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Modeling human health risks of airborne endotoxin in homes during the winter and summer seasons

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ABSTRACT

Endotoxin, a component of gram-negative bacterial cell walls, is a pro-inflammatory agent that induces local and systemic inflammatory responses in normal subjects which can contribute to the risk of developing asthma and chronic obstructive lung diseases. A probabilistic approach linking models of exposure, internal dosimetry, and health effects was carried out to quantitatively assess the potential inhalation risk of airborne endotoxin in homes during the winter and summer seasons. Combining empirical data and modeling results, we show that the half-maximum effect of the endotoxin dose (ED50) was estimated to be 707.9 (95% confidence interval (CI): 308.8–1287.0) endotoxin units (EU) for body temperature change, 481.8 (95% CI: 333.2–630.3) EU for elevation of neutrophils, and 1174.5 (95% CI: 816.0–1532.9) EU for elevation of the cytokine, interleukin-6. Our study also suggests that airborne endotoxin in homes may pose potential risks, and a higher risk for elevation of neutrophils and cytokine interleukin-6 appeared in winter season than in summer. Our study offers a risk-management framework for discussion of future studies of human respiratory exposure to airborne endotoxin.

1. Introduction

Endotoxin is a component of the cell walls of gram-negative bacteria, and is known as lipopolysaccharide (LPS) in its pure form. The potent immune stimulatory capacity of endotoxin is mostly attributed to its lipid A moiety, which is highly conserved across different bacterial species (Thorn, 2001). Endotoxin is found in the air and house dust, and occurs as a contaminant of organic dusts and aerosols in the environment. Therefore, it is a ubiquitous toxin potentially capable of affecting large number of people. In humans, acute exposure to endotoxin induces blood and lung inflammatory responses in which neutrophils and macrophages are involved (Sandström et al., 1992; Michel et al., 1995), resulting in respiratory symptoms such as fever, shaking chills, and severe asthma (Rylander et al., 1989; Michel et al., 1991; Jagielo et al., 1996). Chronic exposure to endotoxin in the workplace such as agricultural settings, in which airborne endotoxin levels can be very high, was related to the risk of developing nonatopic chronic obstructive pulmonary diseases (Smid et al., 1992; Schwartz et al., 1995). In residential settings associated with lower levels than in occupational environments, there is also endotoxin contaminating house dust that could be an important determinant of asthma severity (Michel et al., 1996; Rizzo et al., 1997). Although some studies have suggested a protective role of endotoxin exposure in infancy, exposure to endotoxin in childhood and later in life appears to have a detrimental effect in both individuals with asthma and other respiratory conditions and in healthy volunteers (Michel et al. 1996; Douwes et al. 2002).

Exposure to high concentrations of LPS *in vivo* can cause catastrophic circulatory collapse and death, therefore, much effort has focused on the deciphering of the LPS signal transduction pathway to identify targets for sepsis therapies (Simpson and Martinez, 2010). Complex mechanisms of endotoxin which act in the human body are typically initiated by binding of LPS to CD14/TLR4/MD2 complex of receptors on macrophages, lymphocytes, or respiratory epithelial cells, resulting in triggering of various intracellular signaling pathways and induce cytokine release, leading to inflammatory reaction (Thorn and Rylander, 1998; Simpson and Martinez, 2010). The magnitude of the inflammatory response depends in part on the amount of endotoxin exposure. Small amounts of endotoxin may cause a local inflammatory response whereas large amounts may result in a massive release of cytokines into the systemic circulation, resulting in shock, disseminated intravascular coagulation and death.

Several immune or hematological parameters such as fever, fever index (FI), forced expiratory volume (FEV_1), cytokines, and polymorphonuclear neutrophil (PMN) have been frequently selected to describe human body responses to clinical endotoxemia (Burrell, 1994; Anderson et al., 2002). Fever is a very common response when endotoxin stimulates host cells to produce endogenous pyrogens (Brooks et al., 2002) that can affect hypothalamus which is the temperature-regulating portion of the brain. Cytokines are small secreted proteins which mediate and regulate immunity, inflammation, and hematopoiesis. They must be produced *de novo* in response

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to an immune stimulus. Endotoxin mediates cell activation of macrophages and activates the complement cascade. It acts as a physiological stimulus for the synthesis of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), interleukins: IL-1, IL-6, IL-8 and non-protein mediators, which in turn, are responsible for most pathophysiological consequences of a bacterial infection (Rietschel et al., 1996). Neutrophils are the most abundant type of white blood cells in mammals and play a significant role in the innate immune system. They form part of the polymorphonuclear cell family together with basophils and eosinophils (Nathan, 2006). An increase in neutrophils have been found both in the airways and blood indicative of a local and systemic inflammatory response after acute inhalation of LPS in humans (Thorn, 2001). Moreover, studies showed that inhalation of endotoxin caused mainly a neutrophil-dominated inflammatory response and the blood PMNs count has been noticed as one of the most sensitive indicators of LPS-induced inflammation (Michel et al., 1997; Thorn, 2001).

