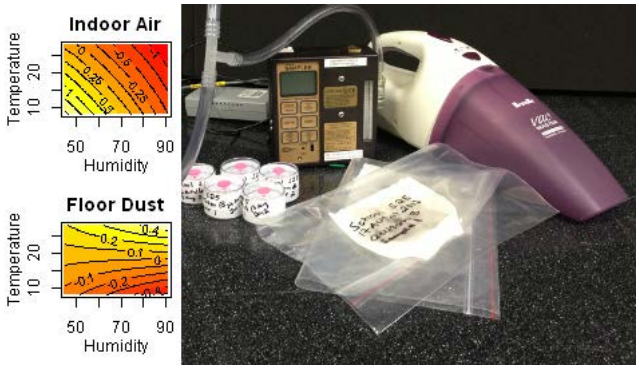
Endotoxins in indoor air and settled dust in primary schools in a subtropical climate

Heidi Salonen[1,2*], Caroline Duchaine[3,4], Valérie Létourneau[4], Mandana Mazaheri[1], Sam Clifford[1,5], Lidia Morawska[1*]

[1] Queensland University of Technology, International Laboratory for Air Quality and Health, 2 George Street, Brisbane, Q 4001 Australia
[2] Finnish Institute of Occupational Health, Developing Indoor Environments, Topeliuksenkatu 41 aA, FI-00250 Helsinki
[3] Université Laval, Department of Biochemistry, Microbiology and Bio-informatics, 2325, rue de l'Université Québec (Québec) G1V 0A6 Canada
[4] Centre de recherche de l'Institut universitaire de cardiologie et de pneumologie de Québec, 2725, chemin Sainte-Foy, Québec (Québec) G1V 4G5 Canada
[5] Centre for Air Quality & Health Research and Evaluation, 431 Glebe Point Rd, Glebe 2037, Australia

(* Corresponding Authors: Phone (+61) 7 3138 2616; fax: (+61) 731389079; e-mail: l.morawska@qut.edu.au/ Phone (+358) 304741; e-mail: heidi.salonen@ttl.fi)

TOC ART



ABSTRACT

Endotoxins can significantly affect the air quality in school environments. However, there is currently no reliable method for the measurement of endotoxins and there is a lack of reference values for endotoxin concentrations to aid in the interpretation of measurement results in school settings. We benchmarked the "baseline" range of endotoxin concentration in indoor air, together with endotoxin load in floor dust, and evaluated the correlation between endotoxin levels in indoor air and settled dust, as well as the effects of temperature and humidity on these levels in subtropical school settings. Bayesian hierarchical modeling indicated that the concentration in indoor air and the load in floor dust were generally (<95th percentile) < 13 EU/m^3 and < 24,570 EU/m^2, respectively. Exceeding these levels would indicate abnormal sources of endotoxins in the school environment, and the need for further investigation. Metaregression indicated no relationship between endotoxin concentration and load, which points to the necessity for measuring endotoxin levels in both the air and settled dust. Temperature increases were associated with lower concentrations in indoor air and higher loads in floor dust. Higher levels of humidity may be associated with lower airborne endotoxin concentrations.

1. INTRODUCTION

Endotoxins are lipopolysaccharide molecules in the outer membranes of gram-negative bacteria and are ubiquitous in indoor and outdoor environments[1-3]. Although there are no commonly established analytical procedures for measuring endotoxin levels[4-6], and it is impossible to establish a clear dose-effect relationship or an exposure limit for the workplace[7], a wide range of indoor studies have found an association between endotoxin exposure and the exacerbation of respiratory allergic diseases, including asthma[8-10]. Even low concentrations of endotoxins may cause respiratory symptoms such as coughing, wheezing and phlegm[3, 11, 12]. Paradoxically, it has also been reported that exposure to endotoxins or other bacterial components in childhood might be a protective factor in allergic diseases, such as asthma and atopy[3, 13, 14].

In indoor environments, endotoxins are mainly measured in house dust using a vacuum cleaner, because it is much easier and cheaper than using active airborne sampling[15-17]. Although endotoxin levels in settled floor dust have been used as a surrogate for personal long-term exposure[12, 18, 19], it is not clear which measure - per gram dust or per square meter - may better reflect the actual exposure of the occupant[20]. Despite the advantages of floor dust sampling, it has also been reported that endotoxin load in settled dust is only a surrogate measure for airborne endotoxins[21] and a poor proxy of inhaled endotoxin exposure, especially in classrooms, where floor dust consists, for a major part, of large and heavy particles (e.g. sand and breadcrumbs)[17]. On the other hand, since environmental air samples represent a snapshot in time, they may be poor surrogates and biased estimators for the actual concentrations they represent[1], and they may not be an appropriate measure to estimate longer-term inhaled exposure and chronic disease risk[21]. Thus, combining the measurement of dust endotoxins with other information about indoor characteristics may provide a better estimate of exposure to airborne endotoxins[15].

Several studies reported that, in residential indoor environments, the levels of endotoxins were generally much lower than the levels reported in occupational industry or farming settings[15, 18, 22, 23], and that endotoxin levels in the environment vary geographically and regionally, in both outdoor and indoor environments[7, 24, 25], and are influenced by meteorology factors such as temperature and humidity[24, 26, 27]. In addition, several other local factors, such as the existence of dampness indoors[17, 24], number of people and pets (especially dog) indoors[28-30], presence of carpets[21, 27], and household cleanliness[29, 31] have been reported to effect on indoor endotoxin levels[24, 32, 33]. However, it should be noted that the effect of several environmental factors on endotoxin levels has been shown in the literature to be inconsistent, and additional studies are needed.

Although much attention has been paid in recent years to the quantification of suspended endotoxins in different workplaces[34], there are very few studies available on the general concentration of endotoxins in indoor air, together with endotoxin loads in settled dust in school environments[12, 15]. Traditionally, schools have been naturally ventilated and passively cooled[35], with carpet or vinyl as

flooring material[36]. Although mechanical ventilation systems dominated over natural ventilation in the twentieth century, natural ventilation has many advantages compared with mechanical systems[35, 37, 38], and may be the future trend in school buildings. Thus, gathering information in relation to naturally ventilated school buildings with different flooring materials is particularly important.

Investigations into the "baseline" level of endotoxins – both in indoor air and in settled floor dust – in school settings without mold and moisture problems, and without other well-known indoor sources of bioaerosols (from farming, pets, cooking etc)[24, 39, 40] will help to characterize normal and abnormal levels of endotoxins, indicate the need for further investigation, and develop and implement corrective measures to improve the indoor air quality. Thus, the overall aims of the present study were: (i) to benchmark the "baseline" concentration of endotoxins in indoor air, together with the load of endotoxins in floor dust, that may be found in carpeted, naturally ventilated, non-moisture/mold damaged school classrooms in a subtropical area during different seasons (winter, spring and autumn); (ii) to quantify correlations between the concentration of endotoxins in indoor air and the load of endotoxins in floor dust, and (iii) to determine the effect of temperature and humidity (seasonality) on endotoxin levels.

