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Electrostatic dust collectors compared to inhalable samplers for measuring endotoxin concentrations in farm homes

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Abstract

Paired electrostatic dust collectors (EDCs) and daily, inhalable button samplers (BS) were used concurrently to sample endotoxin in 10 farm homes during 7-day periods in summer and winter. Winter sampling included an optical particle counter (OPC) to measure $PM_{2.5}$ and $PM_{2.5-10}$. Electrostatic dust collectors and BS filters were analyzed for endotoxin using the kinetic chromogenic *Limulus* amoebocyte lysate assay. Optical particle counter particulate matter (PM) data were divided into two PM categories. In summer, geometric mean (geometric standard deviation) endotoxin concentrations were 0.82 EU/m³ (2.7) measured with the BS and 737 EU/m² (1.9) measured with the EDC. Winter values were 0.52 EU/m³ (3.1) for BS and 538 EU/m² (3.0) for EDCs. Seven day endotoxin values of EDCs were highly correlated with the 7-day BS sampling averages ($r=0.70$; $p<0.001$). Analysis of variance indicated a 2.4-fold increase in EDC endotoxin concentrations for each unit increase of the ratio of $PM_{2.5}$ to $PM_{2.5-10}$. There was also a significant correlation between BS and EDCs endotoxin concentrations for winter ($r=0.67$; $p<0.05$) and summer ($r=0.75$; $p<0.05$). Thus, EDCs sample comparable endotoxin concentrations to BS, making EDCs a feasible, easy to use alternative to BS for endotoxin sampling.

Keywords

bioaerosol; button sampler; electrostatic dust collector; lipopolysaccharide; optical particle counter

Introduction

Coarse particulate matter ($PM_{2.5-10}$) is the mass concentration of airborne particulate matter (PM) whose aerodynamic diameters are within the range of 2.5 to 10 μm . Fine particulate matter ($PM_{2.5}$) is the mass concentration of airborne PM whose aerodynamic diameters are less than 2.5 μm (Duquenne *et al.*, 2013; Schwarze *et al.*, 2006). Several studies have indicated that endotoxin, a lipopolysaccharide component of Gram-negative bacteria, adheres to the surface of coarse particles leading to alveolar macrophage activation (Soukup *et al.*, 2001; Schins *et al.*, 2004; Monn *et al.*, 1999). *In vitro* studies showing stronger inflammatory effects for exposure to $PM_{2.5-10}$ compared to $PM_{2.5}$ supports a role of endotoxin in the inflammatory process (Thorne *et al.*, 2015; Monn *et al.*, 1999; Soukup *et*

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et al., 2001; Schins *et al.*, 2004; Becker *et al.*, 2005; Thorne *et al.*, 2005). Endotoxin is associated with recurrent wheeze, nonallergic asthma, bronchitis, organic dust toxic syndrome, and accelerated lung function decline when inhaled (Douwes *et al.*, 2003; Milton *et al.*, 1995; Vogelzang *et al.*, 1998). However, exposure to low levels of endotoxin at an early age may lead to a protective effect against atopic sensitization and atopic wheeze (Riedler *et al.*, 2001; Klintberg *et al.*, 2001).

Seasonality and time of day may also affect endotoxin and PM_{2.5-10} in the home. Endotoxin concentrations have been found to be four times higher in summer than in winter (Bari *et al.*, 2014) and 40 times higher when occupants are home compared to when away (Madsen *et al.*, 2012). Chen and Hildemann (2009) found that PM_{2.5-10} and endotoxin concentrations were higher during the day than at night (Chen *et al.*, 2009). Humans are sources of microorganisms and their activity has a significant impact on indoor bioaerosol concentrations (Madsen *et al.*, 2012).

Airborne endotoxin concentrations are typically sampled with an inhalable dust sampler. Inhalable samplers ‘actively’ sample aerosol with an efficiency matching that of a human nose and nasopharynx, known as the inhalable particulate fraction (WHO, 1999). A variety of samplers have been used for endotoxin measurements including: the button sampler (BS), the IOM open-face cassette sampler, Gesamtstaub-probenahmesystem sampler (GSP), personal aerosol sampler 6 (PAS-6), and Personen Getragenes Probenahmesystem (PGP) sampler (Duquenne *et al.*, 2013). The BS, one type of inhalable sampler, consists of a stainless-steel, curved-surface covered with 381 μm , circular inlets designed to sample inhalable dust onto a 25-mm filter at a flow rate of 4 L min⁻¹ (Aizenberg *et al.*, 2000). Button samplers are used to sample exposures over short time periods from a few hours to 24 h. They are fairly costly (~\$250), require deployment by trained personnel, and require an air pump to actively pull air through them, necessitating calibration equipment and a battery or AC power. These factors, typical of active air sampling, make BS inconvenient for long-term (multi-day or multi-week) and high site number sampling.

Alternatively, passive electrostatic dust collectors (EDCs) can be used to measure endotoxin concentrations without the need for external power (Noss *et al.*, 2008). Electrostatic dust collectors consist of a custom polypropylene folder that holds two electrostatic cloths for passive sampling. The cloths are a combination of electronegative materials, polyester and polypropylene, hydroentangled and coated with a proprietary formulation that easily attracts particles (Fereshtehkhrou *et al.*, 2005; Gamble, 2001). Electrostatic dust collectors are deployed on a horizontal surface to sample dust from the air and can be deployed in large numbers because they are inexpensive, can be deployed easily by following simple written instructions, and require no electricity. Electrostatic dust collectors have recently been used to sample endotoxin in a variety of domestic and industrial environments including farm homes, an animal companion hospital, schools, apartments, and farms (Samadi *et al.*, 2010; Jacobs *et al.*, 2013; Madsen *et al.*, 2012; Noss *et al.*, 2008; Zahradnik *et al.*, 2011; Kilburg-Basnyat *et al.*, 2015a).

