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Analysis of Settled Dust in Homes and Fungal Glucan in Air Particulate Collected during HEPA Vacuuming

Virginia R. Salares^a Carol Ann Hinde^b J. David Miller^c

^aCanada Mortgage and Housing Corporation, Ottawa, Canada

^bBuchan, Lawton, Parent Ltd, Ottawa, Canada

^cDepartment of Chemistry, Carleton University, Ottawa, Canada

Key Words

Dust · Exposure · Mold · Air particulates · Fungal glucan · HEPA vacuuming

Abstract

Six occupied single-family dwellings in Ottawa, Canada were subjected to weekly or bi-weekly professional cleaning using a HEPA filter-equipped vacuum cleaner and following a strict cleaning protocol based on floor area and type of floor. Sieved weights of dust were collected from each cleaning. Statistically significant reductions of fine dust loading occurred only after four sequential cleanings. As dust loading of the floor was reduced, exposure to dust mite allergens, endotoxins, and fungal glucans in dust decreased. During the first cleaning (when loadings were highest), airborne particles were collected using a MOUDI sampler (0.18–18 µm size fractions). The fungal polysaccharide β-(1, 3)-D-glucan was present in approximately equal proportions in each size fraction. This demonstrates that the majority of the fungal exposure was not to intact spores, but to fungal fragments much smaller than spores that have greater efficiency of deeper

penetration into the lungs. This study also shows the importance of ensuring that cleaning interventions are effective before they can be related to health outcomes.

Introduction

The importance of fine settled dust and population health for allergic disease has long been recognized. Important components of fine dust for allergy include endotoxin, fungal glucan, and various allergens (e.g., dust mites, animals, insects). By the early 1950s, it was understood that sinks of accumulated settled dust were important components of exposure to allergens [1,2]. The importance of this from a health perspective was discussed in a review by Colloff et al. [3]. They indicated that “it is likely that allergen recovered/unit surface area or airborne allergen measurement will be most acceptable. It is unlikely that allergen expressed on a weight: weight basis gives sufficient information on patient exposure where manipulation of the domestic environment has taken place.” For respiratory disease, this prediction has been shown to be the case. In large population studies, when indicators of biological contamination are expressed on a

per unit area basis, more robust indications of health have been seen. Douwes et al. [4] found that both endotoxin and (1, 3)-D-glucan per square meter of living room floor were significantly associated with peak expiratory flow (PEF)-variability, but not when expressed per gram of sampled dust, particularly in atopic children with asthma symptoms. The amount of any contaminant per square meter is related to the amount of dust sampled [5–7]. A reliable value for weight of settled dust per unit floor area or in soft furniture is predictive of asthma. Elliott et al. [8] found that associations of varying strengths were seen for wheezing and asthma for mass loading of dusts on floors, beds, and soft furniture. These associations persisted when adjusting for allergen and endotoxin exposures. Glucan, endotoxin, and various allergens have been well documented as commonly co-occurring in settled dust from Canadian homes [9–12].

Personal exposure to particulates indoors is driven by the personal dust cloud around each person. For example, depending on climate, endotoxin accumulates indoors from pets and outdoor sources [6,11]. Rabinovitch et al. [13] showed that personal exposure to endotoxin did not correlate with, and, was much higher than area measurements. This is a generalizable phenomenon for indoor sourced particulate contaminants. Personal exposure indoors is determined by mass loadings of dust and room activity [14–17]. Aside from allergens, endotoxin and glucan, Canadian house dust can contain high concentrations of metals, flame retardants, perfluorosulfonates, and particulate matter (PM) from wood stoves and automobile traffic [12,18–23]. Higher mass loadings of dust would lead to higher personal exposures to these contaminants as well. Some of these contaminants (e.g., metals, endotoxin) are mainly in the finest dust and others (fungi, dust mites) are found in fine to larger particles.

A number of studies have found that the use of High Efficiency Particulate Air (HEPA) filter-equipped vacuum cleaners in homes reduces hospitalization due to asthma attacks and improves caregiver quality of life [24,25]. However, it was not discerned in early studies how much cleaning effort would be needed to reduce fine particle burdens with technology accessible to homeowners prior to symptom assessment. Furthermore, the potential effect of interventions on cleaners is also important. It is known that vacuuming results in increased exposure to some allergens [26] and PM₁₀ resulting from a mixture of the action of the vacuum cleaner and walking [27].