2. MATERIAL AND METHODS

2.1. Study design, location and classroom characteristics

This was a cross-sectional study, which was carried out between October 2010 and August 2012, in a total of 25 randomly selected primary schools (S01-S25) in the Brisbane Metropolitan Area (BMA), South-East Queensland, Australia, as part of a large epidemiological project titled "Ultrafine Particles from Traffic Emissions and Children's Health (UPTECH)". According to the selection criteria for the schools, there were no major local air pollution sources, including infrastructure projects such as roads, tunnels and building construction, in the vicinity of the schools, other than road traffic. All selected schools were built more than 10 years ago, constructed of concrete or wood, with no central air-conditioning system. The school buildings were ventilated primarily via opened windows and doors, and ceiling fans. Two classrooms used by 8-11 year old children from each school were selected for the measurements. This study was conducted at S04-S07 and S18-S21 in autumn (March-May), S08-S12 and S22-S25 in winter (June-August), and S01-S03 and S13-S17 in spring (September-November), as defined by the Australian government[41]. All measurements were done during teaching periods, according to the Queensland Department of Education, Training and Employment. In Brisbane, schools are closed over most of the summer period (December-February) and therefore, no measurements were conducted during summer time.

Prior to sampling, a "walk-through" assessment was carried out to determine indoor and outdoor sampling locations. Room characteristics, with regards to cleanliness, moisture damage and other possible bioaerosol sources, were assessed visually in each studied classroom before the

measurements were conducted. In addition, building and room characteristics (e.g. number of students, room area, cleaning schedule and floor type) were recorded via a questionnaire and information form. Although the size of the classrooms (40-120 m^2, average 68 m^2) and number of pupils (16-29, average 23) varied between schools, the room area per classroom, as well as the number of students in each measured classroom (which generally differed by 0-2 students within each school) was similar within each of the measured schools. The daily cleaning schedule during the measurement period included carpet vacuum cleaning and desk wiping in each classroom. Vacuum cleaning was conducted before or after school hours and desk wiping was often done once a week. In classroom B at school S6, desk wiping was conducted on the same day (before) as endotoxin measurements. There was no visible moisture/mould or known moisture problems in any of the building structures at the time of the measurements. The floors of the classrooms were carpeted and there were no animals or pot plants inside the rooms. There was no kitchen (cooking) within the measured buildings and the school canteens were located in other buildings.

2.2. Sampling and instrumentations

Endotoxins in indoor air and floor dust were collected from two teaching classrooms at each school. Endotoxins were sampled using two different methods. Two *air samples* were collected using glass fiber filters mounted in 37 mm closed face cassettes (SKC, 225-709) for 8 hours at 2 L/min in each classroom. At a rate of 2 L/min, 37 mm closed face cassettes efficiently sampled particles with an aerodynamic diameter of up to 10µm. Samples were collected between the hours of 8 am and 7 pm (8h sampling in each classroom) during normal room activities. Classrooms were occupied between 9 am and 3 pm. The sampling of airborne endotoxins was conducted close to the children's desks, at least 1 meter from the nearest wall and at the height of about 1.0 m from the floor, which is in the children's breathing zone when they were seated. The flow rate of the SKC-pump (Airchek sampler, model 224-PCXR8, serial no. 944390) was regularly checked using a Gilian Gilibrator-2TM Primary Flow Calibrator (Range 20 cc – 6LPM P7N8-289-1) before each set of measurements. The airborne endotoxin concentrations were expressed as endotoxin units (EU) per cubic meter of air. No outdoor control was performed but 1–2 field blank filters were taken for quality control of air sampling at each school. Blank filters were subjected to all precalibration, postcalibration, storage and assay procedures and the average blank value was subtracted from the measured endotoxin value for each sample.

Dust samples were collected using a Breville Vac Master vacuum cleaner with a filter (Kimpech Science Wipers). Before sampling, both the inside and outside of the vacuum cleaner were disinfected with 70% alcohol. Dust was collected from a 1 m^2 area in each classroom for five minutes and in close proximity to children's desk/working area. Dust samples were collected between the school hours of 9 am and 3 pm. After sampling, cassettes and filters were put into a plastic bag and frozen (-20°C) until analyzed, together with the field blank filter. The choice of -20 °C was based on earlier experiences and published studies in relation to the impact of storage temperatures for

endotoxin measurements[34, 42-44]. The loads of endotoxin in floor dust were expressed as in EU/m^2. Endotoxins were extracted by vortexing (Maximum speed, Multi-Pulse Vortexer, Clas-Col, Terre haute, Ind) filters for one hour in 20mL (30mL for dust samples) of sterile pyrogen-free salt solution (0.9% NaCl) plus 0.025% Tween 20. Solutions were then centrifuged at 500 g for 5 minutes and supernatants were used for endotoxin quantification. Endotoxin measurements for both indoor air and dust samples were performed in duplicate using the LAL assay (Associates of Cape Cod, Woods Hole, MA) as previously described[42]. Briefly, filter extraction solutions were diluted and an inhibition/enhancement test was performed prior to measurement. Blank filters were extracted for filter controls. Control values were subtracted from the sample values. The detection limit of the method was 0.33 EU/m^3 for air samples and 0.47 EU/m^2 for dust samples. Samples with concentrations below the detection limit were assigned a value of half of the detection limit[21]. The efficiency, reproducibility and sensitivity of this method for airborne endotoxin quantification have been evaluated and proven by previously reported studies[34, 42-44].

Outdoor and indoor temperatures were measured at three sampling locations at each school. Measurements were conducted 24/7 using a pSENSE portable CO_2 Metre and a TSI IAQ Monitor (Model 8551) in the indoor locations (classrooms). Outdoor relative humidity and temperature were also measured concurrently for 24/7 using a Monitor Sensors µSmart Series weather station.

2.3. Statistical analysis

Endotoxin load (EU/m^2) in floor dust was modeled as count data with a Poisson likelihood. The mean load, *y*, within each classroom, *j*, at each school, *i*, was modeled with a hierarchical linear model[45]:

$$\begin{aligned} y_{ij} &\sim \text{Poisson}(\lambda_{ij}) \\ \log(\lambda_{ij}) &= \alpha_i \\ \alpha_i &\sim \mathcal{N}(\alpha_0, \tau_0) \\ \alpha_0 &\sim \mathcal{N}(0, 10^{-6}) \\ \tau_0 &\sim \Gamma(0.001, 0.001) \end{aligned} \quad (1)$$

The hierarchical model assumed a mean school level, with the separate classrooms treated as replicates (as there was only one measurement per classroom). The school level means were drawn from a distribution of school means whose mean had a weakly informative prior. All variance parameters were given weakly informative Gamma priors. Prediction at an unobserved school, labeled "S26", could be achieved by sampling α_{26} from the hierarchical prior.

Airborne endotoxin concentrations (EU/m^3) were also modeled as count data, but were non-negative, non-integer values close to zero. As such, the Poisson was an inappropriate distribution. The log-Normal distribution did not permit values of zero and the Normal approximation to the Poisson was a poor choice because the counts were close to zero. The "ones trick"' in JAGS (Just Another

183 Gibbs Sampler, Plummer (2003)) was used to specify a custom distribution which was a continuous
184 analogue of the discrete Poisson distribution. The likelihood of this pseudo-Poisson random variable
185 was:

186 $$f(y,\lambda) = \frac{\lambda^y e^{-\lambda}}{\Gamma(y+1)} \quad (2)$$

187 where the gamma function, Γ, generalizes the factorial function ($x!$) to real numbers (excepting
188 negative integers). Because there were two replicates in each classroom, an additional hierarchy level
189 was added to center the classroom level mean around a school level mean. The Bayesian hierarchical
190 model was then given as:

191 $$\begin{aligned} y_{ijk} &\sim f(y_{ijk}, \lambda_{ijk}) \\ \log(\lambda_{ij}) &\sim \mathcal{N}(\alpha_i, \tau_\lambda) \\ \alpha_i &\sim \mathcal{N}(\alpha_0, \tau_0) \\ \alpha_0 &\sim \mathcal{N}(0, 10^{-6}) \\ \tau_0, \tau_\lambda &\sim \Gamma(0.001, 0.001) \end{aligned} \quad (3)$$

192 where k represents the two replicates in each classroom. The parameters, λ_{ij}, were Poisson rate
193 parameters, so the predicted values of airborne endotoxin concentration were drawn from a Poisson
194 distribution.