Endotoxin concentrations measured with EDCs have been compared to those measured with active samplers. The PM₁₀ Harvard impactor, vacuum dust sampling and EDCs were

compared in the same study by being deployed in both farm (n=9) and nonfarm homes (n=7) in the Netherlands (Noss *et al.*, 2008). Electrostatic dust collectors correlated well with the Harvard impactor ($r=0.70$) and with floor dust sampling ($r=0.65$). Electrostatic dust collectors have recently been deployed in a companion animal hospital side-by-side to PAS-6 samplers, an inhalable dust active personal sampling device (Samadi *et al.*, 2010). PAS-6 samplers were moderately correlated with EDC endotoxin concentrations ($r=0.70$). However, EDC endotoxin concentrations have yet to be compared to an inhalable active sampling device in farm homes in the United States, whose farming practices are widely different to European practices. This study compared endotoxin concentrations of EDCs to BS. Button samplers are well-established, active inhalable fraction air samplers designed to sample 95% of particles at 1 μm and 50% of particles up to 100 μm of aerodynamic size.

The goal of this study was to compare endotoxin concentrations measured with EDCs and BS over two seasons in farm homes and to explore whether exposure data from EDCs can be used to estimate airborne concentrations. Button samplers, like other active samplers, typically require field workers to set-up the samplers. Thus, deploying BS side-by-side with EDCs, we sought to compare airborne PM concentrations, endotoxin concentrations from active sampling, and endotoxin loading onto EDCs over a 7-day period in farm homes. The inclusion of an optical particle counter (OPC) allowed for an additional comparison of size-specific airborne PM concentrations to BS and EDC endotoxin concentrations over the sampling period.

Methods

EDC and BS Assembly

Electrostatic dust collector cloths were heated for 6 h at 160°C to degrade preexisting endotoxin present from cloth manufacturing and packaging. The EDC cloths were then placed into plastic, non-conductive EDC folders custom-made according to our specifications. Electrostatic dust collectors were assembled under endotoxin-free conditions to prevent endotoxin contamination prior to deployment. Following assembly, each EDC folder was held closed with a rubber band and placed into a new Ziploc® bag. The area of each EDC cloth exposed during sampling was 0.0205 m².

Prior to use, BS were washed overnight in a solution of 0.1% E-Toxa-Clean® in nanopure water. Following overnight treatment, BS were rinsed 10 times with tap water, 5 times with distilled water, and once with nanopure water. The BS were assembled under endotoxin-free conditions using pre-weighed type A/E glass fiber filters (Pall Corp., Ann Arbor, MI) of the same lot (lot T93258). After assembly, the outlets of each BS were covered and each BS was placed into a new plastic bag until deployment. Following deployment, glass fiber filters were post-weighed and stored at -20°C until endotoxin analysis.

EDC and BS Round 1

In Round 1, sampling was conducted at 10 farm homes in summer (July 30th, 2010 to Aug. 19th, 2010). These homes, located within 25 km of Maquoketa, IA, had livestock (usually cattle) operations and/or row crops (corn and soybeans) on their property and housed one to

six occupants. Over a 7-day period, a single EDC holding two cloths was deployed while seven BS were serially deployed for each 24-h period. Sampling was performed in the room where the occupants spent the most of their awake time, typically a living room.

Music stands (Hamilton KB95E Encore Symphonic) were placed in each home with the stand platform adjusted horizontally and to a height of 135 cm. The stands were placed 2.5 cm apart (Figure 1) and were placed to avoid disturbances, direct ventilation, and other devices emitting heat. Each BS, pump (model 224-PCXR8; SKC, Inc., Eighty Four, PA), and battery was changed each morning for each home. All homes were visited between 6:00 and 10:00 am for the 7 days. Pumps were connected to BS with 150 cm of tubing and the pumps rested at the bottom of each music stand in a Pelican[®] box. A new BS was used for each 24-h period in each home. Pre- and post-calibrations were performed on each pump and battery, before and after each 24-h sampling period using a Gillian Gilibrator-2 (Sensidyne Inc., Clearwater, FL). Pumps were set to 4.0 L min⁻¹.

There were 2 field blank EDCs placed in randomly-selected homes. Ten field blank BS were deployed, using one blank BS per home per season. Each field blank, both EDC and BS, were exposed for 3–5 min which was the amount of time needed to set-up the next round of BS sampling in the assigned homes. The blank EDCs and BS were then placed back into their respective Ziploc[®] bags and transported back to lab and stored.

In the first round of sampling, several pumps failed to run the full 24-h sampling period and were removed from use. As a result, the length of sampling ranged from 867 to 1440 min. However, pumps were set-up in the morning to guarantee a sample from the daytime hours because endotoxin concentration is far higher during the day. Pump failure during night hours was estimated to have minimal effect on the data (see Results) since nighttime exposure concentrations were much lower due to the inactivity in the home (Chen *et al.*, 2009; Seedorf *et al.*, 1998).

EDC and BS Round 2

In Round 2, sampling was conducted in winter (Nov. 27th 2012 and Jan. 1st, 2013) in the same homes and following the same procedures as in Round 1 with the following changes. The pump used to maintain a flow rate of 4.0 L min⁻¹ was changed to an Omni Personal Pump (BGI, Inc.; Waltham, MA) because they were able to reliably sample for the full 24-h period. Calibration measurements were conducted before and after BS deployment using a BS calibration adaptor (SKC, Inc.) and a Gillian Gilibrator-2. An optical particle counter (OPC, model 1.108; Grimm, Ainring, Germany) was used to measure particle counts by size every 5 min into 15 different particle size bins ranging from >20 µm to >0.3 µm aerodynamic diameter. The OPC was added to the protocol to help determine particle sizes and particle counts present during sampling in an effort to evaluate temporal changes in PM. The OPC was deployed on a music stand, at a 20° angle to maintain a near vertical OPC stance and to prevent the OPC from falling during sampling. The OPC was placed next to the music stand holding the BS and EDC. The OPC inlet attachment was at approximately the same height as the BS. The set-up for the EDC, BS, and OPC is displayed in Figure 1.