The objectives of this study were two-fold. The first was to determine the extent of cleaning necessary to reduce fine particles by 90% in a number of homes. The second

objective related to exposures of people doing the cleaning in intervention studies.

Methods

House Selection

Over a six-week period, six average carpeted houses with children and/or pets were cleaned by a professional cleaner once or twice a week with a HEPA vacuum cleaner. The houses were not selected due to a suspected or existing problem. Participation was motivated by the offer of free professional cleaning during the study period and no honoraria were offered.

Vacuuming Protocol

The cleaning protocol ensured a consistent level of cleaning between each house cleaned and each visit. The protocol provided the method and duration of vacuuming carpet and hard-surface floors in each room in each house. All finished rooms in the homes were vacuumed including the accessible areas of finished basements. The cleaning schedule was once a week over a period of 6 weeks. Two houses received a second vacuuming each week to determine the impact of more frequent cleaning on the amount of dust collected. Each room was vacuumed completely in one direction then completely in the other direction. The cleaning times varied according to floor type: 60 s/m² for wall-to-wall carpet, 45 s/m² for hard flooring with loose carpets, and 30 s/m² for hardwood/vinyl/laminate/ceramic floors. The entire floor space in each room was cleaned including behind doors, and where feasible, furniture was moved. An individualized spreadsheet was prepared for each house and provided to the cleaner. This spreadsheet listed the required duration of cleaning of each room to be cleaned. The cleaner used a stopwatch to time the vacuuming in each room, continuing until the time was complete, and logged the actual time spent to clean each area on the sheet. The average time of cleaning was 2.1 h (1.8–2.9 h); this was within 10% of the times calculated for each house using the established floor and surface area criteria. All vacuuming was carried out using a Miele S558 HEPA vacuum (Miele Limited, Vaughan, Ontario).

After each house cleaning, the vacuum bag and primary filter were removed and sealed in clean, appropriately sized plastic ziplock bags for analysis. Before removing the bag and in preparation for the next vacuuming, the vacuum cleaner was carefully cleaned by combing and vacuuming the brushes and reversing the airflow to

Table 1. Characteristics of the houses studied

	House 1	House 2	House 3	House 4	House 5	House 6
House type	Detached bungalow	Detached two-storey	Detached bungalow	Detached two-storey	Detached bungalow	Row
Construction	1950s	1947	1950s	1920s	1998	2001
Renovation	1980s	1991	1990s	1950s		
Floor area	125 m ²	220 m ²	145 m ²	115 m ²	170 m ²	155 m ²
Floor type	Mainly carpet	50% carpet	Area carpets	Mainly carpet	Mainly carpet	50% carpet
Occupants	2 seniors	3 adults, 1 child	2 adults, 2 children	2 adults	1 senior	2 adults, 2 children
Pets	None	1 dog	None	2 cats	None	2 cats

dislodge dust from the hose and wand. To ensure consistency, one member of the study team was assigned to retrieve the bag and filter from the last house, clean the vacuum, and install a pre-weighed new filter and vacuum bag. The samples were stored under dry air conditions at room temperature and delivered to the laboratory.

Vacuum Dust Analysis

The vacuumed floor dust from the first cleaning was sieved into three fractions: <150 µm, 150 to <300 µm, and >300 µm (stainless steel test sieve models # 50 and 100; meeting ASTM E-11 specifications, Fisher Scientific, Ottawa) and weighed using a Sartorius balance (A120-S 4; accurate to 0.1 mg). Endotoxin and glucan were analyzed in the <300 µm fraction. Endotoxin concentration in the dust was determined using the *Limulus* amoebocyte lysate (LAL) chromogenic method according to the manufacturer's instructions (Associates of Cape Cod; Falmouth, MA). The dust was extracted by adding 25 mg dust to 1 mL of 0.5N NaOH solution, mixed in vortex mixer, and then shaken on a rotary shaker for 2.5 h. Extraction and analysis for glucan was performed as described previously and glucan was calculated as curdlan-equivalents [9,28]. The dust mite allergens Der f1 and Der p1 were extracted from 100 mg dust with borate buffer and determined with a monoclonal-based enzyme immunoassay according to the manufacturer's instructions (Indoor Biotechnologies, North Carolina) [29] with a Molecular Dynamics plate reader (Union City, CA). Since cleaning does not change the composition of residual particulates, house dust mites and glucan were measured only at the time of the first cleaning. Dust endotoxin was measured in all samples in order to gather the relative impact of the two dominant sources of endotoxin loading noted above.

