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AIRBORNE ENDOTOXIN IN DIFFERENT BACKGROUND ENVIRONMENTS AND SEASONS

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Abstract: Endotoxin is a cell wall component from Gram-negative bacteria, and inhaled endotoxin contributes significantly to the induction of airway inflammation and dysfunction. Background levels of endotoxin have not yet been extensively described. In this study, airborne endotoxin was measured with a standardized protocol in 5 types of background environment (169 samples) in Denmark from October to May. Endotoxin levels in a greenhouse (median = 13.2 EU/m^3) were significantly higher than in the other environments. The air from biofuel plants (median = 5.3 EU/m^3), the air on congested streets (median = 4.4 EU/m^3) and on an agricultural field (median = 2.9 EU/m^3) had higher endotoxin contents than the air in industrial areas (median = 1.3 EU/m^3) or in towns (median = 0.33 EU/m^3). Levels in industrial areas were significantly higher than in towns. A literature study revealed background levels of endotoxin on different continents between 0.063–410 EU/m^3, with median or mean values between 0.063–3.6 EU/m^3. Endotoxin concentrations in towns and industrial areas were higher in April and May than in autumn and winter, and were higher in October than in winter. These data of exposure in background environments and of seasonal variation are helpful for public health practitioners, epidemiologists and industrial hygienists.

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INTRODUCTION

Bioaerosols occur ubiquitously as inhalable mixtures of air and microorganisms and their parts, viruses, and parts of plants and animals. Endotoxin is a cell wall component from Gram-negative bacteria, and is composed of lipopolysaccharides (LPS) as a main constituent, protein and phospholipids. Environmental bacteria (and therefore also endotoxin) in soil and water can be aerosolised by wind, splashing rains, sea spray, and mechanical disturbance [16]. Inhaled endotoxin contributes significantly to the induction of airway inflammation and dysfunction (e.g. [25]), and many occupational studies have shown positive associations between endotoxin exposure and respiratory disorders including asthma-like syndrome, chronic airway obstruction, organic dust toxic syndrome, byssinosis, bronchitis, and increased airway responsiveness. On the other hand, recent studies suggest that environmental exposure to endotoxin may protect against the development of allergic diseases [33]. Thus, independent studies from different countries have shown evidence of a protective association between exposure to endotoxin in early childhood and allergic diseases and asthma later in life.

For airborne fungi, variations in outdoor concentrations throughout the year have been described in some areas of the world [29] and also variations during a day [20]. For endotoxin this has not been very well described. Thus, in

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Table 1. Environments in Denmark where endotoxin has been measured.

Place	n	Description	Place in Denmark	Season of sampling
Towns	53	Mainly residential areas.	All over Denmark	January–April and November–December 2003–2004
Streets	5	Congested streets.	In the centre of Copenhagen	May 2005
Industry	68	Small industrial areas where biofuel plants were present. Measurements were performed upwind of the biofuel plants.	All over Denmark	January–February and October–December 2004–2005
Air from biofuel plants	20	Small industrial areas with biofuel plants. Sampling was performed 250-350 m downwind of the plant.	All over Denmark	January–May and October–December 2004–2005
Greenhouse	5	Greenhouse with pot plants.	Northern Jutland	November 2004
Field	18	A field 200 m from a field where pig slurry was distributed at time of sampling.	Northern Jutland	April 2005

a review, the authors write that the endotoxin content in ambient airborne particles has not yet been extensively studied, and neither has seasonal variation nor the content of endotoxin in polluted air versus that of unpolluted air [16]. In this study, we have, with a standardized protocol, measured the concentrations of airborne endotoxin in the period January–May or September–December in different environments in Denmark, including towns, small industrial areas, agricultural fields, greenhouses and around biofuel plants. The cold periods were chosen since epidemiological studies of exposure to bioaerosols and health effects are often performed outside the main pollen season, which in Denmark is in January–April and September–December.

There is no standard method for sampling bioaerosols and this has often been stressed as a problem [5, 21, 33]. In this study, we used GSP samplers to sample the bioaerosols and the Limulus kinetic assay to quantify endotoxin, and compared results from other papers where the same methods were used. In addition, we collected data of outdoor references from other studies.