EDC and BS endotoxin extraction

After EDCs were collected, they were placed into a 50 ml tube and stored at -20°C until endotoxin analysis. Electrostatic dust collectors were extracted in 10 ml endotoxin-free water (Lonza, Inc.; Walkersville, MD) and continuously shaken for 1 h at room temperature. The extracts were then transferred to 15 ml tubes and the recovered volume was recorded. The extracts were centrifuged at 4°C at 600 xG and transferred to a new tube. The pellets were discarded.

After BS filters were collected and post-weighed, they were placed into 15 ml centrifuge tubes and stored at -20°C until analysis. Button sampler filters were extracted in 5 ml of endotoxin-free water (Lonza, Inc.), shaken continuously for 1 h at room temperature, centrifuged at 4°C at 600 xG, and then transferred to a new tube discarding the pellet. Filters were assayed using 4-fold serial dilutions.

LAL Assay

EDC and BS eluates were analyzed using a modification of the kinetic chromogenic *Limulus* amoebocyte lysate (LAL) assay (Kinetic-QCL; Lonza, Inc., Walkersville, MD), as previously described (Kilburg-Basnyat *et al.*, 2015). Each round of sampling used the same lot of reagents (Round 1: lot HL0476; *Escherichia coli* E50:643; Lonza, Inc.; 13 EU/ng; Round 2: lot 000027184; *E. coli* E50:643; Lonza, Inc.; 10 EU/ng). Several samples from the first round were repeated with the second round of sampling and verified consistency between the two lots. Standard curves were compared across lots and were similar in range and shape. All samples and standard dilutions were prepared in endotoxin-free heat-treated borosilicate glass tubes. A 12-point standard curve from 0.024 to 50.0 EU/ml was generated using 2-fold serial dilutions of endotoxin standards (*E. coli* O55:B5; Lonza, Inc.). Samples were assayed in endotoxin-free microtiter plates (Costar no. 3595; Corning, Inc., Corning, NY). The standards and samples were analyzed using a microplate reader running SoftMaxPro 5.4 software (SpectraMax 340, Molecular Devices, Sunnyvale, CA) with photometric measurements taken at 37°C every 30 s for 90 min at 405 nm. The same microplate reader was used for all samples. The minimum acceptable r^2 value was 0.995 for the standard curve.

Statistical Analysis

Round 1 EDC values were blank corrected by using the average of the four field blanks. EDC and BS data from one household were unavailable because the equipment was removed due to a complaint by a resident. After the removal of one household, the final sample sizes for round 1 of sampling was $n=63$ for BS and $n=18$ for EDCs.

In round 2, two of the four EDC field blank cloths were below the limit of detection (LOD) and were assigned a value of zero with the assumption that no endotoxin had settled onto the cloths during the 3–5 min exposure period. The four blank EDC cloths were averaged and used for blank correction. For round 2 of sampling, the total sample size analyzed for endotoxin concentrations was $n=70$ for BS filters and $n=20$ for EDC cloths. Paired EDC cloths (within the same EDC) were averaged for endotoxin analysis.

Over both seasons, 2/8 of the EDCs were <LOD of 0.17 EU/sample and assigned a value of zero. The mean for all blank EDCs was 0.73 EU/sample and the mean of all EDC samples over both seasons was 20 EU/sample. For BS, 12/19 filter blanks were <LOD of 0.08 EU/filter and assigned a value of zero. For the BS filters sampled in homes over both seasons, the mean was 6.2 EU/sample. There was over a 50-fold increase in endotoxin on the BS filters compared to the blank filters obviating the need for blank correction. However, EDCs were blank corrected.

All EDC, BS, and PM values were determined to be log-normally distributed by using the Kolmogorov-Sminov normality test. For all statistical analyses, variables with p-values <0.1 were retained in the model but p-values less than 0.05 were considered significant and p-values below 0.01 were considered highly significant. All analyses were performed using SAS version 9.3 (SAS Institute, Inc. USA).

Endotoxin concentrations were compared between EDCs and BS across seasons in farm homes. In order to compare the endotoxin concentrations, a multiple linear regression model using backwards elimination was used to determine the variables that were most associated with EDC endotoxin concentrations. The variables considered were BS endotoxin concentrations and season. The variables (BS or season) that remained significant were left in the linear regression model.

The relationship between endotoxin concentrations sampled using EDCs and BS and PM concentrations sampled using the OPC across two seasons were compared. Pearson Correlation coefficients were determined between the different groups (EDC, BS, PM_{2.5}, and PM_{2.5-10}).

The relationship between EDCs, BS, and OPC endotoxin and PM concentrations over the 7-day period in farm homes was assessed. An ANOVA model was used to determine if BS and OPC measurements were associated with EDC endotoxin concentrations. BS endotoxin values were averaged over the 7-day period. Optical particle counts per volume were converted to $\mu\text{g}/\text{m}^3$. The assumed mean mass density was $1.4 \text{ g}/\text{cm}^3$ which was the average of plant ($1.5 \text{ g}/\text{cm}^3$), pollen ($1.4 \text{ g}/\text{cm}^3$) and bacteria ($1.24 \text{ g}/\text{cm}^3$) PM (Baron *et al.*, 2005). The OPC values were also averaged over a 7-day period and were divided into PM_{2.5-10} and PM_{2.5} groups. The PM_{2.5-10} was summed from bins of mid-diameter sizes of 3.46 to 8.66 μm . The PM_{2.5} was summed to include bins from 0.35 to 2.45 μm mid-diameter size. The variables analyzed were PM_{2.5-10}, PM_{2.5}, and two ratios (PM_{2.5-10} / PM_{2.5} and PM_{2.5} / PM_{2.5-10}). All the variables were initially considered for the model and R-square values were used to determine the best PM variable for the model. The p-value of <0.1 was used to determine a PM concentration variable when analyzing the relationship between EDCs, BS, and OPC concentrations.

