Indoor Air 2012; 22: 24–32
wileyonlinelibrary.com/journal/ina

INDOOR AIR
doi:10.1111/j.1600-0668.2011.00740.x

Geographical variation and the determinants of domestic endotoxin levels in mattress dust in Europe

Abstract Endotoxin exposures have manifold effects on human health. The geographical variation and determinants of domestic endotoxin levels in Europe have not yet been extensively described. To investigate the geographical variation and determinants of domestic endotoxin concentrations in mattress dust in Europe using data collected in the European Community Respiratory Health Survey follow-up (ECRHS II). Endotoxin levels were measured in mattress dust from 974 ECRHS II participants from 22 study centers using an immunoassay. Information on demographic, lifestyle, and housing characteristics of the participants was obtained in face-to-face interviews. The median endotoxin concentration in mattress dust ranged from 772 endotoxin units per gram (EU/g) dust in Reykjavik, Iceland, to 4806 EU/g in Turin, Italy. High average outdoor summer temperature of study center, cat or dog keeping, a high household crowding index, and visible damp patches in the bedroom were significantly associated with a higher endotoxin concentrations in mattress dust. There is a large variability in domestic endotoxin levels across Europe. Average outdoor summer temperature of study center, which explains only 10% of the variation in domestic endotoxin level by center, is the strongest meteorological determinant. The observed variation needs to be taken into account when evaluating the health effects of endotoxin exposures in international contexts.

C.-M. Chen[1,2], E. Thiering[1], G. Doekes[3], J.-P. Zock[4,5,6], I. Bakolis[7], D. Norbäck[8], J. Sunyer[4], S. Villani[9], G. Verlato[10], M. Täubel[11], D. Jarvis[7,12], J. Heinrich[1]

[1]Helmholtz Zentrum München, German Research Centre for Environmental Health, Institute of Epidemiology, Neuherberg, Germany, [2]Department for Pediatric Pneumology Allergy and Neonatology, Hannover Medical School, Hannover, Germany, [3]Institute for Risk Assessment Sciences, Division Environmental Epidemiology, Utrecht University, Utrecht, The Netherlands, [4]Centre for Research In Environmental Epidemiology (CREAL), Barcelona, Spain, [5]Municipal Institute of Medical Research (IMIM-Hospital del Mar), Barcelona, Spain, [6]CIBER Epidemiología y Salud Pública, Barcelona, Spain, [7]Respiratory Epidemiology and Public Health Group, Imperial College London, UK, [8]The Dept of Medical Science, Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden, [9]Section of Medical Statistics and Epidemiology, Department of Health Sciences, University of Pavia, Pavia, Italy, [10]Epidemiology and Medical Statistics, University of Verona, Verona, Italy, [11]Department Environmental Health, National Institute for Health and Welfare, Kuopio, Finland, [12]MRC-HPA Centre for Environment and Health, King's College London, London, UK

Key words: Endotoxin; House dust; Geographical variation; ECRHS; Summer temperature; Home characteristics.

C.-M. Chen
Department of Pediatric Pneumology, Allergy and Neonatology
Hannover Medical School
Carl-Neuberg-Str. 1
D- 30625 Hannover
Germany
Tel.: +49 511 532 9294
Fax: +49 511 532 8185
e-mail: Chen.Chih-Mei@mh-hannover.de

Received for review 5 April 2011. Accepted for publication 19 August 2011.

Practical Implications

The incoherent observations of the health effects of endotoxin may be partly owing to the geographical heterogeneity of endotoxin exposure. Therefore, the observed variation should be considered in further studies. Measurements of indoor endotoxin are recommended as an indicator for the level of exposures of individual domestic environments.