MATERIAL AND METHODS

Environments. The chosen background areas are described in Table 1. The different environments were selected because people are expected to spend time in these areas during non-working hours. Exposure to wind from biofuel plants was included because biomass releases large amounts of endotoxin [14, 15], and because there is a likelihood that neighbours to plants handling organic materials are exposed [7]. People spend a lot of time in their homes, but homes were not included in this study as this aspect has been described in other studies [23].

Sampling of airborne endotoxin. Inhalable bioaerosols were sampled at heights of 1.5 m using GSP (CIS by BGI, INC Waltham, MA) inhalable samplers (airflow 3.5 l min^{-1}) for 4–6 hours, between 08:00–14:00. Pump calibration was checked at least every second hour. The samplers were mounted with Teflon filters (pore size 1.0 mm). The samplers were generally placed with the opening in the same direction as the wind in different environments, but in one environment (called wind from the biofuel plant) the samplers were placed with the opening towards the wind from the plant (Tab. 1). Field blanks (no air pulled through the filter) were collected on each sampling day to verify that the filters were not contaminated.

Determination of endotoxin by the Limulus method. Dust was extracted with 6.0 ml sterile 0.05% Tween 20 aqueous solution by orbital shaking (300 rpm) at room temperature for 60 min and centrifuged (1000x g) for 15 min. The supernatant was analysed (in duplicate) for endotoxin by the kinetic Limulus Amboecyte Lysate test (Kinetic-QCL endotoxin kit, BioWhittaker, Walkersville, Maryland, USA). A standard curve obtained from an Escherichia coli O55:B5 reference endotoxin was used to determine the concentrations in terms of endotoxin units (EU) (10.0 EU ≈ 1 ng). The endotoxin concentrations are presented as time weighted averages (TWA).

Literature review of background level of endotoxin. A total of 50 papers concerning exposure to endotoxin in occupational settings and homes were studied to find data of background or reference levels of endotoxin and to find the incidence of papers with background measurements. The papers were published in the period 1982–2005 and were collected randomly from a list from PubMed where the 3 search words ‘endotoxin’, ‘exposure’ and ‘airborne’ were used. Results of background levels of endotoxin from these papers are mentioned in Table 2.

Statistical analysis. Statistical analyses were performed with SAS (version 8e, SAS Institute, Cary, NC). The endotoxin data were log-normally distributed and were thus log-transformed prior to statistical analysis. To compare the endotoxin concentrations in different environments and at different times of year PROC GENMOD (GENeralized linear MODels) was applied.

Table 3. Exposure to endotoxin in different environments where GSP-samplers have been used for sampling airborne dust and the Limulus Kinetic Assay has been used to quantify airborne endotoxin.

Environment	Exposure EU/m^3		Personal (P) or Stationary (S) sampling	References
	Interval	Median(s) or geometric means		
Lumber mill workers[a]	<2.5–347.5	21	P	[4]
Pig farmers[a]	10–8800	464	P	[27, 26]
Pig farmers[a]	0.08–16720	610	P	[26]
Poultry farmers[a]	152–13080	2060	P	[26, 27]
Greenhouse[a]	0.4–101	2.8	P	[17, 26]
Biofuel plant[b]	–	259-573	S	[13]
Insulation workers[b]	–	2.3-1300	P	[1]
Domestic waste collectors	<4–7182	23.7-53.4	P	[34]
Wastewater treatment workers	0.6–2093	27	P	[30]

[a]Exposure data are converted from ng to EU according to the conversion factors mentioned in each paper; [b]Interval not mentioned – only median values.

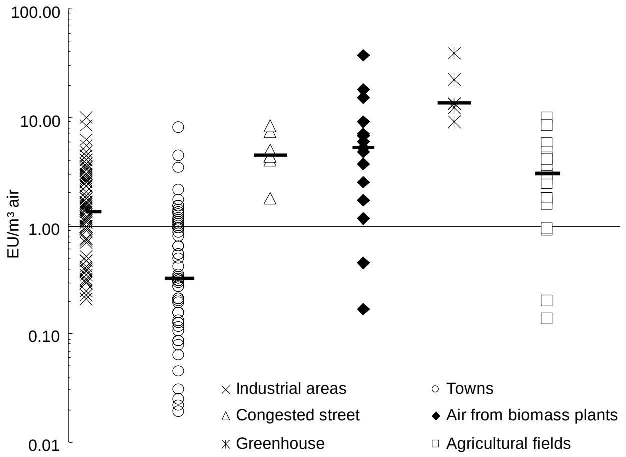

Figure 1. Airborne concentrations of endotoxin (EU/m^3, log 10 scale) in different background environments. The bars (–) indicate median values.