Aerosol Dust Collection and Analysis

Aerosolized particle samples were collected from each house during the entire length of the first time cleaning (Table 1). Size-segregated samples were taken

using a Model 110 (nonrotating) MOUDI sampler (Micro-Orifice Uniform Deposit Impactor) placed 1 m above the largest contiguous space in active use (usually the living room). This sampler divided the ambient particulate into 8 aerodynamic size ranges, each characterized by a 50% cut point as follows: 18, 5.6, 3.2, 1.8, 1.0, 0.56, 0.32, and 0.18 µm. All eight impactor stages were loaded with tared 47 mm diameter Savillex #1141 Teflon PTFE filters (5 mm pore size, 0.1 mm thickness). Air was drawn through each MOUDI with an oilless motor-mounted rotary vane GAST R-G557X pump calibrated to deliver 30 L/min (the pump was calibrated using a Dwyer rotameter). At the completion of the cleaning of each cleaning cycle, the Teflon membrane samples were removed from the MOUDI, replaced in sterile Petri dishes and stored for later analysis for fungal glucans [28].

Statistical Analysis

Statistical analyses (ANOVA, basic statistics) were done on the settled dust weight reductions using repeated measures over time on untransformed data using SYSTAT version 12 (San Jose, CA).

For the purposes of data presentation, the large fraction (L) is >300 µm, the fine dust fraction (F) is the sum of 150–300 µm and <150 µm (from which allergen, endotoxin and endotoxin data were obtained), and the small fraction (S) is <150 µm.

Results

The characteristics of the houses are shown in Table 1. A walk-through inspection by a knowledgeable member of the study team found signs of condensation or minor water leaks in the basement and/or in other locations typical of many houses in Ontario. House 1 had a musty odor noticeable upon entering the house. House 3 also had a musty odor localized near a cold storage area. No moldy

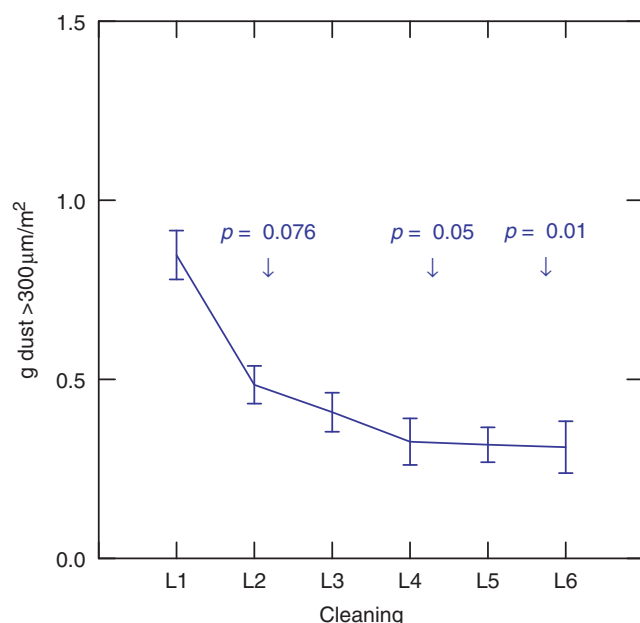


Fig. 1. Changes in the large (> 300 μm ; L) settled dust fraction over the six cleanings in six homes (mean $\pm$ SD); *p*-values indicate at which cleanings there were statistically significant changes by ANOVA.