Introduction

The term endotoxin refers to bacterial lipopolysaccharide (LPS), which is a toxic glycolipid that can be found in the outer membrane of mainly Gram-negative bacteria. It also represents one of the microbial molecular signals responsible for the activation of immune response. In case of limited infections, individuals' innate immune response to LPS helps to clear invaded microbes. However, in sepsis, a systemic hyperinflammatory response owing to LPS may subsequently lead to multiple organ failure and death (Ianaro et al., 2009). Lipopolysaccharide is ubiquitous, being a major constituent of organic dust, and high exposure occurs in rural areas as well as occupational environments (for example in biofuel plants and cotton textile manufacturing) (Madsen, 2006; Simpson et al., 1999). Exposure to endotoxin at the work place may cause acute or chronic health effects including inflammatory reactions, systemic effects, decreased lung function, and irreversible chronic bronchitis (Liebers et al., 2006). Exposure to a lower level of endotoxin in domestic environments during childhood, on the other hand, has been associated with a decreased risk of allergic sensitization and disorders in preschool and school-age children (Braun-Fahrlander et al., 2002; Gehring et al., 2002; Gereda et al., 2000). These observations correspond to the hygiene hypothesis, which suggests that early life exposure to infections and microbes reduces the risk of allergic diseases (von Mutius, 2007; von Mutius et al., 2000; Schaub et al., 2006). Laboratory experiments in mice provide further evidence that low-level pre- and postnatal exposure to endotoxin induces cytokines, which shifts the infants' developing immune system to a predominantly TH1 type response that protects children from developing allergy (Gerhold et al., 2006).

Endotoxin exposures have manifold effects on human health, which may be determined based on the dosage and the timing of exposure. As there is a wide geographical variation in the prevalence of allergic symptoms and diseases in Europe (Asher et al., 2006; Eder et al., 2006), it is of interest to investigate the geographical variation in exposures to different environmental stimuli, including endotoxin. In recent years, several European studies investigated the health effects of domestic endotoxin exposure. Some of them have been multicenter studies using standardized protocols to collect and analyze the levels of endotoxin in house dust and have provided opportunities to compare the geographical variations in domestic endotoxin distribution within limited areas of Europe (Bottcher et al., 2003; Gehring et al., 2008; Giovannangelo et al., 2007b; Schram et al., 2005). All of these studies observed regional differences in domestic endotoxin level. A study that compared domestic endotoxin level in the Netherlands, Germany, and Sweden reported that on average, the endotoxin concentration in German children's mattresses is 1.6 times higher than in Sweden (Giovannangelo et al., 2005). The International Study of Asthmas and Allergy in Children (ISAAC II) reported a fivefold difference in endotoxic concentration in house dust between Rome, Italy and Linköping, Sweden (Gehring et al., 2008); however, only four European study centers were included in this investigation. The comparisons between studies, on the other hand, are difficult and sometimes inadequate. For example, there are at least seven studies that have been conducted in different cities in Germany, and these studies covered north (Hamburg), south (Munich), east (Berlin and Leipzig), and middle part of the Germany (Erfurt), as well as city, suburban, and rural areas in the country (Bischof et al., 2002; Braun-Fahrlander et al., 2002; Gehring et al., 2004a,b; Heinrich et al., 2001; Lau et al., 2005; Schram et al., 2005; Waser et al., 2004). However, five of these studies sampled house dust from the living room floor, and five collected samples from mattress. The size of the floor or mattress surfaces that was sampled and the amount of sampling time also varied. Furthermore, most of these studies focused on investigating the health effects of endotoxin exposure, and detailed information on the measurements of endotoxin level was often not included in the publications.

In the follow-up phase of the European Community Respiratory Health Survey (ECRHS II), endotoxin levels were measured in mattress dust samples collected in 22 study centers from 10 European countries using standardized operational procedures for sample collection and storage. All samples were analyzed in a central laboratory. We took advantage of this unique framework to investigate the geographical variation in domestic endotoxin level in Europe and the determinants of the variation observed. The results of this investigation add to our existing knowledge on geographical variation and the common determinants of endotoxin levels in European households.