RESULTS

Endotoxin was present in all 169 samples (Fig. 1) and the concentrations were significantly different in the different background environments ($p<0.0001$). Thus, the endotoxin levels in the greenhouse (median = 13.2 EU/m^3) were significantly higher than in the other environments. The air from biofuel plants (median = 5.3 EU/m^3), the air on congested streets (median = 4.4 EU/m^3) and on an agricultural field (median = 2.9 EU/m^3) had a higher endotoxin content than the air in industrial areas (median = 1.3 EU/m^3), and in towns (median = 0.33 EU/m^3). Levels in industrial areas were significantly higher than in towns.

Seasonal differences were seen in towns ($p=0.0161$), and thus significantly higher concentrations of endotoxin were found in March and April than in November and December (Fig. 2). In industrial areas, significant variations between the months were also seen in endotoxin concentrations ($p<0.0001$). The endotoxin concentrations were significantly higher in May than in January, February, March and December. Furthermore, the endotoxin concentrations were higher in April than in January, February and March, and was higher in October than in January (Fig. 3). There were too few data of endotoxin in wind from the biofuel plants to carry out a comparison between seasons.

In a review of 50 papers about exposure to endotoxin in homes and occupational settings, reference levels of

Table 2. Background concentrations of endotoxin measured as reference concentrations in different studies using a Limulus Assay and different aerosol sampling methods.

Environment	Country	n	Months of measurements or temperatures in the period	Endotoxin concentration EU/m^3		Reference
				Interval	Median, geometric mean (GM) or mean	
Outside a textile plant[a]	Taiwan	3	April–May	4–410	–	[31]
Urban area	Massachusetts, USA	32	All year	–	GM = 0.51	[23]
Suburban area	Massachusetts, USA	35	All year	–	GM = 0.39	[23]
Air intake in an office building	St. Louis, USA	14	July–September	0.73–2.1	GM = 1.2	[8]
Outside (upwind) paper recycling plants[b]	Denmark	19	April–October	0.21–5.6	Median = 1.3	[2]
Outside (upwind) a composting facility[a]	Illinois	10	September–November	0.1–3.59	Mean = 1.4	[9]
Outside jute mill[a]	India	2	September–October	–	Mean = 0.063	[18]
Upwind of industrial wastewater treatment plants[a]	Finland	8	0–18ºC	<0.48–36	Median = 3.6	[11]

[a]Exposure data are converted from ng to EU according to the conversion factors mentioned in the papers.
[b]This paper was selected because we knew that outdoor references were presented and this paper is not part of the study of incidence of papers with outdoor reference measurements.

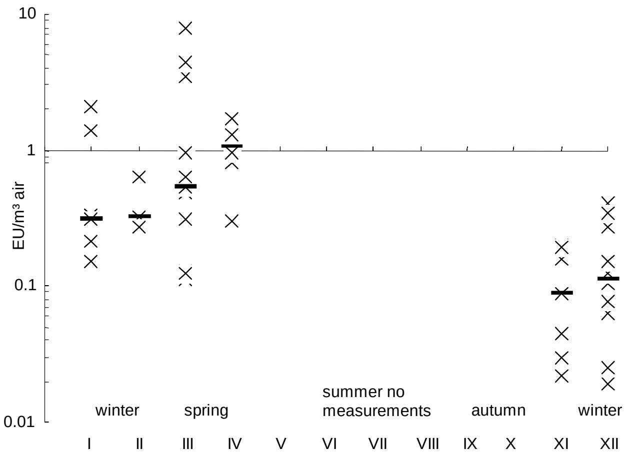

Figure 2. Airborne concentrations of endotoxin (EU/m^3, log 10 scale) in towns in different months. The bars (–) indicate median values.