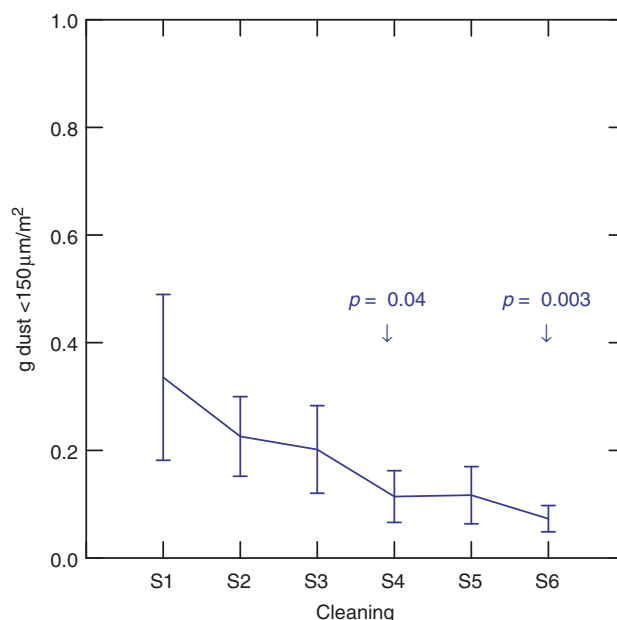


Fig. 3. Changes in the small (> 150 μm ; S) settled dust fraction over the six cleanings in six homes (mean $\pm$ SD); *p*-values indicate at which cleanings there were statistically significant changes by ANOVA.

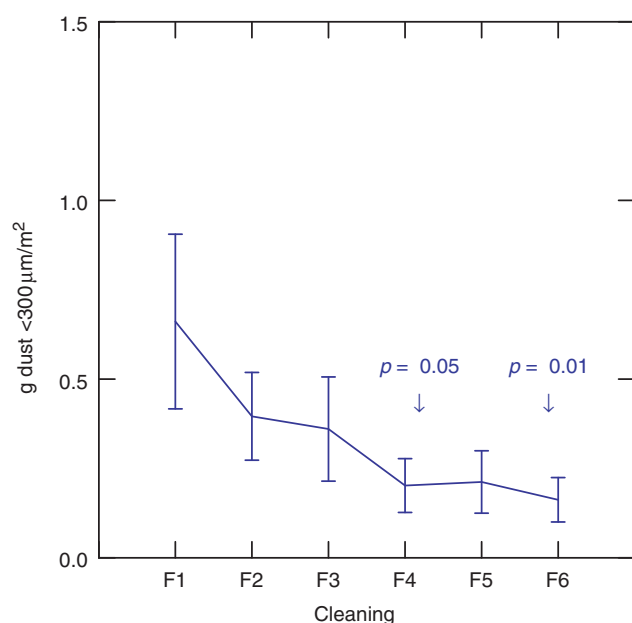


Fig. 2. Changes in the fine (< 300 μm ; F) settled dust fraction over the six cleanings in six homes (mean $\pm$ SD); *p*-values indicate at which cleanings there were statistically significant changes by ANOVA.

or musty smell was detected in the other houses. The occupants of houses 5 and 6 appeared to be controlling moisture by operating a dehumidifier in the basement year-round.

The homeowners reported cleaning their houses 4–5 times per month with portable vacuum cleaners.

Dust was fractionated into > 300 μm , 150–300 μm , and < 150 μm samples. The fine dust collected from the floors of the six homes ranged from 75 to 900 g per house. Geometric Mean mass loadings for cleaning intervals 1–6 for the large fraction were (1) 0.835, (2) 0.473, (3) 0.393, (4) 0.300, (5) 0.301, and (6) 0.275 g/m². For the fine fraction, Geometric Mean values respective of each sequential cleaning were (1) 0.487, (2) 0.311, (3) 0.239, (4) 0.148, (5) 0.137, and (6) 0.113 g/m². For the small fraction, the values were (1) 0.326, (2) 0.175, (3) 0.132, (4) 0.081, (5) 0.073, and (6) 0.055 g/m². These values were all highly correlated. Total masses collected for six cleanings were 16.16, 7.17, and 11.96 g/m² for L, F, and S, respectively.

The effect of cleaning interventions for the different size fractions is shown in Figures 1–3. For the large fraction (Figure 1), after the first cleaning, the reduction reached statistical significance at the 4th cleaning, followed by a slight, but significant further decrease at the 5th cleaning; the second cleaning approached significance ($p = 0.076$). For the fine fraction (Figure 2), after the first cleaning, the reductions did not reach statistical significance until the 4th cleaning ($p = 0.05$) and a further statistically significant reduction at the 6th cleaning ($p = 0.01$). For the small fraction (Figure 3), statistical significance was reached by the second cleaning ($p = 0.04$) and a further statistically

