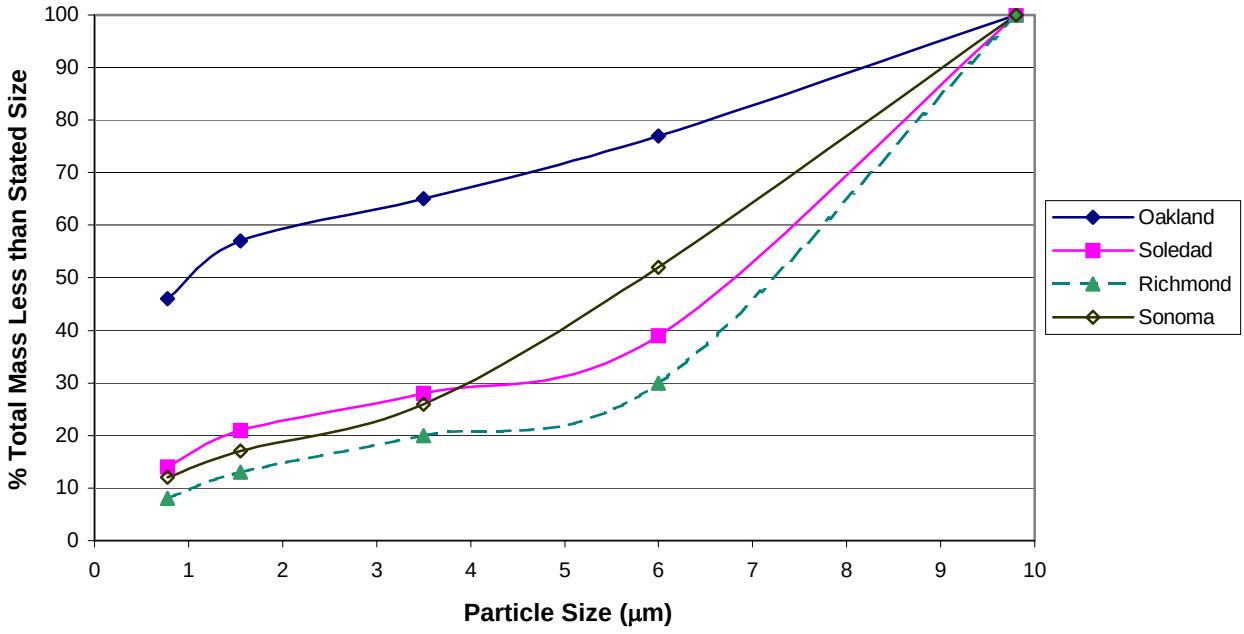

Figure 4. Particle size distributions for Sites 1-3 and 6. Note that these size distributions pertain to all dust collected in each sample. See Figure 5 for endotoxin particle size distribution.

Although mean mass concentrations (µg of dust/m^3) in each environment did not vary significantly among samplers, mean sample masses (mg of dust/filter) did vary. This was expected because higher flow rate samplers collect a larger volume of air per unit of sampling

time than lower flow rate samplers, thus, the amount of dust collected in a larger volume sample should be proportionately greater. Figure 5 shows masses on filters split by sampler type at Site 5, as an example of this difference. Mean mass collected and volume of air sampled by the Harvard-type impactor was ~5 times that of the cyclone/filter cassette and closed-face filter at Site 5 and by nearly as much at Site 4 because, although the sampling times were identical, the sampling rates differed in this proportion (10 versus 2.75 and 2 L/min). Sample masses for the Harvard-type sampler were similarly in excess of those for the cyclone/filter cassette sampler at Sites 1-3.