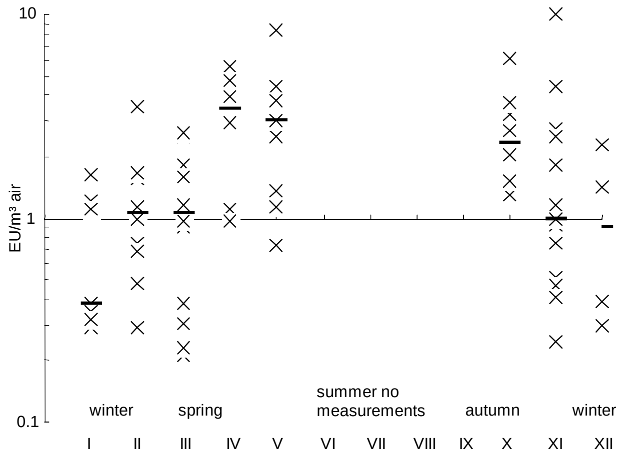

Figure 3. Airborne concentrations of endotoxin (EU/m^3, log 10 scale) in industrial areas in different months. The bars (–) indicate median values.

endotoxin, were only mentioned in 6 papers (Tab. 2). These papers showed levels between 0.063–410 EU/m^3. In 4 of the 6 studies and in the paper of Breum *et al.* [2] the median endotoxin level was between 0.4–1.4 EU/m^3.

DISCUSSION

Background levels of endotoxin are only rarely mentioned in papers of exposure to endotoxin in occupational settings and homes, and in a review of 50 papers, reference levels of endotoxin were only mentioned in 6 papers (Tab. 2). In this study, we measured endotoxin in 53 air samples from Danish towns and we found a median endotoxin level of 0.33 EU/m^3. This level is very close to that found in urban and suburban areas in Massachusetts (Tab. 2). In 68 samples from industrial areas we found a median value of 1.32 EU/m^3 - at the same level found in St. Louis and in Denmark outside office buildings and paper recycling plants, but it is very different from that found in industrial areas in Taiwan and India (Tab. 2).

Background levels of endotoxin in this study were measured by exactly the same methods in different environments. The method of sampling aerosols and the method of quantification of endotoxin has often been mentioned to have an influence on the measured level [5, 21, 33, 34, 34]. The investigations mentioned in Table 3 are studies where the GSP samplers and the kinetic Limulus assay have been used, as in this study. In this study, the highest endotoxin concentration was in the greenhouse, and the concentration was in the interval found for exposure of Spanish greenhouse workers mentioned in Table 3.

The wind emitted from the biofuel plants had a significantly higher content of endotoxin - namely a median of 5.27 EU/m^3 - than the wind entering the biofuel plants, but it was lower than found in some occupational settings, including on biofuel plants (Tab. 3). Increased levels of microorganisms are seen earlier in the neighbourhood of e.g. composting centres [6, 7]. In a study by Schiffman *et al.* [28], healthy humans in an exposure chamber were exposed to air emissions from a swine confinement atmosphere containing different components, including endotoxin (7.4 EU/m^3), in a level close to that we found in the neighbourhood of the biofuel plants. In that study, no effects on physical symptoms were seen, but the exposed subjects were more likely to report nausea, headaches and eye irritation. The authors suggest that this may be due to the exposures or to learned warning signals of potential health effects. In another study, Pacheco *et al.* [22] conclude that airborne endotoxin is associated with respiratory symptoms in reaction to mice in non-mouse-sensitised laboratory workers. These people were exposed to endotoxin in mean levels of up to 566 pg/m^3 (= 5.66 EU/m^3), which is close to that found in the air from biofuel plants and on congested streets. An exposure of about 5 EU/m^3 is considered low compared to what can be found in occupational settings (Tab. 3) and compared to the fact that a health-based exposure limit of 50 EU/m^3 (8-TWA) has been proposed in the Netherlands by the Dutch Health Council. Similarly, the other endotoxin concentrations found in this study are low compared to the suggested exposure limit. Whether the found levels of endotoxin are of importance in relation to health effects is not yet possible to conclude, but knowledge of these background levels are of importance because some of them are close to that described as being associated with health effects in some occupational settings [10, 22].

The endotoxin concentrations in towns and industrial areas were higher in April and May than in autumn and winter. Furthermore, the endotoxin concentration was higher in October than in winter. Long *et al.* [12] have studied endotoxin content in airborne dust, and in their study with 13 outdoor aerosol samples higher endotoxin contents per g dust were found in spring than in winter and autumn. The higher concentration of airborne endotoxin in spring and in October than in winter is probably related to the presence of leaves on the trees because endotoxin is present on the leaves [3], to the higher bacterial growth in the warmer months and to the higher wind speed in spring and autumn. In occupational settings, several studies have been performed concerning exposure to bioaerosols as affected by season. For example, Thorn [32] studied exposure to endotoxin of household waste collectors and did not find any seasonal variation. Similarly, Passman [24] found no clear evidence of seasonality in concentration of the fungus *Aspergillus fumigatus* downwind from a sewage composting plant. In contrast, Nielsen *et al.* [19] found seasonal variations in exposure to endotoxin for biowaste collectors with the lowest exposure level during spring. In airborne bedroom dust, Park *et al.* [23] found higher endotoxin levels in spring than in winter. The data set of wind from biofuel plants was too small to study seasonal differences in content of endotoxin.

