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Endotoxin, cat, and house dust mite allergens in electrostatic cloths and bedroom dust

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ABSTRACT

Environmental exposure to endotoxin, Fel d I (cat) allergen and Der p I (house dust mite) allergen have been associated with asthma symptoms and have been measured in the environment using various sampling methods, including the electrostatic dust collector. The objectives of this study were to investigate whether levels of endotoxin and allergens were detectable in electrostatic dust collectors and to examine the correlation of allergen and endotoxin levels between electrostatic dust collectors and vacuum sampling methods (floor dust and mattress dust). Electrostatic cloths, bedroom floor dust and mattress dust samples from a subset of 60 homes were randomly selected from the Health of Occupants of Mouldy Homes study for allergen and endotoxin analysis. Fel d I and Der p I allergens were analyzed by double monoclonal antibody ELISA and endotoxin by the kinetic Limulus amoebocyte lysate assay. An enhanced ELISA method was used to analyze Der p I in the electrostatic cloths. Endotoxin was detected in all samples, however Fel d I and Der p I were not detected in all electrostatic dust collector samples (detection in 53% and 15% of cloths respectively). No correlations were found between cloth and dust samples for endotoxin or Der p I, but moderate-to-strong correlations were found between all three sampling methods for Fel d I ($r_s = 0.612$ – 0.715 , $p < 0.001$). Poor correlation was found between floor dust and mattress dust samples for Der p I ($r_s = 0.256$, $p = 0.048$). Electrostatic dust collectors may provide a way to measure airborne dust and allergen. Given the moderate-to-low correlations with vacuum dust sampling, this may present a unique measurement system which, when collected alongside traditional vacuum dust sampling, could provide additional exposure measures. Further studies are required to correlate endotoxin and allergen levels measured by electrostatic dust collector with air sampling and to explore the relationships between these bioaerosols, environmental factors and asthma.

KEYWORDS

Der p I allergen;
electrostatic dust collector;
endotoxin; Fel d I allergen;
floor dust; mattress dust

Introduction

Indoor endotoxin exposure from inhaled air has been associated with asthma-related symptoms, wheezing in infants, and poorer respiratory function.^[1] Other indoor bioaerosol exposure, such as exposure to Fel d I (cat) allergen or Der p I (house dust mite) allergen has also been associated with asthma symptoms in asthmatics who are sensitized to the allergen.

To measure endotoxin and allergen exposure, surrogate sampling of vacuumed floor and/or mattress dust sampling is commonly used.^[2–4] Mattress dust sampling may be a source of indoor bioaerosol exposure, as the inhaled air of a sleeping person is close to the mattress. A person also spends a significant amount of time in bed.^[2] However, studies have shown poor correlation between endotoxin and allergen levels measured by active airborne

sampling and by floor dust sampling, and airborne samples may be more representative of endotoxin and allergen levels in inhaled air.^[5,6] A weak correlation exists between endotoxin measured from mattress dust samples and airborne samples,^[7] and no correlation has been found between mattress dust sampling and airborne sampling for Der p I.^[8] Previous studies have found detectable levels of Fel d I from air sampling and mattress dust sampling, but did not assess a correlation between these sampling methods.^[9,10] The electrostatic dust collector (EDC) is a passive, settled dust sampling method that has recently been used as a surrogate to active airborne sampling.^[11–13] EDCs have the advantage over active airborne sampling in that they are inexpensive, not labor intensive, can be left for long periods of time and are easy to install,^[11] so studies using EDCs are able to collect a large number of

samples over longer periods. Various studies have measured endotoxin, Fel d I, and Der p I in farm homes, urban homes, schools, and other settings, using floor dust and EDC sampling.^[6,11–13] However, to our knowledge, no single study has compared Fel d I, Der p I, and endotoxin levels in bedroom floor dust, mattress dust, and EDC samples in urban homes. Comparing these two sampling methods (EDC and vacuum dust sampling) increases our understanding of what exposure information reservoir sampling provides compared with airborne or potential inhalation sampling.