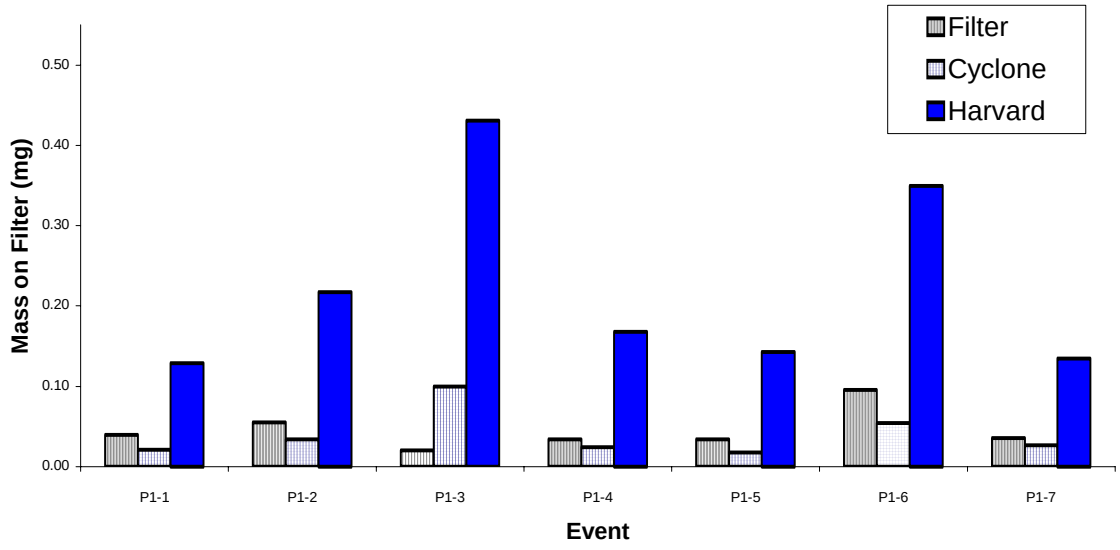

Figure 5. Mass on Filters at Site 5, Albany, CA.

This anticipated trend is important because endotoxin data from Phase 1 (Sites 1–3) indicated that samples collected by the lower flow rate devices (namely the filter cassette and the cyclone/filter cassette, even for 2-week sampling periods) were not sufficient in mass for endotoxin levels to be detected beyond background levels for blank filters (as analyzed in the laboratory of Dr. Daniel Lewis, the National Institute for Occupational Safety and Health (NIOSH), Morgantown, VA).

Phase 1 endotoxin results indicated that endotoxin in samples of less than 0.25 mg in mass may not be detectable. The cyclone/filter cassette and the filter cassette did not collect 0.25 mg of material in any of the Phase 1 or Phase 2 24-hour trials. The Harvard-type impactor

generally, but not always, collected higher masses. Among Sites 1, 4, and 5, where 24-hour Harvard-type samples were collected, sample masses from Sites1 and 4, the dustier environments, met or exceeded 0.25 mg in 4 out of 5 trials. However, at Site 5, a cleaner environment, only 28% of samples achieved this mass. Phase 1 endotoxin data was helpful in recommending a higher sample volume than had been used in the first 5 trials as well as different filter preparation for Site 6. Forty-eight-hour Harvard-type samples collected at Site 6 met or exceeded >0.25 mg in 6 out of 7 trials. Mean mass collected during these 48-hour sampling periods at Site 6 was 0.30 mg. Figure 6 shows endotoxin concentrations for 2-week samples taken in Phase 1 with the cascade impactor, Harvard-type impactor and cyclone/filter cassette. The cascade impactor and cyclone/filter cassette agreed closely on endotoxin levels for particles <4 μm (0.2 EU/m^3 and 0.1 EU/m^3, respectively). Richmond (Site 3) values were markedly lower and may be attributable to the fact that no activity occurred in the test room, which was unoccupied for the duration of the sampling period.

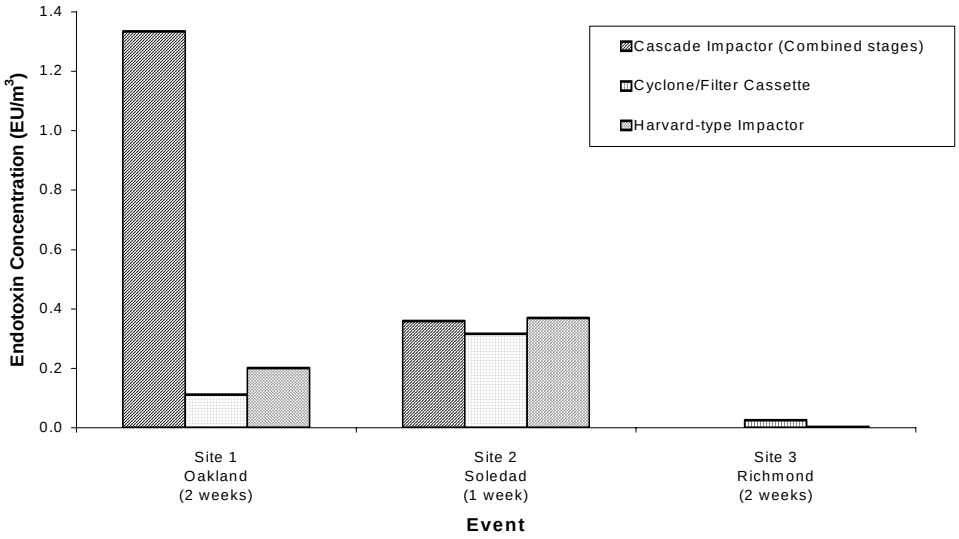

Figure 6. Endotoxin Concentrations (EU/m^3) for Phase 1.