Method

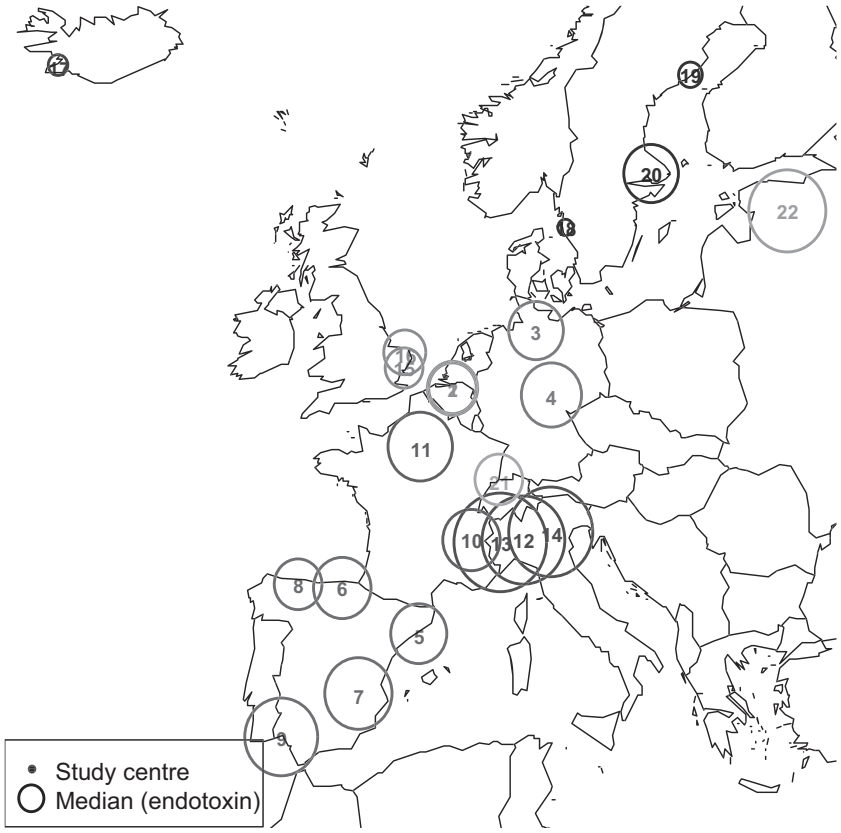
Study design

The ECRHS, (1990–1993) was designed as a multicenter cross-sectional study to estimate variations in the prevalence of asthma, asthma-like symptoms, airway responsiveness, and allergy and their known or suspected risk factors in adults living in Europe (Burney et al., 1994). A group of participants were randomly selected (random sample); in addition, a group of participants who had reported respiratory symptoms were selected (symptomatic sample). In 1999–2001, the participants were followed up as a multicenter cohort (ECRHS II, 2002) in 29 European centers using questionnaires to determine the incidence of and risk

factors for the development of allergic disease, atopy, and rapid loss of lung function. The average follow-up response rate was 60% (Norback et al., 2011). Most of this can be summarized briefly referring to the paper by Burney et al. (1994). Twenty-two centers from 10 European countries agreed to take part in a detailed assessment of home exposures, including collecting dust samples from mattress (see Figure 1) (Heinrich et al., 2006). The current investigation is part of the European Union funded project 'Health effects of indoor pollutants: Integrating microbial, toxicological and epidemiological approaches (HITEA)'. In the HITEA project, a total of 999 house dust samples from the ECRHS II random sample of all 22 participating study centers were randomly selected. The concentration of endotoxin from 974 samples with sufficient amount of dust was analyzed.

Dust sampling and extraction

Domestic endotoxin exposure was assessed by analyzing mattress dust samples collected from each participant's bed, between July 2000 and November 2002. A subset of homes in each center was visited in random order by trained field-workers covering all seasons. The number of samples taken in each season of each study center is provided in Table 1. To standardize dust sampling, a short video demonstrating the dust sampling procedure was shown to all field-workers during a locally arranged training meeting. However, it is important to note that this does not completely eliminate the possibility of intra-field-worker variations. A mattress area under the bed linen where the participant usually slept was marked by a template of 80 cm by 125 cm (1 m^2) and was vacuumed for 2 min with an Electrolux Mondo vacuum cleaner (1300 W) (Electrolux Mondo, Bedfordshire, UK) and an attached ALK dust collection filter (ALK-Abello, Hørsholm, Denmark). Mattress covers or protectors that had been in place for at least 3 months were left on the mattress. Within the next 3 days, samples were frozen at a temperature of −20°C for 24 h and subsequently stored at room temperature in darkness until transported with a silica gel desiccant to the central laboratory. In the central laboratory, the dust samples were sieved (1 mm) to remove larger particles and to obtain a more homogeneous quantity of fine dust for extraction (Zock et al., 2006). In 2008, homogeneous fractions (50–70 mg) of the stored dust samples were transferred to preweighed 10-ml polystyrene vials with screw cap (Sterilin, no 8602-124). The aliquots were sent within days to Utrecht University