195 To characterize the relationship between endotoxin indoor air concentration and floor dust
196 load, a meta-regression was performed on the estimates of $\log \lambda_{ij}$ from both models. For the dust
197 measurements, d_{ij} represented the mean and $\tau_{d_{ij}}$ represented the precision of the estimates of λ_{ij}.
198 Similarly, a_{ij} and $\tau_{a_{ij}}$ were defined for the airborne measurements. The meta-regression model was
199 then given as:

200 $$\begin{aligned} d_{ij} &\sim \mathcal{N}\left(\theta_{ij}, \tau_{d_{ij}}\right) \\ \theta_{ij} &\sim \mathcal{N}(\beta_0 + \beta_1 \alpha_{ij}, \tau_\theta) \\ \alpha_{ij} &\sim \mathcal{N}\left(a_{ij}, \tau_{\alpha_{ij}}\right) \\ \beta_0, \beta_1 &\sim \mathcal{N}(0, 10^{-6}) \\ \tau_\theta &\sim \Gamma(0.001, 0.001) \end{aligned} \quad (4)$$

201 where the meta-analysis estimate, θ_{ij}, contained an estimate of the background level of floor dust
202 endotoxin, β_0, and the rate at which dust endotoxin occurred with respect to airborne endotoxin, β_1. A
203 95% credible interval for β_1, which contained zero, indicated that, at a level of 95%, there was no
204 relationship between the indoor air concentration and floor dust load.

205 To investigate the effect of seasonality in endotoxin floor dust load and airborne
206 concentration, the Poisson mean was modeled for both the Poisson and pseudo-Poisson models in (1)
207 and (3), as follows:

$$\begin{aligned} \log(\lambda_i) &= \gamma_{s_i} \\ \gamma_s &\sim \mathcal{N}(\theta_s, \tau_g) \\ \theta_s &\sim \mathcal{N}(\alpha_0, \tau_\theta) \\ \alpha_0 &\sim \mathcal{N}(0, 10^{-6}) \\ \tau_\theta, \tau_g &\sim \Gamma(0.001, 0.001) \end{aligned} \quad (5)$$

where s_i is the season of observation (summer, autumn, winter, spring) for school *i*. These hierarchical priors model the school-level mean as being centered on the season-level mean θ_s. The schools were measured during the following seasons: autumn S04-07, S18-21; winter S08-12, S22-25; spring S01-03, S13-17. The quantity $\delta_s = \theta_s - \alpha_0$ can be calculated to determine the difference between the season mean and the overall mean, indicating the effect of the season. If the 95% credible interval for a δ_s contains zero then there can be said to be no difference between the season and the overall mean at a 5% level. Even though no measurements were taken during summer, the hierarchical prior provides an estimate of the season level effect for summer.

The regression models for the effect of humidity and temperature on endotoxin concentration and load were linear Poisson (or pseudo-Poisson) regression models with an interaction for humidity and temperature, as follows:

$$\begin{aligned} y_i &\sim f(y_i, \lambda_i) \\ \log(\lambda_i) &= \beta_0 + \beta_T T_i + \beta_H H_i + \beta_{TH} T_i H_i \\ \beta_0, \beta_T, \beta_H, \beta_{TH} &\sim \mathcal{N}(0, 10^{-6}) \end{aligned} \quad (6)$$

with weakly informative Normal priors on all fixed effects coefficients. Here the index, *I*, refers to the observations, as there were no school or classroom level effects to be considered. The Poisson model above was used for the floor dust measurements and the model for airborne measurements was of the same form, but replaced the Poisson likelihood with the pseudo-Poisson likelihood. Regression was performed with standardized covariates (subtracting the mean, dividing by the standard deviation). This centered the covariates around zero and eliminated any issues of covariate scale to do with the interaction term and the priors of the regression coefficients. The means and standard deviations of temperature and humidity were 18 and 4.7 ° C, and 67.1% and 11.6%, respectively.

3. RESULTS AND DISCUSSION

Due to the small number of samples taken at each school, endotoxin concentration and load within each classroom were characterized by predictive modeling from the hierarchical models. This modeling provided a distribution for likely concentrations and loads based on what is known about all of the measurements together, as well as any school level effects that were observed. In addition to modeling the concentrations and loads at S1-25, predictions were made at an unobserved school, S26, based purely on the hierarchical prior, as no data was collected there. The cumulative density function of the predicted values at S26 was similar to the empirical cumulative density function of the pooled data from all classrooms, but was less sensitive to variation within the data, particularly at the upper

quantiles. The 95% credible intervals of the posterior predictive concentration distributions for each classroom cover the data for most schools (Figure 1). For the endotoxin load in floor dust, only one observation was made in each classroom, the classroom level effect was ignored and the posterior mean of the predictive load distribution was between the two observations at that school. The posterior predictive load distribution at S01-03 and S26 was informed solely by the prior and covered the observed data.

3.1. Predictive modeling

The modeled indoor air concentrations of endotoxins in the 25 schools and the endotoxin load in floor dust for 22 schools are presented in Table 1, summarized as quantiles of the predictive distribution for S26. Results showed that 30% of the modeled endotoxin concentrations in indoor air were under the detection limit. The median (P_{50}) concentration was approximately 1 EU/m^3 and P_{70} and P_{95} of the endotoxin concentrations in indoor air were ≤ 3, and ≤ 13 EU/m^3, respectively. Figure 1 presents the predicted concentrations of endotoxins in the schools. Since no dust samples were collected at S1-3, the predictions for endotoxin load in the dust for S01-03 were the same as those at S26, which were based on samples from the hierarchical prior only. These are important findings, as predictors of endotoxin levels from vacuumed house dust have been well described in several indoor studies[30, 31, 46, 47], however the airborne sampling of indoor endotoxin levels is less common.

3.2. The concentration of endotoxins in indoor air

The results showed that endotoxin concentrations varied widely between schools and even within one school building. Concentrations of airborne endotoxins over 1.0 EU/m^3 and over 2.0 EU/m^3 were detected in 17 schools (in 77% of the studied schools) and 12 schools (in 48% of the studied schools), respectively. The average measured concentration of airborne endotoxin was below detection limit for S03, S17 and S22, and the highest value (19.88 EU/m^3) was found at S12. To the best of our knowledge, there are only two studies[48, 49] reporting endotoxin concentrations in school settings, and no studies have previously measured endotoxin concentrations in naturally ventilated subtropical school settings. In those previous studies, concentrations of endotoxins were expressed as $PM_{2.5}$ (Menetrez et al.[48]: AM 9.2 EU/m^3; Rabinovitch et al.[49]: 0.07 EU/m^3) and thus, were not directly comparable with our results (dust sampled with 37 mm closed face cassettes). Nor were our results were directly comparable with previous PM_{10} studies from other indoor settings. In comparison with other indoor studies, where dust sampled with 37 mm closed face cassettes, the predictive mean concentration of endotoxins in the present study, at S26 (3.7 EU/m^3) for example, was over 30 folder higher than the mean endotoxin concentrations reported by Myatt et al.[50] in their office building study in Boston area, and 8.5 times lower than that reported by Rao et al.[51] in moderately/heavily water-damaged houses.