Endotoxin concentrations from BS and PM concentrations sampled using OPC were compared to determine which combination of PM concentrations was most associated with the BS. Weekend and weekday differences of BS were also considered because activity during a work week may significantly differ from the weekend, when occupants may be present in the home more. A mixed model was used with a repeated effect for within

household measurements and an unstructured covariance matrix to test for an association between BS and OPC measurements. Daily BS measurements were compared to 24-h averages of PM measurements. The particles were separated into two groups, $PM_{2.5-10}$ and $PM_{2.5}$, as described above. The same OPC variables as described were initially considered. Akaike's information criterion (AIC) was used to determine the best PM variable to use in the model. The variables analyzed were $PM_{2.5-10}$, $PM_{2.5}$, and two ratios ($PM_{2.5-10} / PM_{2.5}$ and $PM_{2.5} / PM_{2.5-10}$). Days of the week were also analyzed in the model using AIC to determine if weekday or weekend had a significant effect on endotoxin concentrations. Electrostatic dust collectors sampled in summer and winter in the same farm homes were analyzed for endotoxin concentrations to determine the difference between seasons. An ANOVA model was also used to determine if there was a significant change in EDC endotoxin concentrations in homes between winter and summer. Button samplers were also analyzed to determine if there was any difference between endotoxin concentrations sampled in the summer compared to the winter. A mixed model with a repeated effect for within household measurements and an unstructured covariance structure was used to determine if there was an association between BS endotoxin values across seasons and between the weekend and weekdays.

Results

Figure 2 displays the relationship between BS and EDC endotoxin concentrations for each season. Season was originally included in the linear regression analysis but had no significant contribution to the regression ($p=0.95$) and was removed. The BS endotoxin concentrations significantly impacted the linear regression analysis ($p<0.001$). Overall, log EDC and BS correlated well with a Pearson correlation coefficient of 0.70 ($p<0.001$). Round 1 of BS endotoxin concentrations had several shortened sampling periods due to pump failures during the nighttime. A Pearson correlation coefficient was used to compare BS and EDCs to determine if the BS sampling time during round 1 created a bias in the BS endotoxin concentrations. The first set of BS data used assumed zero endotoxin concentration sampling at night and increased the sampling period to 1440 min. The second set of BS data utilized the actual sampling times for each set of BS endotoxin data. The correlation between BS and EDC data did not change despite using the two different sets of BS endotoxin data indicating that the bias for nighttime hours was minimal. As a result, the sampled volume using the actual BS deployment time for each day was used for all analyses.

Pearson correlation values for EDCs and BS during summer and winter, compared to $PM_{2.5}$ and $PM_{2.5-10}$ are displayed in Table 2. Pearson correlation coefficients for EDC vs. BS were slightly higher during the summer ($r=0.75$; $p=0.02$) compared to the winter months ($r=0.67$; $p=0.03$). As expected, the winter BS endotoxin concentrations were highly significantly correlated with $PM_{2.5-10}$ and $PM_{2.5}$ ($r=0.96$ and $r=0.92$, respectively). Less expected was the finding that winter EDC concentrations were significantly correlated with $PM_{2.5}$ ($r=0.72$; $p=0.02$) but were not significantly correlated to $PM_{2.5-10}$ ($r=0.56$; $p=0.09$).

An ANOVA model was used to evaluate the relationship between BS sampled endotoxin concentrations and OPC particle size on EDC endotoxin concentrations. Table 1 displays the back-transformed estimates and standard errors. The fine to coarse PM ratio ($PM_{2.5} /$

PM_{2.5-10}) had the best fit compared to the other candidate variables relating to particle size (i.e. PM_{2.5}, PM₁₀, PM_{2.5-10} / PM_{2.5}) with an R-squared value of 0.656. The effect of the PM_{2.5} / PM_{2.5-10} ratio ($p=0.0802$) on EDC endotoxin concentrations did not reach statistical significance. A 1-unit increase in the PM_{2.5} / PM_{2.5-10} ratio was associated with a 2.4-fold increase in EDC endotoxin values. This indicates that as PM_{2.5} increases and PM_{2.5-10} decreases, EDC endotoxin concentrations increase. A 10-fold increase in BS endotoxin concentration was associated with a highly significant 12-fold increase in EDC endotoxin values ($p=0.0082$).

Table 3 displays the descriptive statistics of EDCs, BS, and the OPC data separated into daily and weekly values over winter and summer. The median was double for 24-h BS endotoxin concentrations sampled in summer compared to winter (0.83 and 0.42 EU/m³, respectively). The median summer EDC values of 627 EU/m² (89.6 EU/m²·day) was 30% higher than the median winter value of 442 EU/m² (63.1 EU/m²·day). There was overlap of the interquartile ranges for EDCs between winter and summer (282 to 794 EU/m² and 536 to 959 EU/m², respectively). Median OPC concentrations for PM_{2.5-10} were higher compared to PM_{2.5} (1.92 and 1.67 µg/m³, respectively). This further indicates a significant difference between BS endotoxin concentrations between seasons and a lack of significant difference between EDC endotoxin concentrations between seasons.

