Environmental Research 145 (2016) 101–108

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Seasonal variation in airborne endotoxin levels in indoor environments with different micro-environmental factors in Seoul, South Korea

Sung Ho Hwang [a], Dong Jin Park [b], Wha Me Park [c], Dong Uk Park [d], Jae Kyoung Ahn [e], Chung Sik Yoon [f,*]

[a] National Cancer Control Institute, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang-si, Gyeonggi-do, South Korea
[b] Occupational Safety and Health Research, Ulsan, South Korea
[c] Institute of Environmental and Industrial Medicine, Hanyang University, Seoul, South Korea
[d] Department of Environmental Health, Korea National Open University, Seoul, South Korea
[e] Research Institute of Standards for Environmental Testing, Seoul, South Korea
[f] Institute of Health and Environment, School of Public Health, Seoul National University, Gwanak ,1 Gwanak-ro, Seoul, South Korea

ARTICLE INFO

*Article history:*
Received 26 August 2015
Received in revised form
18 November 2015
Accepted 20 November 2015
Available online 6 December 2015

*Keywords:*
Endotoxin
Microbial laboratory
Animal laboratory
Seasonal variation
Temperature
Illumination

ABSTRACT

This study evaluated the variation over a year in airborne endotoxin levels in the indoor environment of five university laboratories in Seoul, South Korea, and examined the micro-environmental factors that influenced endotoxin levels. These included temperature, relative humidity, $CO_2$, CO, illumination, and wind velocity. A total of 174 air samples were collected and analyzed using the kinetic limulus amebocyte lysate assay. Endotoxin levels ranged from $<0.001$ to 8.90 EU/m$^3$, with an overall geometric mean of 0.240 EU/m$^3$. Endotoxin levels showed significantly negative correlation with temperature ($r=-0.529$, $p<0.001$), $CO_2$ ($r=-0.213$, $p<0.001$) and illumination ($r=-0.538$, $p<0.001$). Endotoxin levels tended to be higher in winter. Endotoxin levels in laboratories with rabbits were significantly higher than those of laboratories with mice. Multivariate regression analysis showed that the environmental factors affecting endotoxin levels were temperature (coefficient $=-0.388$, $p<0.001$) and illumination (coefficient $=-0.370$, $p<0.001$). Strategies aimed at reducing airborne endotoxin levels in the indoor environments may be most effective if they focus on illumination.



## 1. Introduction

Endotoxins, which are lipopolysaccharide (LPS), are ubiquitous in the environment and are an important structural component of the outer membrane of gram-negative bacteria (Beutler and Rietschel, 2003). Exposure to endotoxins was found to cause and exacerbate asthma and wheezing in both children and adults (Thorne et al., 2005; Abbing-Karahagopian et al., 2012). Endotoxin is also implicated in the impairment of lung function (Rabinovitch et al., 2005; Liebers et al., 2008) and the pathogenesis of pulmonary diseases (Loh et al., 2006) and acute lung injury (Thorn, 2001). Studies on healthy human subjects have shown an acute dose-related inflammatory response to inhaled LPS, with a rise in the blood levels of cytokines such as neutrophils, tumor necrosis factor-alpha, and interleukin-6 (Alexis et al., 2004; Doyen et al., 2012).

A large number of laboratory workers handle potentially dangerous biological materials as part of their daily routine. In Korea, about 165,000 students handle microorganisms in university laboratories facilities, and about 82,000 graduate students are likely to be exposed to pathogens. Exposure to endotoxins primes target cells and enhances the inflammatory response to secondary stimulus from other pollutants (Alexis et al., 2004). Despite its important health effects limited studies have reported the level of airborne endotoxins (Mueller-Anneling et al., 2004; Nilsson et al., 2011; Cheng et al., 2012). Notably, airborne endotoxin was found to be associated with respiratory symptoms to mice in nonmouse-sensitized scientists and technicians (Pacheco et al., 2003).

Therefore, indoor air quality in university laboratories is of considerable importance in terms of the health of laboratory workers. Although there are reports of airborne endotoxin levels in homes, saw mills, and other workplaces (Gereda et al., 2001; Rusca et al., 2008; Madsen et al., 2009; Garcia et al., 2013), few studies have examined endotoxin levels in university laboratories (Pacheco et al., 2006; Hwang et al., 2011). In addition, various factors have been reported to be correlated to airborne endotoxin

* Correspondence to: Occupational & Environmental Health Lab, Department of Environmental Health, School of Public Health, Seoul National University, Gwanak, 1 Gwanak-ro, Seoul 151-742, South Korea.
*E-mail address:* csyoon@snu.ac.kr (C.S. Yoon).

http://dx.doi.org/10.1016/j.envres.2015.11.025
0013-9351/

levels, such as building conditions (e.g., the presence of animals and cooling systems) (Gereda et al., 2001), sensitization to allergens in children (Gehring et al., 2002), season (Carty et al., 2003), allergens in mice facilities (Pacheco et al., 2006), children with asthma (Lai et al., 2015), and symptoms related to work (Rusca et al., 2008). However, there has been little discussion about airborne endotoxin levels in indoor laboratories; in particular, studies involving continuous monitoring over the course of an entire year are scarce (Tager et al., 2010).

The aims of this study were to assess airborne endotoxin levels over the course of a year in five university laboratories in Seoul, including animal laboratories, and to identify the environmental factors that influence the endotoxin levels, such as temperature, relative humidity, $CO_2$, CO, illumination, and wind velocity.