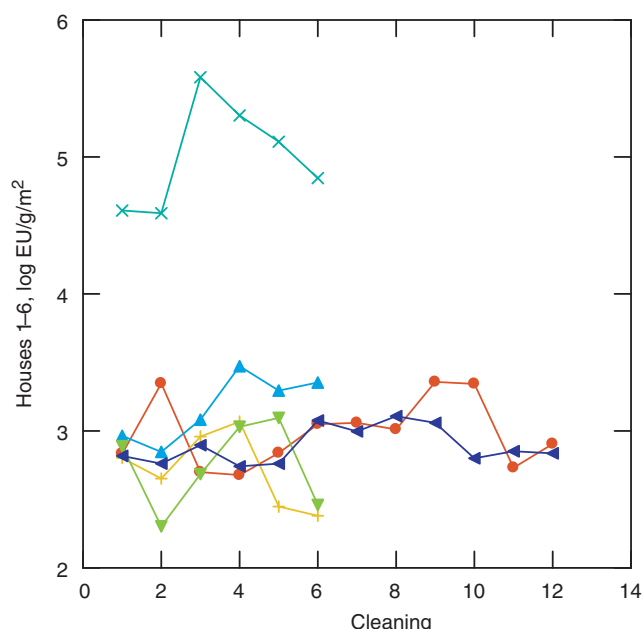


Fig. 4. Endotoxin loading in the fine fraction (> 300); there were six cleanings of six homes and for a two, six additional cleanings.

significant reduction again at the fifth cleaning ($p=0.003$).

Of the two house dust mite allergens, Der f1 was present more commonly and in greater amounts. The concentration was $73.6 \mu\text{g/g}$ or $0.09 \mu\text{g/g/m}^2$ (house 1), $15.5 \mu\text{g/g}$ or $0.07 \mu\text{g/g/m}^2$ (house 2), $8.9 \mu\text{g/g}$ or $0.03 \mu\text{g/g/m}^2$ (house 3), $0.06 \mu\text{g/g}$ or $0.001 \mu\text{g/g/m}^2$ (house 4), $8.9 \mu\text{g/g}$ or $0.07 \mu\text{g/g/m}^2$ (house 5), and $13.3 \mu\text{g/g}$ or $0.09 \mu\text{g/g/m}^2$ (house 6). Der p 1 was detected in houses 3 and 5 at 0.01 and $0.02 \mu\text{g/g}$, respectively.

For fungal glucan, expressed as curdlan equivalents, the values were 85.03, 9.65, 14.08, 7.02, 23.16, and 10.32, respectively, in houses 1–6. The high glucan level found in house 1 is compatible with the observation of a musty odor during the cleaning visits.

Endotoxin loading (EU/g/m^2) is shown in Figure 5. Mean values at the time of the first cleaning averaged $594.6 \pm 187.9 \text{ EU/g/m}^2$ or ca. $59 \pm 19 \mu\text{g/m}^2$. With one exception, the values were constant throughout the experiment. One house had significantly higher endotoxin loadings and a spike at cleaning three (Figure 4). There were no pets in this house. During the first cleaning, the determinant of the high value was higher loadings of fine dust overall. By the third cleaning, this was well on the way downwards. On investigation, the spike was a result of a dog brought into the house for a couple of days.

Glucan concentrations determined in the particulate samples in the 8 size fractions of the MOUDI sampler are