	Study Centre	N	Median (EU/g)	(25th, 75th) percentile	Geometric Mean (EU/g)
1	Antwerp S	33	2496	(1038, 5700)	2439
2	Antwerp C	21	2346	(1550, 4622)	2869
3	Hamburg	70	2728	(1344, 6609)	3255
4	Erfurt	70	3022	(2169, 5315)	3531
5	Barcelona	45	2754	(1728, 6037)	3059
6	Galdakao	57	2873	(1530, 6017)	2769
7	Albacete	45	3393	(1869, 5215)	3327
8	Oviedo	50	2294	(1069, 3567)	2108
9	Huelva	12	3749	(1153, 10970)	3661
10	Grenoble	62	2836	(1315, 5132)	2685
11	Paris	54	3218	(1857, 5740)	3599
12	Pavia	20	4234	(2826, 8715)	4812
13	Turin	5	4806	(1554, 4924)	3019
14	Verona	23	4340	(2943, 8105)	4763
15	Ipswich	38	1765	(863.7, 2993)	1671
16	Norwich	52	2012	(994, 5022)	2460
17	Reykjavik	58	772	(417.7, 1755)	885
18	Gothenbourg	50	542.5	(393.8, 1503)	820
19	Umea	75	1014	(752.5, 1701)	1217
20	Uppsala	33	2675	(1356, 4132)	2317
21	Basel	30	2299	(919.5, 4014)	2224
22	Tartu	71	3942	(2482, 8057)	5173

Fig. 1 Geographic distribution of endotoxin levels in mattress dust across 22 European study centers of European Community Respiratory Health Survey. The number inside the circle identifies the study center, and the size of the circle illustrates the median endotoxin level in endotoxin units per gram of sieved dust

Table 1 Number of house dust samples taken in each season of each study center

	Autumn	Winter	Spring	Summer
Antwerp	14	23	5	12
Barcelona	15	18	7	5
Basel	13	16	0	1
Erfurt	8	21	22	19
Galdakao	20	12	11	14
Gothenbourg	na	na	na	na
Grenoble	27	17	6	12
Hamburg	28	4	9	29
Huel./Albac	20	17	5	15
Ips./Norwich	9	23	17	41
Oviedo	18	16	6	10
Paris	15	23	4	12
Pav/Tur/Ver	11	24	8	5
Reykjavik	14	0	15	29
Tartu	11	36	22	2
Umea	24	21	20	10
Uppsala	10	10	6	7

for endotoxin analysis. First, 5–40 ml (0.05%, v/v) Tween-20 in pyrogen-free water was added, suspensions were incubated in an end-over-end roller for 1 h at room temperature, and after centrifugation (15 min, 1000 *g*), the upper 10% of supernatant was harvested and stored at −20°C for endotoxin analysis. The endotoxin concentration was measured at a 1:500 or 1:1000 dilution in the Limulus amebocyte lysate (LAL) assay (batch no. FL147M) in pyrogen-free water calibrated with *Escherichia coli* LPS standard (batch no. GL0006, with a potency of approximately 12–14 EU/ng), as described previously by Spaan et al. (2008). The analysis results were expressed as endotoxin units per gram (EU/g) of sieved dust. All samples contained endotoxin above the limit of detection (50 EU/g).

Questionnaire information

A face-to-face interview was conducted with each participant in the survey. Information including number of households in the building, heating and ventilation habits, floor covers, and pet keeping was obtained via the interview. During the home visits, trained field-workers also assessed home characteristics such as number of rooms, presence of dampness and mold on the walls or ceilings in several rooms, size of mattress, and types of bedding. In addition, participants were asked about the number of people living in the home (household crowding index) (Melki et al., 2004), age of the mattress, frequency of vacuuming the bedroom floor and the mattress, and their educational level. Detailed descriptions of the collection of home characteristic and personal data were reported previously (Zock et al., 2006).

Meteorological data

Meteorological data for the year 2001 were obtained from national or local meteorological institutes. The average monthly temperature was calculated, and the temperatures of the coldest and hottest months were used to determine winter and summer temperatures, respectively. The annual mean relative humidity was calculated for each city except for the two British centers, Ipswich and Norwich.