Measurable amounts of endotoxin were not collected on all stages of the cascade sampler at Sites 2 and 3, therefore, this sampler was not used at Sites 4 and 5. Thus, legitimate endotoxin particle-size-distribution data was only available from Site 1. Figure 7 shows the particle size

distribution of endotoxin for Site 1, Oakland. As shown in Fig. 7, 49% of detected endotoxin was associated with particles between 3.5 and 6 μm in diameter.

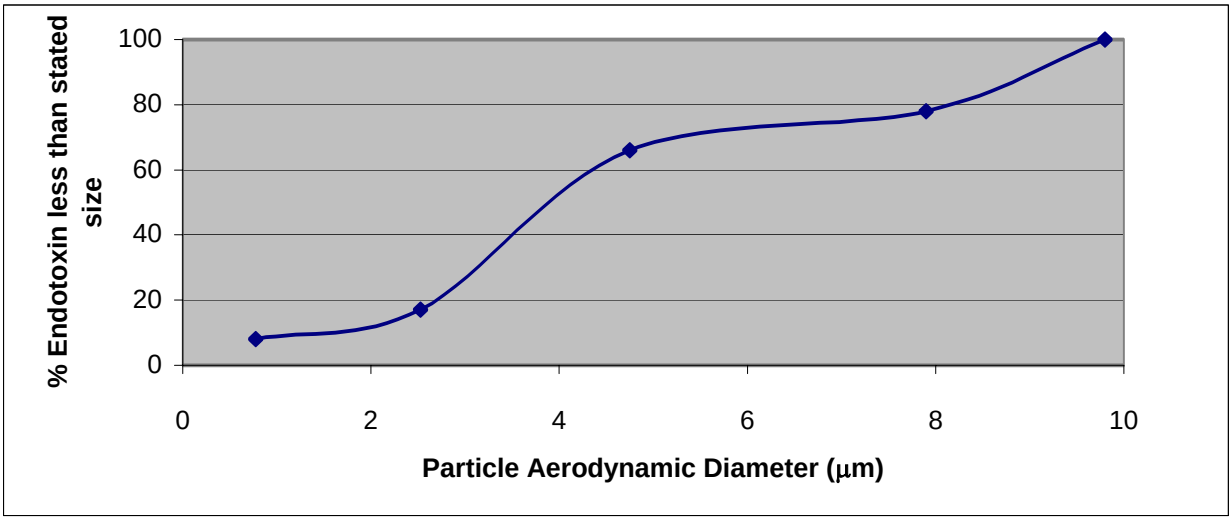

Figure 7. Particle size distribution of ambient endotoxin, Site1, Oakland.

Discussion

The success of each of the tested methods was measured by its ability to (1) collect sufficient dust to meet the limit-of-detection requirement for endotoxin, (2) perform this task efficiently, (3) minimize disturbance to residents, and (4) be supervised by study participants without difficulty or compromising the integrity of the samples.

The lower flow rate samplers, i.e., the cyclone/filter cassette, the filter cassette without cyclone, and the cascade impactor, suffered from an inability to perform tasks (1) and (2). Though these samplers are highly suited for residential applications in terms of their ease of use, they failed to collect enough material in 24 hours for endotoxin analysis in the lower-concentration, rural environments. As the goal of this study was to develop a method that was suitable for use in such environments, this finding is particularly significant. These samplers would have to be operating for prolonged periods, nearly 10 days, to accumulate the minimum sample mass sufficient for analysis that was established by Phase-1 endotoxin results. Such a long sampling time imposes several significant limitations on a study. Longer sampling times increase the chance of disturbances to residents and, likewise, the opportunity for experimental

errors due to sampler disturbance. Over time, volunteers may find it difficult to comply with the tasks asked of them and it is likely that potential participants who would agree to allow a sampler in their homes for short periods would not agree to a longer study. A lengthy sample time also would hinder studies that seek to include a large population within a limited timeline if sampling equipment is limited in quantity.