CONCLUSIONS

This study provides public health practitioners, epidemiologists and industrial hygienists with background levels of endotoxin. Even though the endotoxin concentrations in this study were not high in comparison to that found in some occupational settings, significant variations were seen between background environments and between seasons. Greenhouse air, air from biofuel plants and air from a highly congested street contained the highest amounts of endotoxin. It is not possible to conclude whether these levels of exposure have any effect on the health of the exposed people since published papers show divergent results concerning effects of exposure to different doses of endotoxin.

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REFERENCES

1. Breum NO, Schneider T, Jorgensen O, Valbjørn Rasmussen O, Skibstrup Eriksen S: Cellulosic building insulation versus mineral wool, fiberglass or perlite: installer's exposure by inhalation of fibers, dust, endotoxin and fire-retardant additives. *Ann Occup Hyg* 2003, **47**, 653-669.
2. Breum NO, Würtz H, Ebbehøj N, Midtgård U: Dustiness and bioaerosol exposure in sorting recyclable paper. *Waste Manage Res* 1999, **17**, 100-108.
3. DeLucca AJ, Palmgren MS: Mesophilic Microorganisms and Endotoxin Levels on Developing Cotton Plants. *Am Ind Hyg Assoc J* 1986, **47**, 437-442.
4. Dennekamp M, Demers PA, Bartlett K, Davies HW, Teschke K: Endotoxin expsoure among softwood lumber mill workers in the canadian province of British Columbia. *Ann Agric Environ Med* 1999, **6**, 141-146.
5. Douwes J, Thorne P, Pearce N, Heederik D: Bioaerosols health effects and exposure assessment: progress and prospects. *Ann Occup Hyg* 2003, **47**, 187-200.
6. Folmsbee M, Strevett A: Bioaerosol concentration at an outdoor composting center. *J Air Waste Manag Assoc* 1999, **49**, 554-561.
7. Herr CE, Zur NA, Jankofsky M, Stilianakis NI, Boedeker RH, Eikmann TF: Effects of bioaerosol polluted outdoor air on airways of residents: a cross sectional study. *Occup Environ Med* 2003, **60**, 336-342.
8. Hines CJ, Milton DK, Larsson L, Petersen MR, Fisk WJ, Mendell MJ: Characterization and variability of endotoxin and 3-hydroxy fatty acids in an office building during a particle intervention study. *Indoor Air* 2000, **10**, 2-12.
9. Hryhorczuk D, Curtis L, Scheff P, Chung J, Rizzo M, Lewis C, Keys N, Moomey M: Bioaersol emmissions from a suburban yard waste compostingfacility. *Ann Agric Environ Med* 2001, **8**, 177-185.
10. Kateman E, Heederik D, Pal TM, Smeets M, Smid T, Spitteler M: Relationship of airborne microorganisms with lung function and leucocyte levels of workers with a history of humidifier fever. *Scand J Work Environ Health* 1990, **16**, 428-433.
11. Laitinen S, Kangas J, Kotimaa M, Liesivuori J, Martikainen PJ, Nevalainen A, Sarantila R, Husman K: Workers' exposure to airborne bacteria and endotoxins at industrial wastewater treatment plants. *Am Ind Hyg Assoc J* 1994, **55**, 1055-1060.
12. Long CM, Suh HH, Kobzik L, Catalano PJ, Ning YY, Koutrakis P: A pilot investigation of the relative toxicity of indoor and outdoor fine particles: in vitro effects of endotoxin and other particulate properties. *Environ Health Perspect* 2001, **109**, 1019-1026.
13. Madsen AM: NAGase activity in airborne biomass dust and relationship between NAGase concentrations and fungal spores. *Aerobiologia* 2003, **19**, 97-105.
14. Madsen AM, Kruse P, Schneider T: Characterization of microbial particle release from biomass and building material surfaces for inhalation exposure risk assessment. *Ann Occup Hyg* 2005, **50**, 175-187.