Statistical analysis

Endotoxin level was log-transformed owing to the skewness of its distribution. The degrees of correlation between potential factors that may determine the amount of measured domestic endotoxin were calculated using the Spearman correlation coefficient or chi-square test. The associations between the potential determinants and endotoxin level measured from the mattress were assessed using generalized linear mixed models (GLMM), which assesses the linearity and the magnitude of the associations. The variability between the European centers was taken into account by random effect (Heinrich et al., 2006). All analyses were performed using R version 1.9.0, with additional packages MASS and mgcv.

Results

Geographical variation in indoor endotoxin concentration

A total of 974 dust samples were successfully analyzed for the current investigation. The measured amounts of endotoxin, expressed as EU per gram sieved dust, stratified by study centers are presented in Figure 1. A broad range of center-specific median values of domestic endotoxin level was observed. Taking the geographical location of the study centers into account, the highest level of endotoxin measured in house dust samples is from the three Italian study centers in the Po Valley (median = 4806 EU/g in Turin study area), which has a subtropical humid climatic. Study centers close to the Mediterranean region also have a higher level of domestic endotoxin (median = 3749 EU/g in Huelva study area), while the two northern European study centers Reykjavik and Gothenburg have a median endotoxin level below 1000 EU/g.

Geographically related determinants

Average winter and summer temperature in the year 2001, longitude, latitude and altitude, as well as annual mean relative humidity in the geographical area of each study center in the year 2001 were considered potential meteorological factors that may influence the level of domestic endotoxin. The ecological analysis of the associations between individual potential meteorological factors and the domestic endotoxin levels by study center is listed in Table 2. Both latitude and annual mean relative humidity are highly correlated with

summer temperature ($r = -0.75$ and -0.69), and both longitude and latitude are highly correlated with winter temperature ($r = -0.79$ and -0.77). Therefore, only average winter and summer temperatures of study centers ($r = 0.5$) were included in the second-stage analysis. To identify the effect of meteorological factors on the geographical variation in domestic endotoxin level by center, a generalized linear mixed model was fitted to the data. After mutual adjustment of the meteorological factors, only average outdoor summer temperature of study center was associated with the measured domestic endotoxin at center level (Table 2). Our results show that 10% of the variation in domestic endotoxin level by center is explained by the variation in summer temperature.

Personal and Home Characteristic–related Determinants

Educational level of the study subject and characteristics of the home and the room where the sampled bed was located were associated with the quantified domestic endotoxin level by a generalized linear mixed model after adjusting for the average summer temperature of the geographical location at center level (Table 3). In the first step of the analysis, having a cat or dog as a pet at home, houses that were built before 1970 (houses more than 29 years old), higher number of residents per household, whether the residents smoked indoor, having the bed in the living room on which the dust was sampled, and having damp patches on the wall or ceiling, or having visible mold in the room which the dust was sampled were associated with increases in the amount of indoor endotoxin. We have also associated cat and dog ownership independently with the amount of domestic endotoxin. The results show that homes of cat owners have on average 1.20 (95% CI = 1.01, 1.41) times higher endotoxin concentration compared to homes without cats, while the association with dog ownership was stronger (GM ratio (95% CI) = 1.38 (1.14, 1.66)). Having wall-to-wall carpets on the bedroom floor was associated with a lower endotoxin concentration in mattress dust. Season of dust sampling, on the other hand, was not statistically significantly associated with the measured endotoxin level. In the next step, all potential influential factors that reached a *P*-value <0.1 were selected for the final model to further investigate the magnitude of the association. Table 4 shows that after mutually adjusting for average outdoor summer temperature of study center and all the factors that are likely to be associated with the amount of domestic endotoxin, having a cat or dog as a pet at home, having a higher number of residents, and having damp patches on the wall or ceiling in the room in which the dust was sampled significantly increased the level of domestic endotoxin. However, the determinants identified in our study can only explain 13% of the variation in the measured endotoxin, while 10% is explained by summer temperature at center level.