The estimates of the effect of seasonality for each of the four seasons had 95% credible intervals which contained zero: summer -0.104 (95% CI: -0.805, 0.523); autumn -0.042 (-0.599, 0.534); winter 0.404 (-0.119, 1.006); and spring -0.259 (-0.845, 0.302). As such, there was no appreciable difference across the seasons.

3.3. Endotoxin loads in floor dust

Endotoxin loads in floor dust varied widely (Table 1), with the highest and lowest mean endotoxin loads detected in S07 (24095 EU/m^2) and S09 (1533 EU/m^2), respectively. The predicted median load of endotoxins in carpeted floor dust for S26 (7786 EU/m^2) was higher than that reported in a recent study of endotoxin loads in floor dust in the Netherlands (GM: 2178-6914)[17] and in schools with tiled floors in Northern Carolina (GM: 2200)[52]. The same North Carolinian study reported carpeted floor dust endotoxin loads six times higher (GM: 48 000) than the load reported in this study[52].

Although higher loads of endotoxins were found in schools (GM: 2178–6914 EU/m^2) than residential environment (GM: 462–1285 EU/m^2) in an urban area in the Netherlands[17], the modeled endotoxin loads in this study were over 4 times lower than in farming households in Germany, Austria and Switzerland[14] and in the Netherlands[16]. In an urban environment, schools - and not homes - may represent the most significant location for endotoxin exposure, due to the higher number of children present in a classroom, compared to a home environment[53]. It has been reported that places of education for the youngest children, such as daycare centers and pre-elementary schools, had endotoxin levels three times higher than those found in elementary schools for older children[54]. Morcos et al.[55] compared endotoxin loads in rural and urban schools, and found that rural schools had higher loads due to the close proximity of farm animals.

It should be noted that due differences in sampling methodologies, such as location, time and size of the sampled surface, together with the use of different measurement units, the comparison between endotoxin studies in floor dust is difficult and sometimes inconclusive[24]. In addition, most studies focused on investigating the health effects of endotoxin exposure, and detailed information about the measurement of endotoxins was often not available in the published paper.

The 95% credible intervals of the season effect contained zero for all seasons: summer -0.062 (95% CI: -0.726, 0.354); autumn 0.034 (-0.340, 0.497); winter -0.056 (-0.521, 0.307); and spring 0.088 (-0.246, 0.663). This indicates that there was no seasonal effect. The 95% CI for summer was the widest, as there were no schools measured in summer, due to schools being closed from mid-December to late January for the summer holidays.

3.4. Correlation between the concentration of endotoxins in indoor air and endotoxin loads in floor dust

Bayesian metaregression indicated that there was no relationship between the measured endotoxin levels in floor dust and air within the classrooms in this study. The mean and 95% credible interval for β_1 (Equation 4) was -0.004 (-0.190, 0.181) and similar findings were reported in several earlier studies[8, 15, 21, 56]. This suggests that it may be necessary to measure both airborne and settled dust endotoxin levels, in order to accurately estimate indoor endotoxin exposure in school buildings.

This study also provided information for future indoor sampling strategies, highlighting the need for standardized sampling methodologies. The harmonization of these methods calls for additional research data based on the different methods, together with the systematic comparison of different methods. In addition, the effect of storage and transport of endotoxin samples should be studied, because these conditions can affect endotoxin concentrations, and these issues have often not been addressed[1, 57, 58].

3.5. Temperature and humidity

In the studied school settings, the 24-hour average temperature ranged from 8°C to 27°C and the 24-hour averaged relative humidity (RH) ranged from 42% to 91%. In the present study an interaction between temperature and relative humidity was included in the model, as relative humidity is dependent on temperature. Regression analysis (Equation 6) showed that floor dust endotoxin loads were fairly insensitive to the average relative humidity during the last 24 hours (Figure 2). For both floor dust and airborne endotoxins, increased RH led to lower concentrations, but only weakly in the case of floor dust. An increase in temperature led to a decrease in airborne endotoxin concentrations, but an increase in the endotoxin load in floor dust. The 95% credible interval for the airborne interaction term contained zero, indicating that most of the variation could be accounted for by temperature and RH separately (see Table S1 in Supporting Information). For floor dust endotoxins, the 95% credible interval for the interaction term was strictly positive and greater in magnitude than the humidity alone, indicating that the effect of RH was only particularly important in modifying the effect of temperature. Statistical results indicated that at a higher RH, the effect of temperature was more pronounced than at lower temperatures.

Although some studies reported that season or seasonality has effect on the indoor concentration[18, 59], or loads of endotoxin[60] there are also studies in which the association was not found[15, 30, 61]. As well as the effect of seasonality on indoor endotoxin levels, the effect of individual meteorology factors – temperature and humidity – on endotoxin levels in both indoor air and dust were contradictory in earlier studies. For example, in the USA, Mazique et al.[21] found that, although residential indoor airborne endotoxin concentrations tended to be higher during the fall and spring seasons (spring: 0.19 EU/m^3; fall: 0.15 EU/m^3; summer: 0.04 EU/m^3; winter: 0.07 EU/m^3), home temperature and humidity were not significantly associated with airborne endotoxin concentrations. In Europe, Bischof et al. [30] and Douwes et al. [20] reported that there was no effect of temperature and relative humidity on endotoxin levels in floor dust. In several other studies, floor dust endotoxin

measurements showed little variation over time, with no significant differences during different seasons[61] or over a six-month period[19]. However, the effect of temperature, as well as humidity, on indoor endotoxin levels (both concentrations and loads) have also been reported worldwide[18, 27, 46, 62, 63]. In addition, it has been suggested that relative humidity may be an important factor controlling endotoxin exposure indoors[18]. However, there is still no clear explanation for the existing associations. Despite this, it is commonly assumed that more humid climates will be associated with higher endotoxin levels (because endotoxins arise from bacteria and bacteria thrive in water), while elevated humidity in the absence of wet surfaces or stagnant water will not achieve water activity levels to support the growth of bacteria (bacteria require water activities of ≥ 0.9731).

A limitation of this study was that each school was only measured during one season. Although we found that temperature and relative humidity were significantly associated with airborne endotoxins and loads of endotoxin in settled dust, the concentrations were not fully explained by the ambient temperature and relative humidity. School buildings were ventilated via opened windows and doors (absence of a known continuous air exchange rate), and disturbance of the incoming air (e.g. air drafts), for example, may have influenced endotoxin concentrations. When comparing seasonality effects between studies, one should also take the geographical locations into account. For example, in Brisbane, the average annual temperature and humidity are higher than in colder areas[64, 65], and the temperature difference between seasons in Brisbane (mean 9 am temperature: summer 26.4 °C, autumn 22.0 °C, winter 15.9 °C and spring 22.7 °C)[65], as well as other subtropical areas, is very small compared to many other parts of the word.