The objectives of this study are to examine (i) whether Fel d I allergen, Der p I allergen, and endotoxin can be measured using EDC sampling and (ii) whether Fel d I, Der p I, and endotoxin levels from EDC samples correlate with levels in floor and mattress dust samples.

Methods

Experimental design

Samples of flooring dust, mattress dust, and EDCs were collected from children's bedrooms in urban homes as part of the Health Research Council funded Health of Occupants of Mouldy Environments (HOME) study during the autumn and winter periods from July 2010 to June 2012, as previously described.^[14] Cloth, bedroom floor dust, and mattress dust samples from 60 of the 450 children enrolled in the HOME study were randomly selected for allergen and endotoxin analysis. The children were aged 1–7 years old and were living in the Wellington region, New Zealand. Due to the quantity of floor or mattress dust needed for extraction and analysis of these three bioaerosols, participants with samples below 100 mg were excluded prior to the random selection of the subset of 60. As part of the main questionnaire participants were also asked questions about cat ownership. The HOME study received ethical approval from the Central Health and Disability Ethics Committee (CEN-09-06-039).

EDC sampling method

Two Pledge Grab-It electrostatic cloths (SC Johnson and Son Inc., Racine, WI) were attached to an alcohol-cleaned core flute plastic shelf using paper binders. Each cloth had a surface area of 0.059 m² (0.203 x 0.292 m). Each EDC was placed at a height of 1.5 ± 0.2 m above the floor, attached to the bedroom wall using 3M removable adhesive strips or nails. The cloths were left in the home to passively collect dust for 4 weeks. At the end of the sampling period, researchers took

down the EDC and placed it in a sterile plastic bag, returned it to the lab, then stored at -20 °C.^[15]

Floor and mattress dust sampling method

At the start of the EDC sampling period, prior to the EDC attachment to the wall, a vacuum cleaner (Jaguar Power Pro, 1500 W) was used to collect the bedroom floor and mattress dust samples for 2 min using methods described previously.^[16] A 1 m² area adjacent to the bed that was clear of furniture and, where possible, closest to the bed head. For the mattress samples, a 1 m² mattress area was sampled for a bed and a 0.897 m² area (1.3 x 0.69 m) was sampled for a cot. Vacuuming of the mattress would start from the top of the mattress (near the head) down until a 1 m² area had been sampled. As the cots were all less than 1 m² in area, the entire mattress was sampled. The dust samples were stored at -20 °C until analysis.

Extraction methods

For Fel d I and Der p I, dust samples were extracted with phosphate-buffered saline with 0.2% Tween-20 (PBS-Tween). For endotoxin, the dust samples were extracted with pyrogen-free water containing 0.05% Tween-20 (PFW-T). Extract aliquots were frozen (-20 °C) until analysis. Electrostatic cloths were cut into quarters. One quarter was extracted with 50 mL of PFW-T for endotoxin analysis. Another quarter was extracted with 25 mL of PBS-Tween for the two allergens.

Analyses of samples

Fel d I and Der p I in dust sample extracts were determined by double monoclonal antibody enzyme-linked immunosorbent assay (ELISA) as previously described.^[17] Fel d I in electrostatic cloth extracts were determined as for the dust samples, while for Der p I, an amplified ELISA method, as described by Tovey et al. was used.^[18]

Dilutions (1 in 150) of dust extracts and undiluted EDC extracts were analyzed for endotoxin using the quantitative, kinetic chromogenic Limulus Amoebocyte Lysate (LAL) assay as previously described.^[19] Allergen concentrations below the limit of detection (LOD) were given half the value of the standard LOD (0.08 ng/mL for Fel d I, 5.00 ng/mL for Der p I measured in floor and mattress dust, and 1.22 pg/mL for Der p I measured by EDC) and were subsequently included in the statistical analyses.

Table 1. Endotoxin, Fel d I, and Der p I levels in bedrooms.