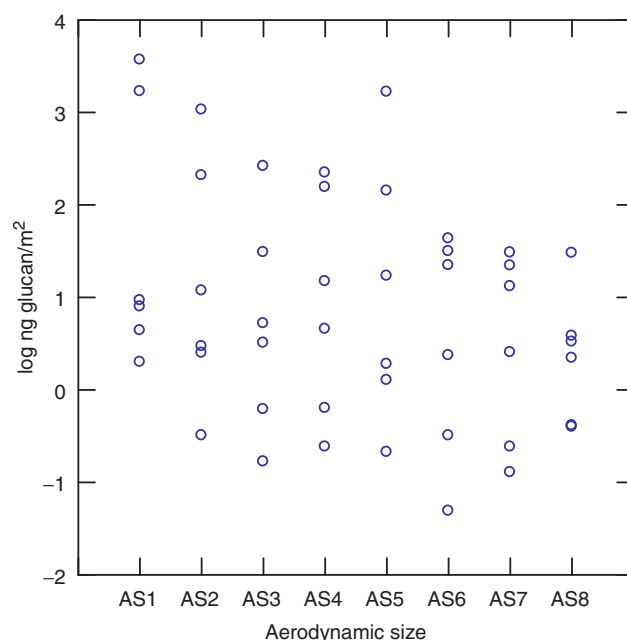


Fig. 5. Airborne glucan concentration measured in six homes with the MOUDI sampler. Open circles represent the value for each of six homes according to aerodynamic size (left to right, aerodynamic sizes representing each stage (18, 5.6, 3.2, 1.8, 1.0, 0.56, 0.32 and $0.18 \mu\text{m}$; AS1–AS8, respectively).

shown in Figure 5. Total airborne glucan averaged 4.32 ng/m^3 (0.89–14.06). Fungal glucan was present in each size fraction from 0.18 to $18 \mu\text{m}$ and was distributed fairly evenly in most homes. Two of the homes had higher airborne glucan concentrations.

Discussion

Krieger et al. [24] reported that providing HEPA filter-equipped vacuum cleaner and education in using it on a regular basis reduced incidence of urgent care for asthmatic children. This result was similar to other studies but the health effects have been generally equivocal [25,30]. There are a number of possible reasons for this, including small sample size and the adequacy of cleaning.

Roberts et al. [31] found that on much used carpets, in between $6\text{--}45 \text{ min/m}^2$ of careful vacuuming with a good quality home vacuum cleaner removes deep dust loading from carpet (mean ca. 20 min; comparison to HVS3 samples). Using professional cleaners with a modern HEPA filter-equipped good quality home vacuum cleaner, our results demonstrate that a steady state was achieved by six rigorous cleanings spread over 6 weeks in the homes cleaned once a week or over 3 weeks in the two homes vacuumed twice a week. Values of what Roberts et al.

[31,32] describe as deep dust loading collected by this method averaged 3.6 g/m^2 , which is comparable to their results. The dust loading reflects the particular cleaning practices and occupancy of the homes studied. Based on the values recovered after dust recovery had become asymptotic using the high effort cleaning described, dust accumulation was calculated to be $\sim 1 \text{ mg/m}^2/\text{day}$ averaged over all homes and all surfaces. Concentrations and loadings of house dust mite allergens and glucan in settled dust were comparable to those from houses in Ottawa [12] and for dust mites and endotoxin to studies elsewhere in Ontario [10]. Weschler [33] noted that settled dust accumulations can have very large surface areas in relation to other surfaces in a building. Particles (on filters or in settled dust) can adsorb (and desorb) relatively large amounts of volatiles depending on their vapor pressures. Accumulations of settled dust collected during the cleaning period when converted to surface areas by his method averaged twice the floor area of each house.

These data provide support for the view that an important reason for equivocal findings from particulate reduction studies is lack of cleaning effort before symptom assessment begins. Effective education on how to clean would be important to a reliable outcome [24,34]. On smooth surface floors, dust mass was correlated with renter-occupied properties and family size and negatively correlated with measures of household income [35]. An additional factor suggested by the work of Roberts et al. [31] and the present work is exposure misclassification. Less intense cleanings than might be required to reliably make allergen loading assessments have been made in

studies assessing allergen burdens in some epidemiology studies, e.g., [5,36].

Occupational exposures including from fungi in settled dust resulting from the use of portable vacuum cleaners without effective HEPA filters is a recognized potential health concern for asthmatics and domestic workers. Although the homes in the present study were chosen to be apparently free of mold damage, most had some signs of water damage or musty odors. Much of the exposure to fungal materials is as fine particles of smaller aerodynamic diameters of viable spores. Foto et al. [28] showed that approximately one third of airborne glucan collected over 5 days in homes came from intact spores, an additional third from spore and hyphal fragments, and the remainder were apparently smaller particles. In addition, they reported that, in mold-damaged homes, there were greater notional concentrations of spore and hyphal fragments compared to undamaged homes. These data are generally comparable to those from Reponen et al. [37]. Samples collected using the MOUDI sampler (Figure 5) support the findings of Foto et al. [28], with roughly similar amounts of airborne glucan found from 0.18 to $18 \mu\text{m}$. Although there are some data on airborne endotoxin using a size-selective sampler (also the MOUDI sampler) [38], such sampling has not apparently been reported.

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