Table 2 Meteorological conditions associated with the center-specific endotoxin concentration in mattress dust

Associations between individual meteorological factors and domestic endotoxin level by study center

Meteorological factors	GM ratio[a] (95% CI)	Range across centers
Winter temperature (unit = 10°C)[b]	1.24 (0.81–1.90)	−10.4 to 11.1°C
Summer temperature (unit = 10°C)[b]	2.84 (1.81–4.45)	11.1 to 26.7°C
Annual mean relative humidity (unit = 10%)	0.88 (0.63–1.24)	62.9 to 85.4
Altitude (per 100 m)	1.01 (0.93–1.10)	5 to 700 m
Longitude (per 10°E)	1.15 (0.93–1.41)	−21.9° to 26.7°
Latitude (per 10°N)	0.70 (0.54–0.92)	37.3° to 64.2°

Associations between meteorological factors and domestic endotoxin level by study center in a multivariable model

Meteorological factors	GM ratio[a] (95% CI)
Winter temperature (unit = 10°C)[b]	0.93 (0.66–1.30)
Summer temperature (unit = 10°C)[b]	3.28 (1.94–5.56)
Altitude (per 100 m)	0.96 (0.88–1.04)

[a]Ratios of the geometric mean by center associated with per unit change in meteorological factors.
[b]Average winter and summer temperature of the year 2001.

Discussion

Our study shows that there is a strong geographical variation in domestic endotoxin level measured from mattress dust in European countries. This geographical variation can only partly be explained by the average summer temperature. Housing and room characteristics including having a cat or dog as pet at home, having a high number of residents per household, and having damp patches on the wall or ceiling in the bedroom were also associated with elevated domestic endotoxin levels. However, the determinants identified in our study can only explain 13% of the variation in the measured endotoxin.

Although dust samples from 22 European countries were collected and analyzed in the current study, the endotoxin level was quantified in less then 100 samples in each center. Consequently, the study design has limited power to detect all determinants that contribute to the inter-center variations. Dust samples were collected between years 1999 and 2001 and were then stored at −20°C until the year 2008 before extraction and quantification. This long-term storage theoretically may lead to decline in the amount of microbes in the samples. However, a previous study showed that storage of house dust at −20°C for up to 10 months does not affect the endotoxin levels in the samples (Fahlbusch et al., 2003). Although we cannot rule out the possibility that long-term storage may have had an effect on the amount of endotoxin reported in this

Table 3 Associations[b] between individual house and room characteristics with the measured domestic endotoxin level

Characteristics of the house and the room	*n*/*N*	GM ratio[a] (95% CI)	*P*-value
Educational level			
Medium	258/967	0.93 (0.78–1.10)	0.378
High	304/967	0.95 (0.80–1.12)	0.536
Have a cat or a dog at home	317/972	1.28 (1.10–1.48)	0.001**
Duration the participants have lived in the current dwelling	–	1.00 (0.99–1.01)	0.823
Age of the dwelling (house built before 1970)	404/956	1.13 (0.98–1.30)	0.095*
No. of households in the sampled building			
5–15	210/541	1.10 (0.81–1.50)	0.547
>15	262/541	0.91 (0.67–1.23)	0.540
No. of people/room living in the sampled home	–	1.44 (1.17–1.78)	0.001**
No. of residents who smoke inside the sampled home	–	1.09 (0.99–1.20)	0.069*
Bed is in the living room	45/969	1.68 (1.20–2.35)	0.002**
Double or triple glazing windows in the bedroom	725/967	0.87 (0.72–1.04)	0.127
Damp patches on the walls or ceilings in the bedroom	68/973	1.70 (1.31–2.21)	0.000**
Visible mold in the bedroom	45/973	1.56 (1.13–2.15)	0.007**
Water leakage at the sampled home in the last 12 months	94/950	1.14 (0.90–1.43)	0.275
Air brick or ventilation aperture in the sampled room	179/969	0.99 (0.81–1.20)	0.906
Season of dust sampling			
Winter (January–March)	281/924	1.06 (0.88–1.27)	0.557
Spring (April and May)	163/924	0.93 (0.75–1.15)	0.482
Summer (June–September)	223/924	1.00 (0.82–1.21)	0.962
Average no. of cigarettes smoked in the sampled home/day			
1–14	33/974	1.28 (0.86–1.90)	0.230
>14	22/974	1.40 (0.87–2.25)	0.171
Open bedroom windows at least 30 min in winter			
Some days	170/971	0.87 (0.70–1.08)	0.193
Most days	82/971	1.05 (0.79–1.40)	0.715
At least once per day	493/971	0.90 (0.74–1.10)	0.319
Carpet in the bedroom			
Partially	377/974	0.97 (0.81–1.15)	0.685
Completely	274/974	0.79 (0.63–0.97)	0.026**
Age of the mattress			
More than a year old but did not have it in the last survey	543/968	0.94 (0.70–1.27)	0.698
Had it since last survey (more than 8 years)	371/968	0.93 (0.69–1.26)	0.647
Use bleach for cleaning			
<1 day/week	182/573	1.01 (0.80–1.26)	0.965
1–3 day/week	122/573	0.92 (0.69–1.21)	0.533
4–7 day/week	69/573	0.97 (0.69–1.38)	0.883

*P-value < 0.05; **P-value < 0.01.