Sample type	n/N ^A	GM (95% CI)
Endotoxin floor dust (EU/g)	60/60	61498 (51182–73893)
Endotoxin floor dust (EU/m ²)	60/60	3092 (2573–3715)
Endotoxin mattress dust (EU/g)	60/60	41137 (31238–54173)
Endotoxin mattress dust (EU/m ²)	60/60	1987 (1503–2627)
Endotoxin cloth (EU/m ²)	60/60	863 (703–1060)
Fel d I floor dust (μg/g)	60/60	9.02 (4.87–16.71)
Fel d I floor dust (μg/m ²)	60/60	0.45 (0.24–0.83)
Fel d I mattress dust (μg/g)	60/60	6.60 (3.53–12.34)
Fel d I mattress dust (μg/m ²)	60/60	0.314 (0.17–0.59)
Fel d I cloth (μg/m ²)	32/60	0.58 (0.32–1.06)
Der p I floor dust (μg/g)	60/60	394.70 (272.40–571.70)
Der p I floor dust (μg/m ²)	60/60	3.82 (2.71–5.41)
Der p I mattress dust (μg/g)	60/60	466.40 (320.10–679.60)
Der p I mattress dust (μg/m ²)	60/60	4.97 (3.43–7.20)
Der p I cloth (μg/m ²)	9/60	3.01 (2.37–3.84)

Endotoxin GMs and 95% CIs are rounded to whole numbers, while Fel d I and Der p I results are rounded to two decimal places

^An/N = Number of samples with detectable levels of endotoxin or allergen out of total number of samples

Statistical analyses

Endotoxin and allergen concentrations across each sampling method were non-normally distributed and were therefore log transformed to normalize their distributions. Geometric means (GM) with 95% confidence intervals (95% CI) were calculated. The Spearman Rank Correlation was used to analyze the correlation between the different sampling methods. A Mann-Whitney U test was used to analyze the relationship between cat ownership and bioaerosol levels. All analyses were done using the statistical software R (Version 3.2.1, R Foundation, Vienna, Austria).

Results

Floor dust samples were collected from 54 households with carpeted floors and six households with hard flooring. Endotoxin, Fel d I, and Der p I were detected using all three sampling methods (Table 1), with variation in the levels of detectability. Fel d I and Der p I were not measurable in all of the EDC samples, where 47% of Fel d I samples (28/60) and 85% of Der p I samples (51/60) were below the limit of detection. Of the 32 households with detectable levels of Fel d I on the cloths, 59% (19/32) owned a cat. A sub-analysis of cat ownership and bioaerosol levels revealed that there was a significant association with Fel d I for all sampling methods and endotoxin measured in cloth (Table 2).

Figure 1 and Table 3 present the results on the correlations between the sampling methods. The highest correlations between sampling methods were found for Fel d I (r_s (floor dust vs. mattress dust) = 0.704, r_s (floor dust vs. cloth) = 0.672, r_s (mattress dust vs. cloth) = 0.613, all $p < 0.001$), while the lowest correlation was

between Der p I mattress dust and cloth ($r_s = -0.056$, $p = 0.669$). Statistically significant correlations were also observed between endotoxin floor dust and mattress dust ($r_s = 0.463$, $p < 0.001$) and between Der p I floor dust and mattress dust ($r_s = 0.265$, $p = 0.040$). Correlations for each bioaerosol did not seem to vary significantly with measurements in μg/g or μg/m².