In addition, simultaneous effects of different local factors may affect the findings and more studies during different seasons and geographical locations are needed to identify these effects. Based on floor dust "stability" findings[19, 61], the long term (over 6 months) average temperature and humidity may be much more significant than the short term average, when studying their effects on floor dust endotoxin levels in different climatic regions.

3.6. Benchmarked baseline concentrations of endotoxins in indoor air and endotoxin loads in floor dust

Based on statistical analysis, the indoor air concentration of endotoxins and endotoxin loads in settled floor dust were generally < 13 EU/m^3 and < 24 570 EU/m^2 (95th percentile), respectively. Although these levels (<95th percentile) of endotoxins are based on a limited number of studied schools, they can guide the future determination of criteria for assessing endotoxins in naturally ventilated, school settings with carpeted classrooms. These levels can be applied for urban school buildings in regions with similar climates, and also when sampling and analysis are carried out using the same method as that used in the present study. Elevated levels may indicate abnormal sources of endotoxins in the school environment, together with the need for further environmental investigations and the implementation of corrective measures, if required. In the future, international harmonization

of the methods for endotoxin exposure assessment, as well as further studies on the simultaneous effect of different local factors, such as cleaning frequency and floor surface material, on endotoxin levels in different climatic regions are needed.

4. ACKNOWLEDGEMENTS

This work was supported by the Australian Research Council (ARC), QLD Department of Transport and Main Roads (DTMR) and QLD Department of Education, Training and Employment (DETE) through Linkage Grant LP0990134. Our particular thanks go to R. Fletcher (DTMR) and B. Robertson (DETE) for their vision regarding the importance of this work. We would also like to thank Prof G. Marks, Dr P. Robinson, Prof K. Mengersen, Prof Z. Ristovski, Prof G. Ayoko, Dr C. He, Dr G. Johnson, Dr R. Jayaratne, Dr S. Low Choy, Prof G. Williams, W. Ezz, F. Salimi, L. Crilley, M. Mokhtar, N. Mishra, R. Laiman, L. Guo, Dr X. Ling, Dr J. Davies, Dr L. Leontjew Toms, F. Fuoco, Dr A. Cortes, Dr B. Toelle, A. Quinones, P. Kidd and E. Belousova, Dr M. Falk, Dr F. Fatokun, Dr J. Mejia, Dr D. Keogh, Prof T. Salthammer, R. Appleby and C. Labbe for their contribution to the UPTECH project. We also thank Dr Salonen's post-doctoral grant organizations involved: the Finnish Work Environmental Fund, the Yrjö Jahnsson Foundation, the Finnish Society for the Promotion of Occupational Health, the Finnish Occupational Hygiene Society, and the Finnish Union of Environmental Professionals. Prof Duchaine was a visiting fellow at ILAQH when participating to this project and is aFRQ-S Senior scholar.

Supporting information available. Posterior means and 95% credible intervals for effects of standardized temperature and relative humidity and their interaction (Table S1) are available free of charge via the Internet at http://pubs.acs.org/.

5. REFERENCES

1. Duchaine, C.; Thorne, P. S.; Mériaux, A.; Grimard, Y.; Whitten, P.; Cormier, Y., Comparison of endotoxin exposure assessment by bioaerosol impinger and filter-sampling methods. *Appl. Environ. Microbiol.* **2001,** *67*, (6), 2775–2780.
2. Thorne, P. S., Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation. *Toxicology.* **2000,** *152*, (1-3), 13–23.
3. Rennie, D. C.; Lawson, J. A.; Senthilselvan, A.; Willson, P. J.; Dosman, J. A., Domestic endotoxin exposure and asthma in children: epidemiological studies. *Frontiers in bioscience (Elite edition).* **2012,** *1*, (4), 56-73.
4. Chun, D. T.; Chew, V.; Bartlett, K.; Gordon, T.; Jacobs, R. R.; Larsson, B. M.; Larsson, L.; Lewis, D. M.; Liesivuori, J.; Michel, O.; Milton, D. K.; Rylander, R.; Thorne, P. S.; White, E. M.; Brown, M. E., Preliminary report on the results of the second phase of a round-robin endotoxin assay study using cotton dust. *Appl. Occup. Environ Hyg.* **2000,** *15*, (1), 152–157.
5. Instanes, C.; Hetland, G.; Berntsen, S.; Løvik, M.; Nafstad, P., Allergens and endotoxin in settled dust from day-care centers and schools in Oslo, Norway. *Indoor Air.* **2005,** *15*, (5), 356–362.
6. Moniruzzaman, S.; Engmanb, L. H.; James, B.; Sigsgaard, T.; Thornee, P. S.; Sundell, J.; Bornehaga, C.-G., Levels of endotoxin in 390 Swedish homes: determinants and the risk for respiratory symptoms in children. *Int. J. Environ. Health Res.* **2012,** *22*, (1), 22-36.