[a]Ratios of the geometric mean associated with housing characters taking into account variations among centers.

[b]The associations are assessed by generalized linear mixed model with study centers as random effect. The models are adjusted for summer temperature at the center level.

Table 4 Determinants of measured domestic endotoxin level in 974 mattress dust samples from 22 European countries in a multivariable model[a]

	GM ratio[b] (95% CI)	*P*-value
Summer temperature by center[c]	1.10 (1.05–1.15)	<0.001
Have a cat or a dog at home	1.25 (1.08–1.45)	0.003
No. of people/room living in the sampled home	1.28 (1.03–1.60)	0.025
No. of residents who smoke inside the sampled home	1.07 (0.98–1.18)	0.149
Bed is located in the living room	1.41 (0.98–2.03)	0.062
Age of the dwelling (house built before 1970)	1.10 (0.95–1.27)	0.203
Damp patches on the walls or ceilings in the bedroom	1.44 (1.02–2.03)	0.036
Visible mold in the bedroom	1.18 (0.78–1.77)	0.436
Carpet in the bedroom		
Partially	0.99 (0.84–1.18)	0.937
Completely	0.83 (0.67–1.03)	0.091
Adjusted R^2 = 0.137		

[a]The associations are assessed by generalized linear mixed model with study centers as random effect.

[b]Ratios of the geometric mean associated with housing characters taking into account variations among centers.

[c]Summer temperature at the center level.

article, this factor is unlikely to affect the association that we report.

Some epidemiological studies have linked exposure to endotoxin in domestic environments to wheezing symptoms and asthma (Celedon et al., 2002, 2007; Park et al., 2001; Thorne et al., 2005), while other studies observed negative associations (Braun-Fahrlander et al., 2002; Gehring et al., 2008; Gereda et al., 2000; von Mutius et al., 2000). The timing and dosage of the exposure and genetic predisposition (Liebers et al., 2008) may contribute to these incoherent observations, but so may the geographical heterogeneity of endotoxin exposure, which to date as has been poorly described in Europe. In our current study, domestic endotoxin was collected and quantified using a standardized method over different study centers, and consequently the variation in endotoxin level owing to design and measurement procedures is minimized. We found that the median of the domestic endotoxin level in Turin, Italy, was six times higher than that in Reykjavik, Iceland. This corresponds to the observation from the ISAAC study that the endotoxin concentration in Rome was 5-fold higher comparing to Linköping. However, it is important to note that only five samples were taken in the Turin study center; therefore, this observed endotoxin level can only be considered as reference. Most of the previous European studies, except one in Germany, have collected dust samples from the living room floor or mattress of the child who was recruited in the study; therefore, it is difficult to compare those previously published results to the results of our current study, which report the endotoxin concentration from adults' mattresses. The German studies that collected dust sampled from mattress of adults reported a median endotoxin level of 3000 EU/g (Chen et al., 2007), which corresponds to the regional level reported in the current study (2728 EU/g in Hamburg and 3022 EU/g in Erfurt). Across the Atlantic, a recent study in United States which assayed 2552 house dust samples nationwide with a wide range of demographic groups also reported a wide geographical variation in domestic endotoxin level; the geometric mean of endotoxin concentration

in the dust in bedding varies from 14 000 EU/g in New England to 31 000 EU/g in Pacific division (Thorne et al., 2009). The endotoxin concentrations in this U.S. study are much higher than the domestic endotoxin concentration reported in Europe because the samples were collected from five to six selected domestic areas for each home, including the kitchen floor.