Discussion

GMs of endotoxin and Fel d I in mattress dust were smaller than those in bedroom floor dust, while conversely, GMs of Der p I were smaller in floor dust. Our results are consistent with previous studies.^[3,20,21] When comparing our EDC results obtained from urban homes with a study of farm houses in America with a 28-day sampling period,^[11] our GM for endotoxin was over three times lower (863 EU/m² compared with 2768 EU/m²), but our GM for Fel d I was seven times higher (0.5813 μg/m² compared with 0.078 μg/m²). For endotoxin, these differences are likely due to the differences in farming homes compared to urban homes; Noss et al. also found endotoxin levels were higher in farm homes compared to non-farming homes.^[6] The high Fel d I levels in our study could be due to many homes containing cats in New Zealand, and the ease of transportability of Fel d I on clothing.^[22] In our study, a third of the households owned a cat and these households had higher levels of cat allergen than households without cats. When comparing our EDC results for Der p I with a Dutch study of children's bedrooms,^[13] our GM was nearly 10 times higher (3.014 μg/m² compared with 0.3339 μg/m²). A potential reason for this difference is a longer sampling period (4 weeks vs. 2 weeks), although as previously investigated by Kilburg-Basnyat et al.,^[11] the rate of bioaerosols captured by EDC sampling decreases from a 2-week to a 4-week sampling period, so the difference cannot be fully accounted for by this. Other reasons may include different analytic methods and that there is a difference in Der p I levels between the two countries. A previous study looking at levels of Der p I in mattress dust in Taiwan mentioned that New Zealand has one of the highest levels of Der p I in the world, and they also had significantly lower GMs of the allergen compared to our study (0.72 μg/g compared with 466.40 μg/g).^[19] Also, when comparing Der p I levels in our floor dust samples with a study conducted in Poland, the GM in our study was far greater than their median of house mite allergens in carpets of urban homes (394.70 μg/g vs. 0.13 μg/g).^[3]

Table 2. Bioaerosol levels and cat ownership.

Sample type	GM (95% CI) in households with cats	GM (95% CI) in households without cats	P-value
Endotoxin floor dust (EU/g)	56636 (47893– 66975)	64083 (52874–77668)	0.414
Endotoxin floor dust (EU/m ²)	2806 (2373–3319)	3245 (2678–3933)	0.414
Endotoxin mattress dust (EU/g)	29785 (20747– 42760)	48344 (33675–59869)	0.215
Endotoxin mattress dust (EU/m ²)	1451 (1008–2089)	2325 (1866–2898)	0.215
Endotoxin cloth (EU/m ²)	1179 (977–1423)	739 (602–906)	0.023
Fel d I floor dust (µg/g)	85.1 (52.4–138)	2.94 (1.86–4.63)	<0.001
Fel d I floor dust (µg/m ²)	4.23 (2.60–6.87)	0.145 (0.0916–0.230)	<0.001
Fel d I mattress dust (µg/g)	39.6 (26.6–59.0)	2.69 (1.48–4.89)	<0.001
Fel d I mattress dust (µg/m ²)	1.89 (1.26–2.84)	0.128 (0.0706–0.232)	<0.001
Fel d I cloth (µg/m ²)	8.08 (5.48–11.9)	0.156 (0.110–0.220)	<0.001
Der p I floor dust (µg/g)	310 (231–415)	445 (297–667)	0.157
Der p I floor dust (µg/m ²)	3.08 (2.29–4.14)	4.26 (2.95–6.16)	0.168
Der p I mattress dust (µg/g)	390 (248–615)	510 (365–713)	0.822
Der p I mattress dust (µg/m ²)	4.18 (2.64–6.64)	5.42 (3.94–7.47)	0.920
Der p I cloth (µg/m ²)	3.12 (2.41–4.03)	2.96 (2.34–3.75)	0.990

All bolded p-values are statistically significant (p-value <0.05)