7. Delfino, R. J.; Staimer, N.; Tjoa, T., Personal endotoxin exposure in a panel study of school children with asthma. *Environ. Health.* . **2011,** *10*, (69), 1-14.
8. Horick, N.; Weller, E.; Milton, D. K.; Gold, D. R.; Li, R.; Spiegelman, D., Home endotoxin exposure and wheeze in infants: correction for bias due to exposure measurement error. *Environ. Health Perspect.* **2006,** *114*, (1), 135-140.
9. Liu, A. H., Endotoxin exposure in allergy and asthma: Reconciling a paradox. *J. Allergy Clin. Immunol.* **2002,** *109*, (3), 379–392.
10. Rylander, R., Review: Endotoxin in the environment–exposure and effects. *J. Endotoxin Res.* **2002,** *8*, (4), 241-252.
11. George, C. L. S.; Jin, H.; Wohlford-Lenane, C. L.; O'Neill, M. E.; Phipps, J. C.; O'Shaughnessy, P. T.; Kline, J. N.; Thorne, P. S.; Schward, D. A., Endotoxin responsiveness and subchronic grain dust-induced airway disease. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2001,** *280*, (2), 203-213.
12. Park, J. H.; Gold, D. R.; Spiegelman, D. L.; Burge, H. A.; Milton, D. K., House dust endotoxin and wheeze in the first year of life. *A. J. Respir. Crit. Care Med.* **2001,** *163*, (2), 322–328.
13. von Mutius, E.; Braun-Fahrlander, C.; Schierl, R.; Riedler, J.; Ehlermann, S.; Maisch, S.; Waser, M.; Nowak, D., Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin. Exp. Allergy.* **2000,** *30*, (9), 1230–1234.
14. Braun-Fahrländer, C.; Riedler, J.; Herz, U.; Eder, W.; Waser, M.; Grize, L.; Maisch, S.; Carr, D.; Gerlach, F.; Bufe, A.; Lauener, R. P.; Schierl, R.; Renz, H.; Nowak, D.; von Mutius, E., Environmental exposure to endotoxin and its relation to asthma in school-age children. *N. Engl. J. Med.* **2002,** *347*, (12), 869-877.
15. Park, J. H.; Spiegelman, D. L.; Gold, D. R.; Burge, H. A.; Milton, D. K., Predictors of airborne endotoxin in the home. *Environ. Health Perspect.* **2001,** *109*, (8), 859–864.
16. Noss, I.; Wouters, I. M.; Visser, M.; Heederik, D. J.; Thorne, P. S.; Brunekreef, B.; Doekes, G., Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Appl. Environ. Microbiol.* **2008,** *74*, (18), 5621-5627.
17. Jacobs, J. H.; Krop, E. J.; Wind, S. D.; Spithoven, J.; Heederik, D. J., Endotoxin levels in homes and classrooms of Dutch school children and respiratory health. Published on October 25, 2012 as doi: 10.1183/09031936.00084612. *Eur. Respir. J.* **2012**.
18. Park, J. H.; Spiegelman, D. L.; Burge, H. A.; Gold, D. R.; Chew, G. L.; Milton, D. K., Longitudinal study of dust and airborne endotoxin in the home. *Environ. Health Perspect.* **2000,** *108*, (11), 1023-1028
19. Gereda, J. E.; Leung, D. Y.; Thatayatikom, A.; Streib, J. E.; Price, M. R.; Klinnert, M. D.; Liu, A. H., Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* **2000,** *355*, (9216), 1680–3.
20. Douwes, J.; Doekes, G.; Heinrich, J.; Koch, A.; Bischof, W.; Brunekreef, B., Endotoxin and B(1->3)-glucan in house dust and the relation with home characteristics: a pilot study in 25 German houses. *Indoor Air.* **1998,** *8*, (4), 255-263.
21. Mazique, D.; Diette, G. B.; Breysse, P. N.; Matsui, E. C.; McCormack, M. C.; Curtin-Brosnan, J.; Williams, D.; Peng, R. D.; Hansel, N. N., Predictors of airborne endotoxin concentrations. *Environ. Res.* **2011,** *111*, (4), 614-617.
22. Donham, K.; Haglind, P.; Peterson, Y.; Rylander, R.; Belin, L., Environmental and health studies of farm workers in Swedish swine confinement buildings. *Br. J. Ind. Med.* **1989,** *46*, (1), 31-37.
23. Liebers, V.; Bruning, T.; Raulf-Heimsoth, M., Occupational endotoxin exposure and possible health effects on humans. *Am. J. Ind. Med.* **2006,** *49*, (6), 474-491.
24. Chen, C.-M.; Thiering, E.; Doekes, G.; Zock, J.-P.; Bakolis, I.; Norbck, D.; Sunyer, J.; Villani, S.; Verlato, G.; Tubel, M.; Jarvis, D.; Heinrich, J., Geographical variation and the determinants of domestic endotoxin levels in mattress dust in Europe. *Indoor Air* **2012,** *22*, (1), 24-32.
25. Gehring, U.; Strikwold, M.; Schram-Bijkerk, D.; Weinmayr, G.; Genuneit, J.; Nagel, G.; Wickens, K.; Siebers, R.; Crane, J.; Doekes, G.; Di Domenicantonio, R.; Nilsson, L.; Priftanji, A.; Sandin, A.; El-Sharif, N.; Strachan, D.; van Hage, M.; von Mutius, E.; Brunekreef, B., Asthma and allergic symptoms in relation to house dust endotoxin: Phase Two of the International Study on Asthma and Allergies in Childhood (ISAAC II). *Clin. Exp. Allergy.* **2008,** *38*, (12), 1911-1920.

26. Tager, I. B.; Lurmann, F. W.; Haight, T.; Alcorn, S.; Penfold, B.; Hammond, K., Temporal and spatial patterns of ambient endotoxin concentrations in Fresno, California. *Environ. Health Perspect.* **2010,** *118*, (10), 1490–1496.
27. Wickens, K.; Douwes, J.; Siebers, R.; Fitzharris, P.; Wouters, I.; Doekes, G.; Mason, K.; Hearfield, M.; Cunningham, M.; Crane, J., Determinants of endotoxin levels in carpets in New Zealand homes. *Indoor Air* **2003,** *13*, (2), 128–135.
28. Johansson, E.; Vesper, S.; Levin, L.; LeMasters, G.; Grinshpun, S.; Reponen, T., Streptomycetes in house dust: associations with housing characteristics and endotoxin. *Indoor Air* **2011,** *21*, (4), 300–310.
29. Ownby, D. R.; Peterson, E. L.; Wegienka, G.; Woodcroft, K. J.; Nicholas, C.; Zoratti, E.; Johnson, C. C., Are cats and dogs the major source of endotoxin in homes? *Indoor Air.* **2013,** *23*, (3), 219-226.
30. Bischof, W.; Koch, A.; Gehring, U.; Fahlbusch, B.; Wichmann, H. E.; Heinrich, J., Predictors of high endotoxin concentrations in the settled dust of German homes. *Indoor Air.* **2002,** *12*, (1), 2-9.
31. Thorne, P. S.; Cohn, R.; Mav, D.; Arbes, S. J.; Zeldin, D. C., Predictors of endotoxin levels in U.S. housing. *Environ. Health Perspect* **2009,** *117*, (5), 763–771.
32. Rennie, D. C.; Lawson, J. A.; Kirychuk, S. P.; Paterson, C.; Willson, P. J.; Senthilselvan, A., Assessment of endotoxin levels in the home and current asthma and wheeze in school-age children. *Indoor Air.* **2008,** *18*, (6), 447–453.
33. Tavernier, G.; Fletcher, G.; Gee, I.; Watson, A.; Blacklock, G.; Francis, H.; Fletcher, A.; Frank, T.; Frank, P.; Pickering, C. A., IPEADAM study: indoor endotoxin exposure, family status, and some housing characteristics in English children. *J. Allergy Clin. Immunol.* **2006,** *117*, (3), 656–662.
34. Duquenne, P.; Marchand, G.; Duchaine, C., Review. Measurement of endotoxins in bioaerosols at workplace: A critical review of literature and a standardization issue. *Ann. Occup Hyg.* **2012,** *57*, (2), 137-172.
35. Liddament, M. W. *The natural ventilation of UK school classrooms.The case for natural ventilation in schools and the origin of the standards* CIBSE School Design Group: Veetech Ltd., 2011.
36. Gilmore, F. *Health considerations when choosing school flooring*; Asthma Regional Council of New England: Dorchester, MA, 2005; p 10.
37. *WHO Publication/Guideline 2009. Natural ventilation for infection control in health-care settings; http://whqlibdoc.who.int/publications/2009/9789241547857_eng.pdf;* 9789241547857; World Health Organization: 2009.
38. Kleiven, T. Natural ventilation in buildings. Architectural concepts, consequences and possibilities. Dissertation. Norwegian University of Science and Technology, Trondheim, 2003.
39. Lehtonen, M.; Reponen, T.; Nevalainen, A., Everyday activities and variation of fungal spore concentrations in indoor air. *Int. Biodegradation.* **1993,** *31*, (1), 25-39.
40. WHO *WHO guidelines for indoor air quality: dampness and mould*; World Health Organization: Copenhagen, 2009; p 248.
41. Australian Government *Australian weather and the seasons. Available on line at: http://australia.gov.au/about-australia/australian-story/austn-weather-and-the-seasons;* 2012.
42. Létourneau, V.; Nehmé, B.; Mériaux, A.; Massé, D.; Duchaine, C., Impact of production systems on swine confinement buildings bioaerosols. *J. Occup. Environ Hyg.* **2010,** *7*, (2), 94-102.
43. Spaan, S.; Doekes, G.; Heederik, D.; Thorne, P. S.; Wouters, I. M., Effect of extraction and assay media on analysis of airborne endotoxin. *Applied and Environmental Microbiology (AEM).* **2008,** *74*, (12), 3804-3811.
44. Spaan, S.; Heederik, D. J.; Thorne, P. S.; Wouters, I. M., Optimization of airborne endotoxin exposure assessment: effects of filter type, transport conditions, extract solutions, and storage of samples and extracts. *Appl Environ Microbiol.* **2007,** *73*, 6134-43.
45. Gelman, A.; Hill, J. *Data Analysis Using Regression and Multilevel/Hierarchical Models. Available online at: http://www.langtoninfo.com/web_content/9780521867061_frontmatter.pdf;* 9780521867061; Cambridge, 2007; p 19.
46. Dales, R.; Miller, D.; Ruest, K.; Guay, M.; Judek, S., Airborne endotoxin is associated with respiratory illness in the first 2 years of life. *Environ. Health Perspect.* **2006,** *114*, (4), 610–614.