Unlike the other samplers, the Harvard-type (MS&T) PM10 impactor was effective in meeting the goals of the desired sampling scheme and is an improvement over past methods for measurement of airborne endotoxin. The standard method for endotoxin sampling in residential settings thus far was measurement of the concentration in settled dust samples. Not only are such bulk samples devoid of any particle size information, the relationship between the concentration of endotoxin in settled dust and air has not been established. In terms of the goal to collect particle-size information on residential endotoxin, this device works as an efficient sampler of particles of the inspirable mass fraction, <10.0 μm. This means that the (PM10) sampler collects all and only those particles that can be inhaled into the respiratory tract and is thus useful in providing human health effects data. In addition, data collected with this sampler can be compared directly to data from many other studies because ambient air quality data on airborne particulate matter is collected in this way and many researchers use PM10 samplers for indoor and outdoor measurements. However, unlike the cascade impactor, the Harvard-type impactor does not subdivide collected material into its constituent size fractions. This limits the specificity with which one may make determinations about the health effects of associated endotoxin exposures. This sampler is useful for gathering general information about how much endotoxin is inhaled by residents, but does not provide specific enough particle size information to identify where these particles are deposited throughout the respiratory tract.

Meeting all four goals of the sampling scheme, the Harvard impactor set-up was able to efficiently collect enough material for endotoxin detection and minimize disturbance to residents while being supervised by study participants without difficulty or compromising the integrity of the samples. Unlike the lower flow rate samplers, the Harvard-type impactor's 10 L/min flow rate was sufficiently high to collect enough material for endotoxin assay in 24–48 hours in most cases. If sampling had been conducted during the warmer months of the year, when airborne endotoxin is expected to be more abundant, it is likely that the margin of advantage of this sampler would be somewhat diminished, i.e., even the lower-flow-rate samplers may have

collected sufficient material. Despite concerns that the 10 L/min flow rate of this sampler might cause a noise disturbance to residents, all volunteers found that the acoustically insulated pump was successful in preventing such disturbances. Additionally, the ease with which this sampler's filters may be changed (uncontaminated) by laypersons is an advantage for this kind of study where several samples may be desired by investigators.

High background levels of endotoxin on the filters presented an unanticipated challenge for the tested methods. The use of filters with high background endotoxin content and the laboratory's limit of detection contributed to blanks occasionally showing higher endotoxin levels than actual field samples. These high blank levels may have been inherent in the filters as they are produced or the filters may have been contaminated during handling. My associates were not aware that the filters used as weighing blanks also would be analyzed as endotoxin blanks. Consequently, handling of the filters was not as careful as it should have been (for example, the filters were left unprotected for some periods and were used to demonstrate the sampling equipment). (Blank filters for subsequent events were kept in closed containers and handled only during weighing. As described earlier, this problem also was addressed for the samples collected at Site 6 by baking the filters before use to eliminate background endotoxin. Thus, although the amount of material collected on these filters was small, it is hoped that endotoxin will be detectable because the background contribution from the filter itself should be lower.)

Given that the particle size range for residential endotoxin has seldom been examined in previous studies, and that only one of my samples was successful to date, it is difficult to assign any significance to the particle size data currently available. The high concentration of endotoxin found on particles between 3.5 and 6 μm in diameter does agree with the particle size data for endotoxin found in certain occupational environments. Attwood *et al*. (1986) found 40% of endotoxin in swine confinement buildings in the 3.5–8.5 μm dust fraction. However, Alwis *et al*. (1999) found a greater proportion of endotoxin from wood dust in the <2.0 μm fraction. It appears that the particle-size distribution of endotoxin at a site may be related to the characteristic size distribution of particles in that environment. If this is the case, data from Site 1 is not characteristic of the particle size distribution for endotoxin that might be found in environments that are less dominated by fine particulate, such as Sites 2, 3, and 6 (Fig. 4). As the Site 1 mass concentration and distribution was unlike the other 3 sites where cascade-

impactor measurements were taken, the particle size distribution of endotoxin at these other sites may show different concentrations of endotoxin in the tested size fractions. Sites 1 and 4 may have been different in particle size distribution and concentration from the other four sites for several important reasons. Investigators at both Sites 1 and 4 noted causes for elevated particle concentrations, including a lit candle in the sampling room at Site 1 and visible settled dust at Site 4. In addition, both of these were multi-story apartment buildings in residential urban areas whereas the other sites were two-story, detached or semi-detached houses in less-urban, even rural residential areas.