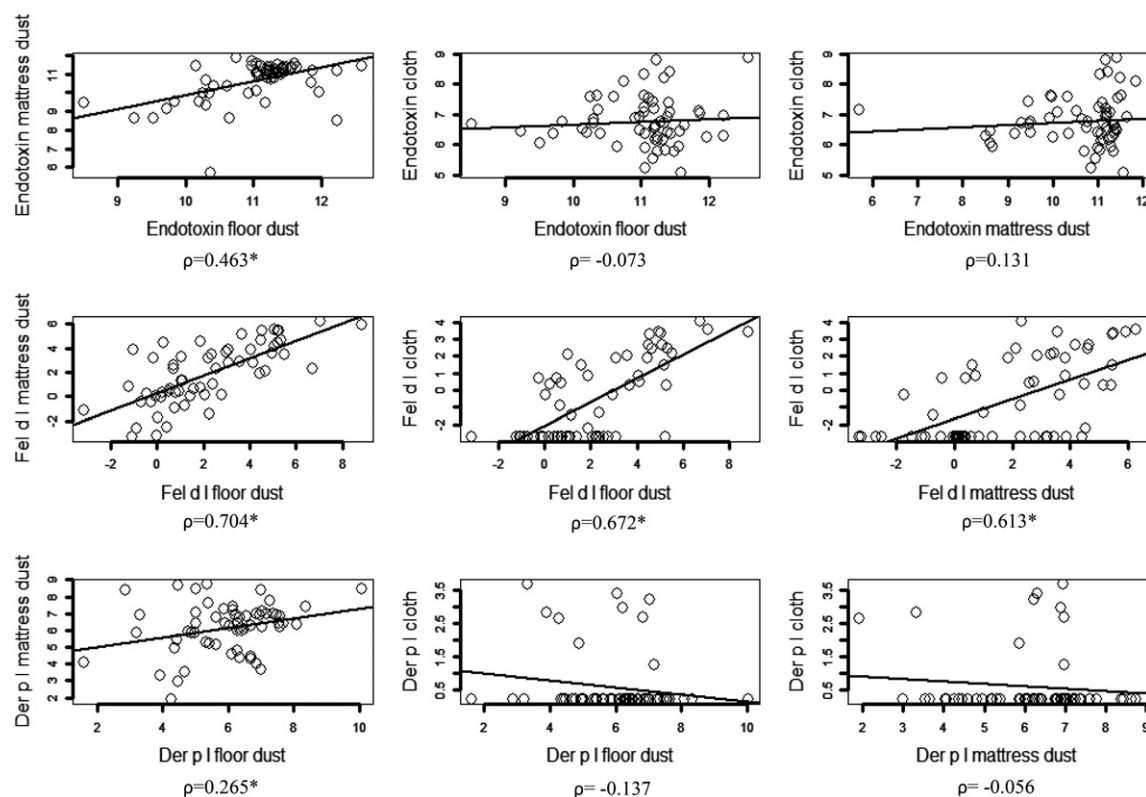


Figure 1. Correlations between log-transformed sample types for endotoxin, Fel d I and Der p I. ρ = correlation coefficient, *p-value is <0.05.

Only a small number of EDC samples had detectable levels of Der p I, even with high-sensitivity analysis. Tovey et al.^[18] found that 57.2% of samples measured by personal airborne exposure (using a combination of electrostatic cloths and adhesive film) had detectable levels of Der p I when analyzed by similar methods. Krop et al.^[13] were only able to detect Der p I in <2% of electrostatic cloths when analyzed with MARIA assay, but with ELISA, a much greater proportion of school EDC samples had detectable levels (N = 117). Different brands of cloth were

used in these studies and may therefore have different collection properties, such as different-sized electrostatic charges, which may influence the amount of Der p I that are attracted to the cloths. The rate of Der p I collection on the cloths over time may also change depending on the length of the sampling period. Kilburg-Basnyat et al. conducted a study investigating the rate of collection on electrostatic cloths over time and found that the increase in endotoxin levels from a 2-week collection period to a 4-week collection period was only 1.38 instead of

Table 3. Correlation between sample types.

Sample types	Correlation (dust samples* in $\mu\text{g/g}$)		Correlation (dust samples* in $\mu\text{g/m}^2$)	
	Rho (ρ)	P-value	Rho (ρ)	P-value
Fel d I floor dust vs. Fel d I mattress dust	0.704	<0.001	0.715	<0.001
Fel d I floor dust vs. Fel d I cloth	0.672	<0.001	0.671	<0.001
Fel d I cloth vs. Fel d I mattress dust	0.613	<0.001	0.612	<0.001
Endotoxin floor dust vs. Endotoxin mattress dust	0.463	<0.001	0.477	<0.001
Der p I floor dust vs. Der p I mattress dust	0.265	0.040	0.256	0.048
Der p I floor dust vs. Der p I cloth	−0.137	0.298	−0.142	0.279
Endotoxin cloth vs. Endotoxin mattress dust	0.131	0.319	0.123	0.351
Endotoxin floor dust vs. Endotoxin cloth	−0.073	0.582	−0.028	0.831
Der p I cloth vs. Der p I mattress dust	−0.056	0.669	−0.053	0.684

All bolded p-values are statistically significant (p-value <0.05)

*Dust samples refer to floor and mattress dust samples only. EDC samples were only measured in $\mu\text{g/m}^2$

double.^[11] However, for Fel d I, they found the differences in levels between a 1-week, 2-week, and 4-week sampling period were not significant. The charge or collection properties of electrostatic cloths used in bio-aerosol studies and the effect of collection period for Der p I may be a useful topic to investigate in the future. Overall, our results would suggest that EDC sampling may not be an adequate surrogate for active airborne sampling of Der p I and another airborne method may be required.