Table 4 displays the results for fixed effects for the mixed model analysis for winter 24-h BS values and OPC values. Table 4 parameter estimates and standard errors are displayed as antilogs because the original data were log-normally distributed and analyzed as log values. The variables included in the mixed model analysis are those that returned the lowest AIC. The PM_{2.5-10} variable was maintained in the model because it had the lowest AIC value compared to the other options (PM_{2.5}, PM_{2.5-10} / PM_{2.5} and PM_{2.5} / PM_{2.5-10}) and was also significant ($p<0.0001$). A 10-fold increase in OPC-sampled PM_{2.5-10} values was associated with an 9-fold increase in BS endotoxin concentrations. Weekdays were also included in the model as it was a significant variable ($p<0.01$). Holding coarse PM equal, BS endotoxin measurements taken on the weekend are 1.2-times higher than weekdays. An ANOVA model was used to determine that there was no significant difference between winter and summer EDC endotoxin values ($p=0.46$). However, a mixed model analysis of daily BS values was associated with a 3-fold increase of endotoxin concentrations in the summer compared to winter (Table 5). This increase may be due to the increase in outdoor endotoxin concentrations during the summer and open windows in the homes allowing outdoor air in during the summer. A variable to contrast endotoxin values between weekends and weekdays was also considered but was removed due to lack of significance ($p=0.15$).

Figure 3 displays PM_{2.5} and PM_{2.5-10} concentrations obtained using the OPC in two homes over a 25-h sampling period overlapping two days. Household 4 was randomly selected, and Household 8 was chosen because it had the highest observed endotoxin concentrations over the 7-day time period. Figure 3a displays Household 4 with a PM_{2.5-10} peak of 20 µg/m³ between 6 and 7 am on Saturday, presumably when home occupants are in the home and active. Household 8 (Figure 3b) experienced a PM_{2.5} peak of 160 µg/m³ measured at 10 am on a Wednesday morning. Household 8 had the highest endotoxin concentrations during both seasons and had high PM values throughout the day. This home had the highest

occupancy and a wood burning stove that was presumably in use during winter sampling. There was a noticeable decrease in PM in the nighttime between 1 am and 6 am.

Discussion

Comparing EDCs to other well-established sampling methods is important to establish EDCs as an effective sampling tool. Endotoxin concentrations sampled using EDCs have not previously been compared to BS endotoxin concentrations. In this study, endotoxin concentrations measured with the EDC correlated moderately well with those measured with the BS over summer and winter ($r=0.70$). Because of this significant association and moderate correlation, the linear relationship between BS and EDCs might be used to estimate endotoxin air concentrations from EDC sampling. Further, EDCs can be used to analyze dust samples for allergens, total protein, and glucans in addition to endotoxin because multiple EDC cloths are deployed simultaneously (Kilburg-Basnyat *et al.*, 2015a; Noss *et al.*, 2010). This may allow for samples to be sent to multiple labs when EDCs are used in large epidemiological studies.

EDCs have previously been successfully compared to other sampling methods. In farm and non-farm homes, endotoxin concentrations were significantly correlated for EDCs vs. PM_{10} Harvard impactors ($r=0.70$) and EDCs vs. vacuumed reservoir dust samples ($r=0.65$) (Noss *et al.*, 2008). EDC endotoxin concentrations were also correlated with PAS-6 samplers ($r=0.70$), another active sampling method (Samadi *et al.*, 2010). Similarly, EDCs and BS endotoxin correlations were significantly correlated across summer and winter indicating that EDCs sample endotoxin concentrations similar to BS. The repeatability of significant correlation across active sampling devices indicates that EDCs may be an alternative or complement to a variety of active sampling methods.

EDC endotoxin concentrations were associated with BS endotoxin concentrations and the $PM_{2.5}/PM_{2.5-10}$ OPC concentration ratio. A 10-fold increase in BS endotoxin concentrations was associated with a 12-fold increase in EDC endotoxin concentrations indicating a strong association between BS and EDC endotoxin concentrations. The finding of a 2-fold increase in EDC endotoxin concentrations with the $PM_{2.5}/PM_{2.5-10}$ ratio is surprising. In comparison, BS were most associated with the $PM_{2.5-10}$ fraction sampled using the OPC. A 10-fold increase in $PM_{2.5-10}$ concentrations was associated with a 9-fold increase with BS endotoxin concentrations. The differences between association of PM fractions to the BS and EDC endotoxin concentrations may indicate a difference in the PM sampling capabilities of the two samplers. EDCs may attract fine particles from a broader volume adjacent to the EDC, whereas coarse particles may only settle due to gravity from the column of air above the EDC. EDC cloths contain polyester, an electronegative material that easily attracts smaller particles but may have difficulty attracting the $PM_{2.5-10}$ fraction which contains the majority of endotoxin (Heinrich *et al.*, 2003; Attwood *et al.*, 1986; Monn *et al.*, 1999). Another explanation may be that the $PM_{2.5-10}$ fraction is not easily extracted from EDCs. However, a study that compared mailed EDCs spiked with dust to dust-only endotoxin concentration samples found no significant difference between endotoxin concentrations (Kilburg-Basnyat *et al.*, 2015b). This indicates that the extraction method is not causing the $PM_{2.5-10}$ fraction bias in the EDCs.

Endotoxin concentrations have previously been associated with larger PM fractions. A study in a Dutch pig farm suggested that the larger PM fraction (3.5 to 8.5 μm) may actually reflect airborne clumps of endotoxin (Wheeler *et al.*, 2011). Weekdays might have higher endotoxin concentrations due to occupant presence in the home. EDC endotoxin concentrations have been found to be 23 times higher when the occupant was home compared to when they were absent (Madsen *et al.*, 2012). Other predictors of endotoxin that may explain the difference between weekay and weekend in the home include the number of people living in the household and the presence of children (Thorne *et al.*, 2009).

The main difference between EDC and BS endotoxin sampling may relate to PM size. The loss of larger particles from EDCs and direct association with the $\text{PM}_{2.5-10}$ fraction could explain why the correlation coefficient of 0.70 between BS and EDC endotoxin concentrations was not higher. Particularly because endotoxin has been most associated with $\text{PM}_{2.5-10}$ in other studies (Wheeler *et al.*, 2011).