We observed that average summer temperature is the strongest meteorological determinant for the geographical variation in domestic endotoxin, and this is probably because higher temperatures typically relate to more active bacterial growth in the environment. Surprisingly, we did not observe an association between relative humidity and regional endotoxin concentration, as one may expect because it is known that growth and proliferation of bacteria depends largely on moisture. However, we did find that having damp patches on the wall or ceiling in the bedroom was significantly associated with higher level of endotoxin in mattress dust. This finding suggests that whenever humidity in the indoor air is not properly managed, e.g., owing to insufficient ventilation or shortcomings in the construction, and condensation/dampness occurs, excessive microbial growth can be a consequence, which can be measured also in elevated levels of endotoxin. Our data suggest that it is not so much the overall relative humidity in the air that relates to endotoxin levels in house dust, but rather how the humidity impacts and affects the building surfaces. On the other hand, damp patches on the wall or ceilings may also indicate housing dilapidation or poor maintenance of the mattress or other furnishings; therefore, the observed association may also indicate that, in general, the mattress endotoxin level is elevated in poor-quality dwellings.

Only 13% of the variation in the measured endotoxin can be explained by the factors identified in our study. This finding is in line with a previous German study in which 7.3% of the variability in endotoxin level measured from mattresses of adults is explained by housing characteristics, season of dust sampling, and study center (Gehring et al., 2004a,b). In a European study, lower percentages of variability in endotoxin level in the mattresses of 4- to 6-year-old children were explained by housing characters and season of dust sampling (4.9% for Swedish samples, 5.0% for Dutch samples, and 6.7% for German samples) (Giovannangelo et al., 2007a,b). Previous studies that investigated domestic endotoxin exposure have associated the amount of endotoxin at home to indoor pets, multiple occupants, central air conditioning, windows frequently opened to outside, season of dust sample collection, and relative humidity. The most consistently reported determinants are indoor pets and multiple occupants (Gehring et al., 2004a; Gereda et al., 2001; Giovannangelo et al., 2007a,b; Heinrich et al., 2001; Waser et al., 2004), and our current investigation has further confirmed these positive associations. Skin and mucous membranes of humans and pets are heavily colonized by a large variety of microbial species that contribute to the commensal human microflora, which has been identified as a major source for bacteria in house dust (Täubel et al., 2009). Multiple household occupants and having pets at home thus increase the amount of endotoxin in the environment. Tobacco smoke, on the other hand, has also been reported to increase air concentrations of endotoxin because it contains LAL-reactive material (Larsson et al., 2004). However, the amount of endotoxin in a cigarette is not high, and the positive association between smoking indoors and the domestic endotoxin levels disappeared after adjusting for other determinants. We also observed higher endotoxin concentrations if the sampled bed is located in the living room. A previous study has shown generally higher endotoxin levels on the living room floor compared to the bedroom floor (Ownby et al., 2010). A bed/mattress that is located in the living area may collect higher amounts of outdoor dust that is carried in by the residents on shoes or clothes, which would be reflected in higher endotoxin levels. However, this association was weakened after adjusting for other determinants, which demonstrates that effect size is relatively low. We did not see any association between the use of bleaching agents and endotoxin levels. However, further investigation into the effects of cleaning habits on endotoxin levels is needed. For example, in a U.S. study, wet mop cleaning was associated with lower domestic endotoxin concentrations (Perzanowski et al., 2006).

In our current study, summer temperature explains 10% of the variation in domestic endotoxin level by center. However, cultural behaviors also vary between centers with different climates. For example, the dog keepers in Southern European countries may let their pets run outside of the house for longer periods compared to the dog keepers in Northern Europe, and the dogs from Southern Europe may therefore bring more endotoxin into the home. The housing character also varies between areas with different climates. It is important to take these differences into account when interpreting the results.

Conclusion

There is considerable variation in mattress dust levels of domestic endotoxin in European countries. Average outdoor summer temperature of study center is the strongest meteorological determinant for the observed geographical variation in mattress dust endotoxin. The incoherent observations of the health effect of endotoxin may be partly due to the geographical heterogeneity of endotoxin exposure. Therefore, the observed variation should be considered in further studies. As demonstrated in the current study, the meteorological factors and housing characteristics explain only a small

part of the variation in indoor endotoxin level. Therefore, measurements of indoor endotoxin are recommended to determine the level of exposures.

Acknowledgements

This work was supported by the European Commission as part of HITEA (Health Effects of Indoor Pollutants: Integrating microbial, toxicological and epidemiological approaches), Grant agreement no. 211488 under the Seventh Framework Programme, Topic ENV.2007.1.2.1.1. Indoor air pollution in Europe: An emerging environmental health issue.

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