Out of the 32 EDC samples (53%) with detectable levels of Fel d I, 59% of them were from households who had owned a cat within the last 12 months. Conversely, out of the 28 cloths (46.7%) with undetectable levels of Fel d I, only one household had owned a cat in the last 12 months, which is interesting to note. This could be because the cat was not allowed to enter the child's bedroom. Fel d I has been found to transfer from cat owners' clothing to clothing worn by non-cat owners, which could explain how detectable levels were found in bedrooms of households who had not owned a cat.^[2] However, as all of the floor and mattress dust samples had detectable levels of Fel d I, these vacuum dust samples may reflect a previous history of cat ownership. Therefore, using EDC sampling as well as floor and mattress dust sampling could provide information on current airborne household exposure to Fel d I. The vacuum dust sampling could provide information on the amount of Fel d I that has accumulated in the floor and mattress over a period of time, while the EDC sampling would provide information on more recent exposure, such as from disturbed surfaces or clothing. It may be useful in future studies to analyze Fel d I with the high-sensitivity method used for Der p I, and doing so is likely to improve the detectability rate in the cloths.

To our knowledge, this is the first study to have compared Fel d I EDC and mattress dust sampling

methods. Fel d I is contained in small (<2.5 μm), airborne particles that are too small to settle and remain airborne on convection currents, which could explain the significant correlations of 0.612–0.715 found between the sampling methods (in $\mu\text{g/m}^2$).^[23] Our results suggest it may be sufficient to measure Fel d I levels using only one sampling method. One study showed Fel d I floor dust samples had an independent correlation with airborne samples after multivariate analysis,^[24] while another study showed that EDC and airborne sampling and EDC and floor dust sampling were moderately correlated ($r = 0.72$ and 0.61 , respectively).^[5]

We found a lower correlation for endotoxin measured by EDC compared with floor dust sampling ($r = 0.463$) than a previous study that found the two sampling methods correlated well ($r = 0.65$).^[6] This may be due to a number of factors, including seasonal influences, geography, house construction materials and flooring type. Noss et al. measured endotoxin levels in living rooms of both urban and farming homes in the Netherlands.^[6] It is unknown if their sampling period was in the same seasons as our study (autumn and winter). We suspect there may have been large differences in the proportion of hard floors and carpeted floors between the studies, as Scandinavian countries do not often have the wall-to-wall carpeting found in most New Zealand bedrooms. Disturbance of hard floors by walking, for example, causes more dust particles to rise into the air than with carpeted floors, as carpet can hold onto the dust more strongly.^[16] Another reason for the lower correlation between the sampling methods may be that endotoxin is site-dependent. Endotoxin is found in the walls of Gram-negative bacteria, and a previous study found that Gram-negative bacteria in floor dust mostly originated from non-human sources, whereas bacteria in mattress dust largely originated from human sources.^[25] The study did not investigate bacterial sources

for airborne samples, but sources for airborne endotoxin levels might differ. For example, they may be more readily influenced by outdoor airborne levels. Noss et al. also found a strong correlation between traditional air sampling and EDC sampling for endotoxin, and little correlation between air and floor dust sampling.^[6] This, together with our current results, suggest that EDC sampling may better reflect airborne endotoxin levels than floor or mattress vacuum dust sampling.