Seasonal variability between endotoxin concentrations in the home have previously been studied. Predictors of summer endotoxin concentrations have included outdoor endotoxin concentrations, mice in the home in the previous 12 months, and mean indoor relative humidity levels (Bari *et al.*, 2014). Outdoor airborne endotoxin concentrations were significantly higher in March and April compared to November and December (Madsen, 2006) and may have influenced indoor airborne endotoxin concentrations. Park *et al.* performed indoor air sampling monthly and determined that airborne endotoxin levels were lower in winter than in spring (Park *et al.*, 2000; Park *et al.*, 2001).

Statistical analyses indicated that BS endotoxin concentrations were 3-fold higher in summer compared to winter while there was no difference between seasons for EDC endotoxin concentrations. However, the GM for 7-day averages in all homes indicated similar increases in endotoxin concentrations in the summer compared to winter for BS (1.6-fold higher) and EDCs (1.4-fold higher). Thus the trend was similar and the lack of a significant difference between seasons for EDC endotoxin concentrations may be due to fewer repeated measures. There was a BS filter for every 24-h while there were only EDC endotoxin measurements available for the 7-day deployment period.

Electrostatic dust collector endotoxin concentrations have previously been compared across winter and springtime in another study. EDCs were deployed in 16 homes during the winter and 11 homes during the springtime. There was no significant difference between endotoxin concentrations ($p=0.32$) (Madsen *et al.*, 2012). Another study using Harvard coarse impactors, an active sampling method for endotoxin sampling, found indoor median endotoxin concentrations 4-fold higher in summer than winter (0.41 vs. 0.12 EU/m^3) (Bari *et al.*, 2014). Our findings followed a similar trend with a doubling of the 7-day median endotoxin concentration for BS in summer versus winter (0.91 vs. 0.49 EU/m^3 , respectively). Thus, active air samplers may be more capable of detecting subtle seasonal differences between endotoxin concentrations than EDCs.

Endotoxin values for BS and EDCs were comparable to previous studies. In Iowa, dichotomous samplers were used to sample 197 rural homes from 2007–2011 (Pavilonis *et*

al., 2013). This study had a range of 0.01 to 4.5 EU/m³ comparable to our range of BS endotoxin values over both seasons (0.01 to 10.74 EU/m³). EDC values were also similar to 7 days sampling from our prior study in which we observed a median of 605 EU/m² and an interquartile range of 403 to 2934 EU/m² (Kilburg-Basnyat *et al.*, 2015a). Our median and interquartile range was similar for summer (737 EU/m²; 536–959 EU/m²) and winter (442 EU/m²; 282–794 EU/m²).

Conclusion

In conclusion, endotoxin concentrations sampled using EDCs and BS in this study of 10 farm homes were moderately correlated. However, BS were most associated with PM_{2.5-10} values and EDCs were most associated with the PM_{2.5}/PM_{2.5-10} ratio indicating that EDCs are more effective at capturing PM_{2.5} than PM_{2.5-10}. BS sampling revealed 3-fold higher endotoxin concentrations in summer than winter while EDCs had no difference between endotoxin concentrations sampled between seasons. This may be due to EDCs having fewer repeated measures than the BS. The significant correlation of endotoxin sampled using EDCs and BS indicates that EDC data can be used to approximate airborne endotoxin concentrations.

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Chapter IV References

- Aizenberg V, Grinshpun S, Willeke K, Smith J, Baron P. Performance characteristics of the button personal inhalable aerosol sampler. *AIHAJ-American Industrial Hygiene Association*. 2000; 61:398–404.
- Attwood P, Versloot P, Heederik D, De Wit R, Boleij JSM. Assessment of dust and endotoxin levels in the working environment of dutch pig farmers: A preliminary study. *Annals of Occupational Hygiene*. 1986; 30:201–208. [PubMed: 3752839]
- Bari MA, Macneill M, Kindzierski WB, Wallace L, Héroux M-È, Wheeler AJ. Predictors of coarse particulate matter and associated endotoxin concentrations in residential environments. *Atmospheric Environment*. 2014; 92:221–230.
- Baron PA, Willeke K. *Aerosol measurement: Principles, techniques, and applications*. 2005
- Becker S, Dailey LA, Soukup JM, Grambow SC, Devlin RB, Huang Y-CT. Seasonal variations in air pollution particle-induced inflammatory mediator release and oxidative stress. *Environmental Health Perspectives*. 2005; 113:1032–1038. [PubMed: 16079075]
- Chen Q, Hildemann LM. The effects of human activities on exposure to particulate matter and bioaerosols in residential homes. *Environmental Science & Technology*. 2009; 43:4641–4646. [PubMed: 19673245]
- Douwes J, Thorne P, Pearce N, Heederik D. Bioaerosol health effects and exposure assessment: Progress and prospects. *Annals of Occupational Hygiene*. 2003; 47:187–200. [PubMed: 12639832]
- Duquenne P, Marchand G, Duchaine C. Measurement of endotoxins in bioaerosols at workplace: A critical review of literature and a standardization issue. *Annals of Occupational Hygiene*. 2013; 57:137–172. [PubMed: 23002277]