538 47. Gereda, J. E.; Klinner, M. D.; Price, M. R.; Leung, D. Y.; Liu, A. H., Metropolitan home
539 living conditions associated with indoor endotoxin levels. *J. Allergy Clin. Immunol.* **2001,** *107*, (5),
540 790–796.
541 48. Menetrez, M. Y.; Foarde, K. K.; Esch, R. K.; Schwartz, T. D.; Dean, T. R.; Hays, M. D.; Cho,
542 S. H.; Betancourt, D. A.; Moore, S. A., An evaluation of indoor and outdoor biological particulate
543 matter. *Atmos. Environ.* **2009,** *43*, (34), 5476–5483.
544 49. Rabinovitch, N.; Liu, A. H.; Zhang, L.; Rodes, C. E.; Foarde, K.; Dutton, S. J.; Murphy, J. R.;
545 Gelfand, E. W., Importance of the personal endotoxin cloud in school-age children with asthma. *J.*
546 *Allergy Clin. Immunol.* **2005,** *116*, (5), 1053-1057.
547 50. Myatt, T. A.; Staudenmayer, J.; Adams, K.; Walters, M.; Rudnick, S. N.; Milton, D. K., A
548 study of indoor carbon dioxide levels and sick leave among office workers. *Environ. Health.* **2002,** *1*,
549 (1), 3; DOI 10.1186/1476-069X-1-3.
550 51. Rao, C. Y.; Riggs, M. A.; Chew, G. L.; Muilenberg, M. L.; Thorne, P. S.; Van Sickle, D.;
551 Dunn, K. H.; Brown, C., Characterization of Airborne Molds, Endotoxins, and Glucans in Homes in
552 New Orleans after Hurricanes Katrina and Rita. *Appl. Environ. Microbiol.* **2007,** *73*, (5), 1630-1634.
553 52. Foarde, K.; Berry, M., Comparison of biocontaminant levels associated with hard vs carpet
554 floors in non-problem schools: Results of a year long study. *J. Expo. Anal. Environ. Epidemiol.* **2004,**
555 *14*, (Suppl 1), 41-48.
556 53. Sheehan, W. J.; Hoffman, E. B.; Fu, C.; Baxi, S.; Bailey, A.; King, E.-M.; Chapman, M. D.;
557 Lane, J. P.; Gaffin, J. M.; Permaul, P.; Gold, D. R.; Phipatanakul, W., Endotoxin exposure in inner-
558 city schools and homes of children with asthma. *Ann. Allergy Asthma Immunol.* **2012,** *108*, (6), 418–
559 422.
560 54. Rullo, V. E. V.; Rizzo, M. C.; Arruda, L. K.; Solé, D.; Naspitz, C. K., Daycare centers and
561 schools as sources of exposure to mites, cockroach, and endotoxin in the city of São Paulo, Brazil. *J.*
562 *Allergy Clin. Immunol.* **2002,** *110*, (4), 582-588.
563 55. Morcos, M. M.; Morcos, W. M.; Ibrahim, M. A.; Shaheen, M. A., Environmental exposure to
564 endotoxin in rural and urban Egyptian school children and its relation to asthma and atopy. *Minerva.*
565 *Pediatr.* **2011,** *63*, (1), 19-26.
566 56. Sohy, C.; Lieutier-Colas, F.; Cassett, A.; Meyer, P.; Pauli, G.; Pons, F.; de Blay, F., Dust and
567 airborne endotoxin exposure in dwellings in the Strasbourg metropolitan area (France). *Allergy.* **2005,**
568 *60*, (4), 541-542.
569 57. Douwes, J.; Versloot, P.; Hollander, A.; Heederik, D.; Doekes, G., Influence of various dust
570 sampling and extraction methods on the measure ment of airborne endotoxin. *Appl. Environ*
571 *Microbiol.* **1995,** *61*, (5), 1763–1769.
572 58. Thorne, P. S., Bioaerosol sampling in field studies: Can samples be express mailed? *Am. Ind.*
573 *Hyg. Assoc. J.* **1994,** *55*, (11), 1072–1079.
574 59. Pavilonis, B. T.; Anthony, T. R.; O'Shaughnessy, P. T.; Humann, M. J.; Merchant, J. A.;
575 Moore, G.; Thorne, P. S.; Weisel, C. P.; Sanderson, W. T., Indoor and outdoor particulate matter and
576 endotoxin concentrations in an intensely agricultural county. *J. Expo. Sci. Environ. Epidemiol.* **2013,**
577 *23*, (3), 299-305.
578 60. Su, H.-J.; Wu, P.-C.; Chen, H.-L.; Lee, F.-C.; Lin, L.-L., Exposure assessment of indoor
579 allergens, endotoxin, and airborne fungi for homes in Southern Taiwan. *Environ. Res.* **2001,** *85*, (2),
580 135-144.
581 61. Ownby, D. R.; Peterson, E. L.; Williams, L. K.; Zoratti, E., M.;; Wegienka, G. R.; Woodcroft,
582 K. J.; Joseph, C. L.; Johnson, C. C., Variation of dust endotoxin concentrations by location and time
583 within homes of young children. *Pediatr. Allergy Immunol.* **2010,** *21*, (3), 533-540.
584 62. Thorn, J.; Beijer, L.; Jonsson, T.; Rylander, R., Measurement strategies for the determination
585 of airborne bacterial endotoxin in sewage treatment plants. *Ann. Occup. Hyg.* **2002,** *46*, (6), 549-554.
586 63. Thorne, P. S.; Kulhánková, K.; Yin, M.; Cohn, R.; Arbes, S. J.; Zeldin, D. C., Endotoxin
587 exposure is a risk factor for asthma the national survey of endotoxin in united states housing. *Am. J.*
588 *Respir. Crit. Care Med.* **2005,** *172*, (11), 1371-7
589 64. McKnight, T.; Hess, D., *Climate Zones and Types. Physical Geography: A Landscape*
590 *Appreciation*. Prentice Hall: Upper Saddle River, NJ, 2000.
591 65. Australian Government Climate statistics for Australian locations. Bureau of Meterology
592 Website; http://www.bom.gov.au/climate/averages/tables/cw_040913.shtml (Accessed 1 May 2013).

6. TABLES

Table 1

Basic statistics and percentiles for the modeled endotoxin concentrations in indoor air (n = 74) and endotoxin loads in floor dust (n = 44) in 25 and 22 school environments, respectively.