A low correlation of 0.265 was found between floor dust and mattress dust samples for Der p I. This finding is consistent with previous studies.^[26,27] No correlation was found between the floor and mattress dust and EDC samples, although we cannot confirm the results as only nine EDC samples had detectable Der p I levels. As 90% of households in our study had carpeted floors, this may have made it harder for the Der p I to become airborne when the floor was disturbed. It would be interesting to investigate this with a larger sample size, a longer sampling time and/or an improved technique for analysing Der p I levels from electrostatic cloths. However, until the techniques improve for EDC sampling, it may be best to use alternative sampling methods to assess airborne Der p I.

One interesting observation was that the correlations calculated from floor and mattress dust measured in $\mu\text{g/g}$ were similar to those measured in $\mu\text{g}/\text{m}^2$. This is useful to know, as studies that analyze results in $\mu\text{g/g}$ only may be comparable to those measured in $\mu\text{g}/\text{m}^2$. One study found a similar observation for Der p I, where the correlations between $\mu\text{g/g}$ and $\mu\text{g}/\text{m}^2$ were 0.8–0.9 ($p < 0.001$), depending on the analysis protocol used.^[27] However, it would be still be wise to confirm this in another study to assess the reproducibility of this finding, especially for endotoxin and Fel d I.

There were several limitations to the study, including that there was only one sampling location from each bedroom. Depending on the size of the bedroom, it is possible that differences occurred in the levels of allergen and endotoxin within a room, due to ventilation, air currents, or proximity to furniture, for example.^[11] It may be useful to analyze endotoxin and allergen levels from multiple cloths from several EDCs placed in different locations within a room. Although each EDC had two cloths placed on it, we analyzed a quarter of one of the cloths and did not compare the bioaerosol levels between the two cloths, as the other cloths were reserved for other bio-contaminant analysis.^[15] Kilburg-Basnyat et al. showed

good correlations for endotoxin and Fel d I between paired EDCs.^[11]

Endotoxin and allergens were analyzed from the EDC samples using single extraction of PFW-T, but double extraction may have yielded higher levels and/or a greater proportion of detectable levels of allergen. Adding 0.05% Tween-20 for extracting endotoxin has been debated in the past, but several studies have since shown that using PFW-T yields higher levels of endotoxin in dust samples.^[28–30] Due to availability, our study used the Pledge Grab-It cloths instead of the Zeeman electrostatic cloths used by many studies.^[5,6,11,31] The two brands of cloths have different properties, such as different sizes and different surface textures. Previous studies using the Pledge Grab-It to measure fungal DNA and ergosterol levels showed that a larger buffer volume and at least two extractions were needed.^[16,32] These differences may have also influenced the difference in our endotoxin GM to that found by Kilburg-Basnyat et al.^[11] It is therefore important to determine the optimal extraction method for endotoxin from the Pledge Grab-It cloths to ensure we have complete extraction.

This study only used a subset of the HOME study samples. Further analyses using all 450 samples would be beneficial to confirm or disprove the findings from this study. Other allergens that have been found to be associated with asthma and asthma-related symptoms, such as pollen allergens and horse allergen, could be analyzed to determine which method is best for sampling them.^[33,34] The HOME study was a matched case-control study of wheezing and non-wheezing children that included a comprehensive assessment of potential confounders.^[14] Therefore, samples from the HOME study could also be used to confirm the relationship between asthma-related symptoms, sampling method and bioaerosol levels, and investigate any associations between bioaerosol levels, sampling method and indoor environmental factors. Although EDC sampling is thought to be a surrogate method for airborne sampling,^[12] we cannot confirm this for Der p I as only nine of the 60 cloths had detectable levels, so further analysis is needed with a larger cohort.

Conclusion

Electrostatic cloths placed in the bedrooms of 60 children for 4 weeks universally contained measurable endotoxin, and almost universally Fel d I (when cats were kept indoors as pets) but rarely contained Der p I. Only Fel d I in cloths correlated with reservoir

levels on bedroom floors and mattresses suggesting that either collection method should reflect exposure. The undetectable levels of Der p I in most cloths makes correlations with reservoir levels difficult to interpret and suggests that cloths are unlikely to be useful in estimating exposure. The poor correlation between cloths and reservoir levels for endotoxin suggests that cloths may give additional information about exposure and further study of endotoxin in cloths compared to air sampling is warranted.

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