- Fereshtehkhoh, S.; Russo, PJ.; Strickland, J.; Wilbur Cecil Policicchio, NJ. Three dimensional structures useful as cleaning sheets. United States: The Procter & Gamble Company; 2005. Vol. US 6936330 B2
- Gamble, P. Swiffer Dusting Sheet. Cincinnati, OH: Procter & Gamble; 2001. Material safety data sheet.
- Heinrich J, Pitz M, Bischof W, Krug N, Borm PJA. Endotoxin in fine (pm2.5) and coarse (pm2.5–10) particle mass of ambient aerosols. A temporo-spatial analysis. *Atmospheric Environment*. 2003; 37:3659–3667.
- Jacobs JH, Krop EJ, De Wind S, Spithoven J, Heederik DJ. Endotoxin levels in homes and classrooms of dutch school children and respiratory health. *European Respiratory Journal*. 2013; 42:314–322. [PubMed: 23100494]
- Kilburg-Basnyat B, Metwali N, Thorne PS. Effect of deployment time on endotoxin and allergen exposure assessment using electrostatic dust collectors. *Annals of Occupational Hygiene*. 2015a; 59:104–115. [PubMed: 25187036]
- Kilburg-Basnyat B, Metwali N, Thorne PS. Performance of electrostatic dust collectors (edcs) for endotoxin assessment in homes: Effect of mailing, placement, heating and electrostatic charge. *Journal of Occupational and Environmental Hygiene*. 2015b In Press.
- Klintberg B, Berglund N, Lilja G, Wickman M, Van Hage-Hamsten M. Fewer allergic respiratory disorders among farmers' children in a closed birth cohort from sweden. *European Respiratory Journal*. 2001; 17:1151–1157. [PubMed: 11491158]
- Madsen AM. Airborne endotoxin in different background environments and seasons. *Annals of Agricultural and Environmental Medicine*. 2006; 13:81–86. [PubMed: 16841877]
- Madsen AM, Matthiesen CB, Frederiksen MW, Frederiksen M, Frankel M, Spilak M, Gunnarsen L, Timm M. Sampling, extraction and measurement of bacteria, endotoxin, fungi and inflammatory potential of settling indoor dust. *Journal of Environmental Monitoring*. 2012; 14:3230–3239. [PubMed: 23152160]
- Milton DK, Amsel J, Reed CE, Enright PL, Brown L, Aughenbaugh GL, Morey PR. Cross-sectional follow-up of a flu-like respiratory illness among fiberglass manufacturing employees: Endotoxin exposure associated with two distinct sequelae. *American Journal of Industrial Medicine*. 1995; 28:469–488. [PubMed: 8533789]
- Monn C, Becker S. Cytotoxicity and induction of proinflammatory cytokines from human monocytes exposed to fine (pm2.5) and coarse particles (pm10–2.5) in outdoor and indoor air. *Toxicology and Applied Pharmacology*. 1999; 155:245–252. [PubMed: 10079210]
- Noss I, Wouters IM, Bezemer G, Metwali N, Sander I, Raulf-Heimsoth M, Heederik DJJ, Thorne PS, Doekes G. B-(1,3)-glucan exposure assessment by passive airborne dust sampling and new sensitive immunoassays. *Applied and Environmental Microbiology*. 2010; 76:1158–1167. [PubMed: 20038709]
- Noss I, Wouters IM, Visser M, Heederik DJJ, Thorne PS, Brunekreef B, Doekes G. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Applied and Environmental Microbiology*. 2008; 74:5621–5627. [PubMed: 18676704]
- Park J-H, Spiegelman DL, Burge HA, Gold DR, Chew GL, Milton DK. Longitudinal study of dust and airborne endotoxin in the home. *Environmental Health Perspectives*. 2000; 108:1023. [PubMed: 11102291]
- Park J-H, Spiegelman DL, Gold DR, Burge HA, Milton DK. Predictors of airborne endotoxin in the home. *Environmental Health Perspectives*. 2001; 109:859. [PubMed: 11564624]
- Pavilonis BT, Anthony TR, O'shaughnessy PT, Humann MJ, Merchant JA, Moore G, Thorne PS, Weisel CP, Sanderson WT. Indoor and outdoor particulate matter and endotoxin concentrations in an intensely agricultural county. *J Expos Sci Environ Epidemiol*. 2013; 23:299–305.
- Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, Carr D, Schierl R, Nowak D, Von Mutius E. Exposure to farming in early life and development of asthma and allergy: A cross-sectional survey. *The Lancet*. 2001; 358:1129–1133.
- Samadi S, Heederik DJ, Krop EJ, Jamshidifard A-R, Willemse T, Wouters IM. Allergen and endotoxin exposure in a companion animal hospital. *Occupational and Environmental Medicine*. 2010; 67:486–492. [PubMed: 20519747]

- Schins RPF, Lightbody JH, Borm PJA, Shi T, Donaldson K, Stone V. Inflammatory effects of coarse and fine particulate matter in relation to chemical and biological constituents. *Toxicology and Applied Pharmacology*. 2004; 195:1–11. [PubMed: 14962500]
- Schwarze P, Øvrevik J, Låg M, Refsnes M, Nafstad P, Hetland R, Dybing E. Particulate matter properties and health effects: Consistency of epidemiological and toxicological studies. *Human & Experimental Toxicology*. 2006; 25:559–579. [PubMed: 17165623]
- Seedorf J, Hartung J, Schröder M, Linkert KH, Phillips VR, Holden MR, Sneath RW, Short JL, White RP, Pedersen S, Takai H, Johnsen JO, Metz JHM, Groot Koerkamp PWG, Uenk GH, Wathes CM. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in northern europe. *Journal of Agricultural Engineering Research*. 1998; 70:97–109.
- Soukup JM, Becker S. Human alveolar macrophage responses to air pollution particulates are associated with insoluble components of coarse material, including particulate endotoxin. *Toxicology and Applied Pharmacology*. 2001; 171:20–26. [PubMed: 11181108]
- Thorne PS, Cohn RD, May D, Arbes SJ, Zeldin DC. Predictors of endotoxin levels in us housing. *Environmental Health Perspectives*. 2009; 117:763. [PubMed: 19479019]
- Thorne PS, Kulhánková K, Yin M, Cohn R, Arbes SJ Jr, Zeldin DC. Endotoxin exposure is a risk factor for asthma: The national survey of endotoxin in united states housing. *American Journal of Respiratory and Critical Care Medicine*. 2005; 172:1371–1377. [PubMed: 16141442]
- Thorne PS, Metwali N, Mendy A, Salo P, Co C, Jaramillo R, Ruse K, Zeldin D. Endotoxin exposure: Predictors and associated respiratory health outcomes prevalent in the u.S. 2015 In preparation.
- Vogelzang PFJ, Van Der Gulden JWW, Folgering H, Kolk JJ, Heederik D, Preller L, Tielen MJM, Van Schayck CP. Endotoxin exposure as a major determinant of lung function decline in pig farmers. *American Journal of Respiratory and Critical Care Medicine*. 1998; 157:15–18. [PubMed: 9445272]
- Wheeler AJ, Dobbin NA, Lyrette N, Wallace L, Foto M, Mallick R, Kearney J, Van Ryswyk K, Gilbert NL, Harrison I, Rispler K, Héroux M-E. Residential indoor and outdoor coarse particles and associated endotoxin exposures. *Atmospheric Environment*. 2011; 45:7064–7071.
- Who. Health, O. A. E. and Health, D. O. P. O. T. H. Hazard prevention and control in the work environment: Airborne dust. Geneva: 1999.
- Zahradnik E, Sander I, Kendzia B, Fleischer C, Brüning T, Raulf-Heimsoth M. Passive airborne dust sampling to assess mite antigen exposure in farming environments. *Journal of Environmental Monitoring*. 2011; 13:2638–2644. [PubMed: 21842065]