15. Madsen AM, Mårtensson L, Schneider T, Larsson L: Microbial dustiness and particle release of different biofuels. *Ann Occup Hyg* 2004, **48**, 327-338.
16. Monn C, Koren HS: Bioaerosols in ambient air particulates: a review and research needs. *Rev Environ Health* 1999, **14**, 79-89.
17. Monso E, Magarolas R, Badorrey I, Radon K, Nowak D, Morera J: Occupational asthma in greenhouse flower and ornamental plant growers. *Am J Respir Crit Care Med* 2002, **165**, 954-960.
18. Mukherjee AK, Chattopadhyay BP, Bhattacharya SK, Saiyed HN: Airborne endotoxin and its relationship to pulmonary function among workers in an Indian jute mill. *Arch Environ Health* 2004, **59**, 202-208.
19. Nielsen BH, Nielsen EM, Breum NO: Seasonal variation in bioaerosol exposure during bio-waste collection and measurements of leaked percolate. *Waste Manage Res* 2000, **18**, 64-72.
20. Okten SS, Asan A, Tungan Y, Ture M: Airborne fungal concentrations in east patch of Edirne city (Turkey) in autumn using two sampling methods. http://www.trakya.edu.tr/Enstituler/FenBilimeri/fenbilder/index.php 2005.
21. Oppliger A, Rusca S, Charriére, Duc TV, Droz P: Assessment of bioaerosols and inhalable dust exposure in Swiss sawmills. *Ann Occup Hyg* 2005, **49**, 385-391.
22. Pacheco KA, McCammon C, Liu AH Thorne PS, O'Neill ME, Martyny J, Newman LS, Hamman RF, Rose CS: Airborne endotoxin predicts symptoms in non-mause-sensitized technicians and research scientists exposed to laboratory mice. *Am J Respir Crit Care Med* 2003, **167**, 983-990.
23. Park JH, Spiegelman DL, Burge HA, Gold DR, Chew GL, Milton DK: Longitudinal study of dust and airborne endotoxin in the home. *Environ Health Perspect* 2000, **108**, 1023-1028.
24. Passman FJ: Recovery of *Aspergillus fumigatus* aerospora from municipal sewage sludge composting operations in the state of Maine. *Mycopathologia* 1983, **83**, 41-51.
25. Pirie RS, Collie PM, Dixon PM, McGorum BC: Inhaled endotoxin and organic dust particulates have synergistic proinflammatory effects in equine heaves (organic dust-induced asthma). *Clin Exp Allergy* 2003, **33**, 676-683.
26. Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham K, Palmgren U, Nowak D: Air contaminants in different European farming environments. *Ann Agric Environ Med* 2002, **9**, 41-48.
27. Radon K, Weber C, Iversen M, Danuser B, Pedersen S, Nowak D: Exposure assessment and lung function in pig and poultry farmers. *Occup Environ Med* 2001, **58**, 405-410.
28. Schiffman SS, Studwell CE, Landerman LR, Berman K, Sundy JS: Symptomatic effects of exposure to diluted air sampled from a swine confinement atmosphere on healthy human subjects. *Environ Health Perspect* 2005, **113**, 567-576.

29. Shelton B, Kirkland KH, Flanders WD, Morris GK: Profiles of airborne fungi in buildings and outdoor environments in the United States. *Appl Environ Microbiol* 2002, **68**, 1743-1753.

30. Smit LAM, Spann S, Heederik D: Endotoxin exposure and symptoms in wastewater treatment workers. *Am J Ind Med* 2005, **48**, 30-39.

31. Su HJ, Chen HL, Huang CF, Lin CY, Li FC, Milton DK: Airborne fungi and endotoxin concentrations in different areas within textile plants in Taiwan: A 3-year study. *Environ Res* 2002, **89**, 58-65.

32. Thorn J: Seasonal variations in exposure to microbial cell wall components among household waste collectors. *Ann Occup Hyg* 2001, **45**, 153-156.

33. Williams LK, Ownby DR, Maliarik MJ, Johnson CC: The role of endotoxin and its receptors in allergic disease. *Ann Allergy Asthma Immunol* 2005, **94**, 323-332.

34. Wouters I, Hilhorst SKM, Kleppe P, Doekes, G, Douwes J, Peretz C, Heederik D: Upper airway inflammation and respiratory symptoms in domestic waste collectors. *Occup Environ Med* 2002, **59**, 106-112.