This research has shown that the limit of detection for endotoxin poses a challenge for the collection of particle size-separating air samples in residential settings. Although correlations between sample mass and the limit of detection for endotoxin are laboratory specific and currently undocumented, the Phase 1 results suggest that sample masses of <0.25 mg may not be sufficient to measure endotoxin concentrations above background levels. The mean mass collected during the 48-hour sampling periods at Site 6 of 0.30 mg suggests that if 24-hour samples had been taken at this site, such samples may not have been sufficiently large for endotoxin analysis.

Overall, this study supports the notion that ambient levels of endotoxin in residential environments are low relative to occupational settings and that considerable attention must be paid to the sample volume in rural and semi-urban environments where particle composition and concentration may differ significantly. The Harvard-type impactor effectively balances this high–sample-volume requirement with the demands of residential sampling. However, the small size of this study in conjunction with the noticeable lack of previous data on this subject leave us with many unanswered questions. This work identified several areas that need further examination to understand conditions that affect the relationship between sample mass and endotoxin detection in similar environments. This work achieved its goal of identifying a suitable methodology for beginning such investigations.

Acknowledgments

My sincerest thanks to my mentor, Dr. Janet M. Macher, of the California Department of Health Services Environmental Health Laboratory, for all of her patient teaching, editing and creativity and to Dr. Matt Orr, my advisor at U.C. Berkeley, for his guidance, encouragement

and willingness to learn with me. Many thanks to Jeff Wagner, also of the California Department of Health Services Environmental Health Laboratory, for his technical assistance and help in planning and executing this study. And, finally, thanks to all of the study participants, Jed Waldman, Loreta Lozada Lynn, Donald Scales, and Ira Tager, for volunteering their residences.

References

Alwis, K., Mandryk, J., and A. Hocking. 1999. Exposure to Biohazards in Wood Dust: Bacteria, Fungi, Endotoxins, and (1→3)-β-Glucans. Applied Occupational and Environmental Hygiene, **14(9)**: 598-608.

Attwood, P., Versloot, P., and D. Heederik, *et al*. 1986. Assessment of Dust and Endotoxin levels in the working environmental of Dutch pig farmers: a preliminary study. Ann. Occupational Hygiene, **30**: 201-208.

Dowes, J., Versloot, P., and A. Hollander. *et al*. 1995. Influence of Various Dust Sampling and Extraction Methods on the Measurement of Airborne Endotoxin. Applied and Environmental Microbiology, **61(5)**: 1763-1769.

Gereda, J. E., Leugn, D.Y.M., Thatayatikom, A., *et al*. 2000. Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitization in infants at high risk of asthma. The Lancet **355**: 1680-1683.

Milton, DK. 1996. Worker exposure to endotoxin, Phenolic compounds, and formaldehyde in a fiberglass insulation manufacturing plant. American Industrial Hygiene Association Journal, **57**: 889-896.

Milton, DK. 1999. Endotoxin and other bacterial cell-wall components. Bioaerosols: Assessment and Control. Harvard School of Public Health. p. 1-23.

Monn, C., and S. Becker. 1999. Cytotoxicity and Induction of Proinflammatory Cytokines from Human Monocytes Exposed to Fine ($PM_{2.5}$) and Coarse Particles ($PM_{10-2.5}$) in Outdoor and Indoor Air. Toxicology and Applied Pharmacology, **155**: 245-252.

Nieuwenhuijsen, M., Kruize, H., and Schenker, M. 1998. Exposure to Dust and Its Particle Size Distribution in California Agriculture. American Industrial Hygiene Journal, **58**: 34-38.

Olenchock, S.A. 1994. Health effects of biological agents: The Role of endotoxins. Applied Occupational and Environmental Hygiene, **9**: 62-64.

Rubow, K.L., V.A. Marple, J. Olin, *et al*. (1987) A personal cascade impactor: design, evaluation and calibration. American Industrial Hygiene Association Journal. **48(6**): 532-538.

Thorne, P., J. DeKoster, and P. Subramanian. 1996. Environmental assessment of aerosols, bioaerosols, and airborne endotoxin in a machining plant. American Industrial Hygiene Association Journal, **57**: 1163-1167.

Wagner, J. and Macher, J. Comparison of passive aerosol sampler to size-selective pump samplers in indoor environments. (Submitted to American Industrial Hygiene Association Journal, February 2001.)

Zucker, B.-A., Draz, A., and Muller, W. 2000. Comparison of Filtration and Impingement for Sampling Airborne Endotoxin. Journal of Aerosol Science **31(6)**: 751-755.