Practical Implications

Endotoxin concentrations measured in farm homes using EDCs compared favorably to BS indicating that EDCs are an effective passive sampling method for sampling airborne endotoxin. Electrostatic dust collectors can be readily deployed and retrieved from households without a need for pumps, calibration equipment or highly-trained staff. Thus, using EDCs in epidemiologic studies facilitates a larger sample size for endotoxin exposure assessment.

Figure 1.

The deployment set-up of the BS and pump, EDCs, an OPC on music stands in each farm home with (A) and (B) being the front view of each stand and (C) is the back view of the EDC/BS music stand.

Figure 2.

The x-axis is the average BS endotoxin concentrations in each home and the y-axis is the average EDC endotoxin concentrations in each farm home in summer (open circle) and in winter (filled square).

Figure 3. $\text{PM}_{2.5}$ and $\text{PM}_{2.5-10}$ concentrations from the OPC displayed in $\mu\text{g m}^{-3}$ along the y-axis for a 25-h sampling period in (a) Household 4 over a Friday-Saturday period and (b) Household 8 from Wednesday to Thursday.

Table 1

Comparison of winter EDC endotoxin concentrations with BS endotoxin concentrations and an aerosol size ratio ($PM_{2.5}/PM_{2.5-10}$) as model parameters.

Parameter Estimates				
Effect	DF	Antilog of the Parameter Estimate	Antilog of the Standard Error	Pr > t
Intercept	1	355	1.58	<0.0001
Log 7-day BS endotoxin average	1	12.2	1.99	0.0082
7-day average of $PM_{2.5}/PM_{2.5-10}$	1	2.37	1.53	0.0802

EDC Endo=355*[(12.2)^{Log 7-day BS Endo Avg}]*[(2.37)^{'PM_{2.5}/PM_{2.5-10}'}]

Table 2

Pearson correlation coefficients for log-transformed endotoxin data

	EDC (Summer)	OPC PM _{2.5} (Winter)	OPC PM _{2.5-10} (Winter)	BS (Winter)
BS (Summer)	0.75 $p=0.02$ n=9	--	--	--
BS (Winter)	--	0.92 $p<0.001$ n=10	0.96 $p<0.0001$ n=10	--
EDC (Winter)	--	0.72 $p=0.02$ n=10	0.56 $p=0.09$ n=10	0.67 $p=0.03$ n=10

Table 3

The descriptive statistics for all the BS samples taken and for the average BS endotoxin values over the 7-day period in addition to EDC 7-day averages in all the homes during summer (a). Table 3b displays the values for winter with the addition of PM_{2.5} and PM_{2.5-10} averages for 24-h and 7-day periods. Note that EDC endotoxin data are expressed per square meter of exposed surface.

A. Summer				
	N	GM	GSD	Median Interquartile Range
24-h average of all homes				
BS (EU m ⁻³)	63	0.82	2.7	0.83 0.50–1.39
7-day average of all homes				
BS (EU m ⁻³)	9	0.98	2.1	0.91 0.58–1.36
EDC (EU m ⁻²)	9	737	1.9	627 536–959

B. Winter				
	N	GM	GSD	Median Interquartile Range
24-h average of all homes				
BS (EU m ⁻³)	70	0.52	3.1	0.42 0.25–0.83
OPC PM _{2.5} (µg m ⁻³)	70	1.94	2.1	1.7 1.14–2.77
OPC PM _{2.5-10} (µg m ⁻³)	70	2.11	3.0	1.91 1.0–3.99
7-day average of all homes				
BS (EU m ⁻³)	10	0.61	2.8	0.49 0.30–0.79
EDC (EU m ⁻²)	10	538	3.0	442 282–794
OPC PM _{2.5} (µg m ⁻³)	10	2.12	2.0	1.67 1.36–2.57
OPC PM _{2.5-10} (µg m ⁻³)	10	2.27	3.0	1.92 1.34–3.94

Table 4

Comparison of airborne endotoxin concentrations sampled in winter using BS with OPC aerosol size and weekend, with weekday =1 and weekend =0, as model parameters.

Results for Fixed Effects			
Effect	Antilog of Estimate	Antilog of Standard Error	Pr > t
Intercept	0.31	1.04	<0.0001
Log 24-h average PM _{2.5-10}	8.90	1.06	<0.0001
Weekday	1.22	1.06	0.0047

BS Endo=0.31*[(8.9)^{log 24-h Avg PM_{2.5-10}}]*[(0.82)^{Weekend}]

Table 5

Comparison of airborne endotoxin concentrations sampled during winter and summer using BS with season, with summer =1 and winter =0, as a model parameter.

Results for Fixed Effects				
Effect	Season	Antilog of the Estimate	Antilog of the Standard Error	Pr > t
Intercept		0.35	1.24	0.0002
Season	Summer	2.66	1.37	0.0065
Season	Winter	0	--	--

BS Endo=0.35*[(2.66)^Season]