	Concentration in indoor air [EU/m^3]	**Load in floor dust [EU/m^2]**
Poisson rate parameter, λ_0	1.48	7740
Data Geometric mean (GM)	1.227	7502
Data Arithmetic mean (AM)	2.725	9436
Data Standard deviation (STDEV)	3.215	6563
Percentiles		
P_5	< LOD	2460
P_{10}	< LOD	3190
P_{20}	<LOD	4420
P_{30}	<LOD	5430
P_{40}	1	6520
Median (P_{50})	1	7710
P_{60}	2	9180
P_{70}	3	10980
P_{80}	5	13620
P_{90}	8	18720
P_{95}	13	24570

LOD: Limit of detection

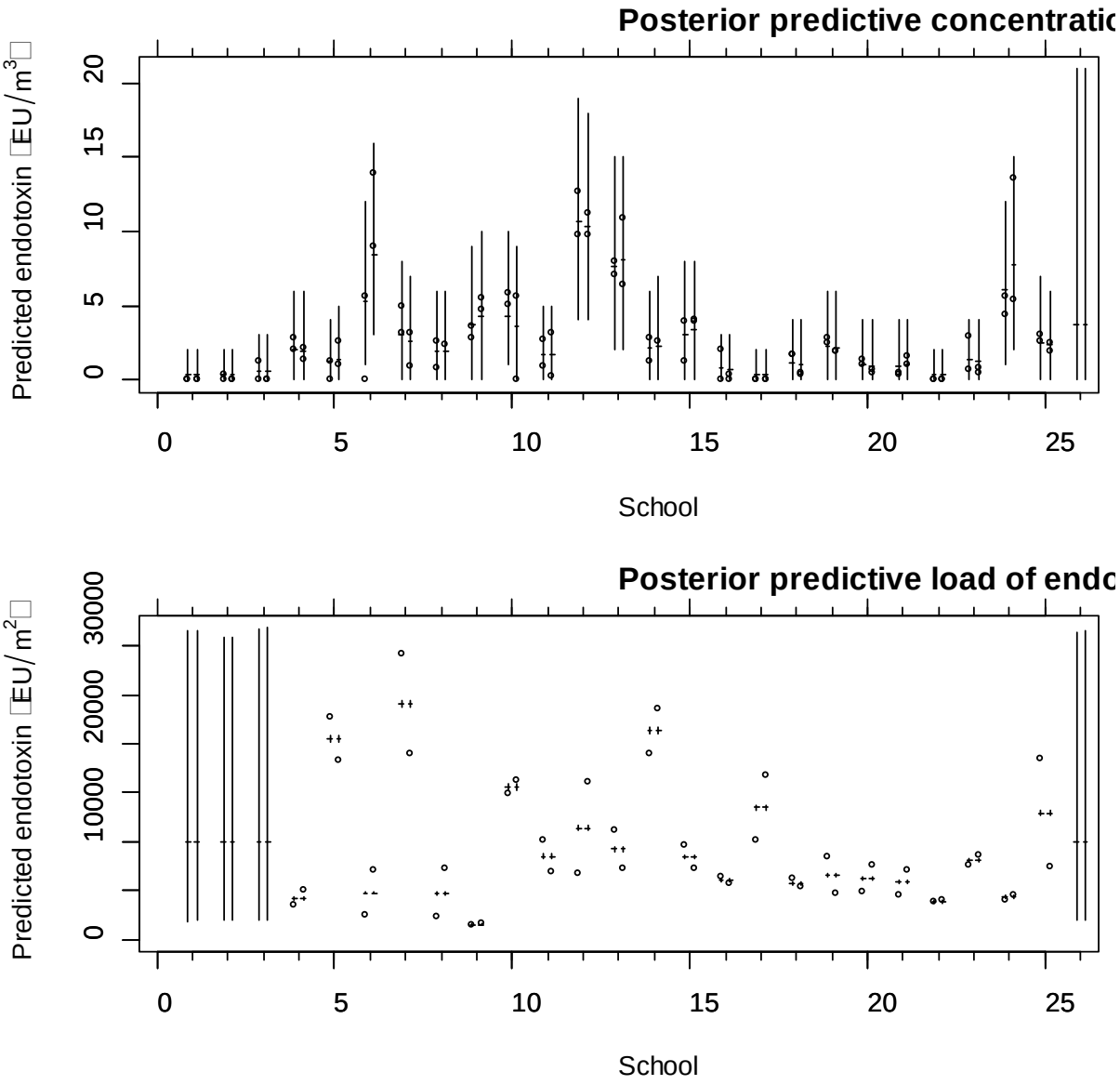
7. FIGURE CAPTIONS

Figure 1. Predictive distributions from the Poisson and pseudo-Poisson hierarchical linear models of concentration of endotoxins in indoor air and endotoxin load in floor dust. Observed concentrations and loads are shown as open circles; the mean predicted values are shown as horizontal lines and the 95% credible intervals are shown as vertical lines.

Figure 2. 24hr average relative humidity and temperature for each of the observations, grouped by season during which the measurement was taken.

612 **Figure 1**

613



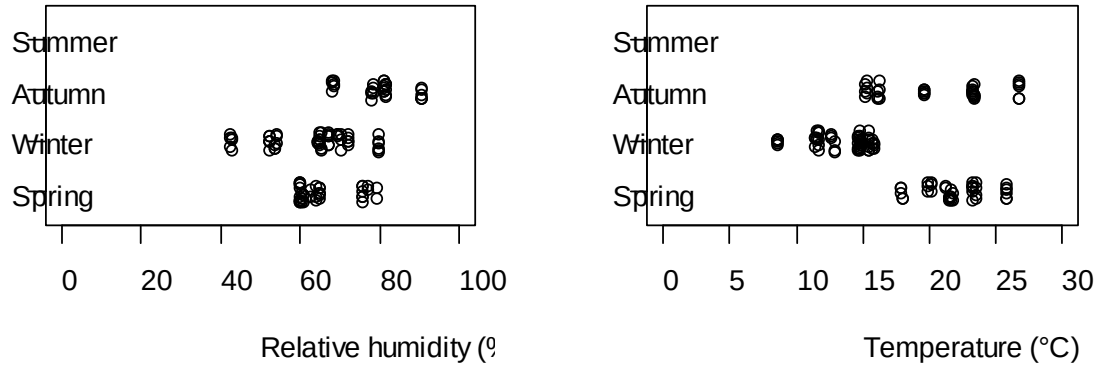
614

615

616

617

618 **Figure 2**



619

620

621

622

SUPPORTING INFORMATION

TABLE S1

AUTHORS:

Heidi Salonen, Caroline Duchaine, Valérie Létourneau, Mandana Mazaheri, Sam Clifford, Lidia Morawska

MANUSCRIPT TITLE:

Endotoxins in indoor air and settled dust in primary schools in a subtropical climate

NUMBER OF PAPERS:

Cover sheet (S1) + 1 page (S2)

Table S1. Posterior means and 95% credible intervals for effects of standardized temperature and relative humidity and their interaction. Italicized entries indicate a 95% CI which contains zero.

	Indoor Air			Floor Dust		
	2.5%	Mean	97.5%	2.5%	Mean	97.5%
β_0	0.632	0.797	0.961	9.189	9.192	9.197
β_T	-0.424	-0.272	-0.119	0.163	0.167	0.171
β_H	-0.570	-0.429	-0.289	-0.015	-0.012	-0.008
β_{TH}	*-0.166*	*0.045*	*0.243*	0.038	0.042	0.045

667