## 2. Materials and methods

### 2.1. Sampling and analysis

In the spring, summer, autumn, and winter (from February 2011 to January 2012), we collected samples from three microbial (A, B, and C) and two animal laboratories (D and E) of similar height (Table 1). Laboratories A and B conducted work on producing amino acids using microorganisms, while the work in laboratory C focused on waste water purification using microorganisms. The main tasks of laboratories D and E were feeding and weighing of mice and rabbits (Table 1).

The frequency of experiment was low when we visited the laboratories. Since people in the laboratory were usually standing or walking for their work and the number of people changed monthly, we could obtain an average of approximately three samples per month in each laboratory. For laboratories A, B, and C, which had a separate study room, the samples were collected from the center of the laboratory.

A total of 174 air samples were collected on regular working days by month and the samples were taken from 100 to 150 cm above the floor. The outdoor levels of endotoxin (36 samples) were also measured on the rooftop of the same building on the campus. During the endotoxin sampling, temperature, relative humidity, $CO_2$, CO, illumination and wind velocity were recorded from each spot using a VelociCalc® air velocity meter model 9555 series (TSI Inc., USA) and meter model IM-2D (Topcon Inc., Japan) to measure illumination. Samples were collected using an air sampler (17G9 GilAir Sampler, Gilian Product Sensidyne, Inc., U.S.A.) onto glass fiber filters (diameter, 37 mm; SKC Inc., USA) at a flow rate of 2.0 L/min ($\pm$5%) for an average of 6 h using an Escort Elf® Pump (Mine Safety Appliances Company, Pennsylvania, USA). The samples were stored at $4 \pm 2$ °C, sent to an analytical laboratory within a week of sampling, and analyzed immediately upon arrival. Detection and quantitation of endotoxin concentrations were analyzed by the kinetic-turbidimetric *Limulus Amebocyte Lysate* (LAL) assay (Associations of Cape Cod, Inc., USA). The entire endotoxin extraction procedure was conducted at room temperature ($25 \pm 2$ °C). An extraction volume of 15 ml of pyrogen free water was added to a test tube, which was then capped and sonicated at a minimum peak frequency of 48 kHz for 1 h (ASTM, 2007). After that, samples were centrifuged at 1000*g* for 15 min and the supernatant was transferred to a pyrogen free test tube. 100μl of each sample was distributed into a pyrogen-free 96 well microplate and incubated at 37 °C for 10 min in an automated microplate reader (Bio Tek ELx 808, Bio Tek Instruments, USA). 100μl of LAL reagent was added to each well and analyzed duplicated at 340 nm using WinKQCL Software (BioWhittaker, Cambrex Co., USA). The Escherichia coli O55:B5 control standard endotoxin (Lonza, USA) was utilized to draw standard curve ranging from 0.005 to 50 endotoxin unit/ml. Only the calibration curve grater WinKQCL of equal to 0.98 was accepted for the further analysis. The positive product control(PPC) recoveries should be within the range of 50–200% and the coefficients of variation (CV) should be less than 10% to be valid. The endotoxin concentrations were expressed as endotoxin unit per cubic meter of air (EU/$m^3$). The assay limit of detection (LOD) was 0.01 EU/mL extract. Values below the LOD were assigned a value of LOD/$\sqrt{2}$ (Hornung and Reed, 1990).

### 2.2. Statistical analyses

All statistical analyses were conducted using SPSS (version 23.0; IBM Inc., USA). Non-parametric statistics were used to test for significant differences in the endotoxin levels in the laboratories and to determine seasonal differences in endotoxin levels because the endotoxin levels were rightly skewed but not followed log-normal distribution. Analyses of variance (ANOVAs) were used to examine the significant differences between the endotoxin levels and seasonal variations. Two groups were defined for each factor (sampling site and illumination) using a *t*-test, as follows: For sampling site, “laboratory room” and “study room” for laboratories A, B, and C and “near the animal cages” and “center of the laboratory” for laboratories D and E; for illumination, $>400$ lx and $<400$ lx for laboratories A–E. Spearman's correlation analyses were used to assess the relationship between endotoxin levels and the micro-environmental factors. Finally, univariate and multiple linear regression mixed effect analysis was performed to determine the environmental factors that affected endotoxin levels the most. The variables with the highest *p*-values above 0.05 were eliminated one at a time, and then the model was refitted until all included variables had *p*-values of 0.01 or less.

## 3. Results

Table 2 shows the mean endotoxin levels and the micro-environmental factors measured. The endotoxin levels of the five

**Table 1**
Characteristics of investigated laboratories in university facilities.

| Lab | Type of Laboratory (floor) | Ventilation system | Area × height ($m^3$) | Structure of laboratory | Experiment |
|---|---|---|---|---|---|
| A | Microbial (1) | Window | 146 × 2.6 | Separate between laboratory and study room | Production of amino-acid with microorganism |
| B | Microbial (6) | Window | 151 × 2.6 | Separate between laboratory and study room | Production of amino-acid with microorganism |
| C | Microbial (5) | Window | 151 × 2.6 | Separate between laboratory and study room | Waste purification with microorganism |
| D | Animal mice (4) | HVAC[a] | 65 × 2.5 | Laboratory only | Weighing, feeding, and cutting fur of mice |
| E | Animal rabbit (5) | Window | 20 × 2.5 | Laboratory only | Feeding rabbit |

[a] Heating, ventilation, and air-conditioning (HVAC).