The high health risk associated with the inhalation of airborne particles has been recognized and documented (Pope et al., 1995; Brown et al., 2002). Epidemiologic investigations showed that particulate matter (PM) in ambient air contributes to the progression of respiratory diseases such as asthma, and leads to an increase in morbidity and mortality from respiratory and cardiac conditions (Fairley 1999; Ostro et al., 2000; Pope, 2000; Samet et al., 2000; Dockery, 2001). In addition, inhalation of airborne particles has been associated with adverse effects on childhood lung function growth, which theoretically could increase lifetime risk for chronic respiratory disorders (Jedrychowski et al., 1999; Gauderman et al., 2002). Several studies have demonstrated that environmental PM which reaches the lungs is phagocytosed by lung macrophages, releasing cytokines, reactive oxygen intermediates, and other inflammatory mediators (Kobzik, 1995; Becker et al., 1996; Ning et al., 2000). Endotoxin is constituent of PM that thought to play a major role in stimulating the release of inflammatory mediators (Becker et al., 1996; Bonner et al., 1998; Ning et al., 2000). Furthermore, exposure to endotoxin may prime macrophages resulting in a more vigorous inflammatory response upon exposure to other anthropogenic components of PM (Imrich et al., 1999; Elder et al., 2000; Long et al., 2001). It is possible that the effect of concurrent exposure to endotoxin and PM could be especially deleterious in patients with preexisting lung inflammation due to infection, chronic bronchitis or other inflammatory lung diseases such as asthma.

There is a growing recognition that health risks associated with airborne particles are influenced by size. The relation between the concentrations and characteristics of airborne particles and the resultant toxic doses and potential hazards after their inhalation depends greatly on their patterns of deposition and the rates and pathways for their clearance from the deposition sites (Lippmann et al., 1980). The distribution of the deposition sites of inhaled particles is strongly dependent on their aerodynamic diameters (Lippmann et al., 1980). Therefore, establishing an approximate aerodynamic particle size distribution for airborne endotoxin is an important factor in determining endotoxin toxicity and its health effects.

There are increasing reports in the literature on endotoxin in domestic environments, where there is an increase in respiratory diseases (Michel, 2000; Gehring et al., 2001; Heinrich et al., 2001). Nevertheless, accumulating evidence from homes suggests that certain levels of endotoxin are potentially detrimental to respiratory health (Park et al., 2000; Kujundzic et al., 2006; Rennie, et al., 2008). Although airborne endotoxin may be more representative of the true exposure (Dassonville et al., 2008), studies of airborne endotoxin levels in homes are less frequent than those on house dust levels. In this context and given the potential health risks posed by airborne endotoxin in homes, the purpose of this study was to use a probabilistic approach to quantitatively assess the potential inhalation risk of airborne endotoxin in homes during the winter and summer seasons. Additionally, uncertainties resulting from the assessment were addressed.

2. Materials and methods

The probabilistic risk assessment framework in the present study was divided into four phases as shown in Fig. 1 and described in detail in subsequent sections.

2.1. Problem formulation

There are relatively few empirical data regarding airborne endotoxin concentrations (Park et al., 2000; Kujundzic et al., 2006; Dassonville et al., 2008; Rennie, et al., 2008) and particle size distribution (Kujundzic et al., 2006) in homes. Therefore, we must rely on a data reanalysis technique together with whatever empirical data are available. In the present study, airborne endotoxin concentrations and particle size distributions in homes were obtained from published literature where available (Kujundzic et al., 2006). Our study focused on seasonal variations in indoor endotoxin exposure in homes, including winter and summer seasons. The major database of adult human subjects exposed to various endotoxin concentrations was adopted from Suffredini et al. (1999). Information on particle size distributions and concentrations of airborne endotoxin was reanalyzed from the published data (Kujundzic et al., 2006). The conversion from nanograms (ng) to endotoxin units (EU) was based on the US FDA's sub lot of the International standard, EC-6, that was assigned a potency of 10 EU ng^{-1} (Malyala and Singh, 2008). Table 1 summarizes the calculated size range-specific percentages of particles and mass concentrations for particulate matter (PM)-bound endotoxin in the present study. Kolmogorov–Smirnov test (K–S test) was used as a goodness-of-fit test of probability distribution by using the TableCurve 2D 5.01 (AISN Software, Mapleton, OR, USA).

2.2. Exposure assessment

Data used for exposure assessment in this study was adopted from Kujundzic et al. (2006). In their study, total airborne bacteria, endotoxin concentrations, and bioaerosol size distribution at homes during the winter and summer seasons were measured (Kujundzic et al., 2006). Hence, we obtained the size-specific endotoxin concentrations (denoted as C_i) by incorporating total measured endotoxin concentration and particle size distribution (0.056–3.2 μm). Moreover, we took into account the bioavailability of human exposure, thus the size-specific endotoxin concentrations were incorporated into the exposure model to calculate the human internal exposure dose (denoted as D).

To obtain the actual internal doses of PM-bound endotoxin through the inhalation pathway, the multiple-path particle dosimetry (MPPD) (CIIT Centers for Health Research, 2006) and exposure (modified from Chio et al. (2007)) models were applied. First, the MPPD model was applied to estimate the deposition fraction (d_F) of various particle sizes inhaled into the different lung regions. The size-dependent deposition fraction (d_F) is the model output using a polydispersive condition of the environmental setting with a particle size range of 0.01–10 μm as the major model input. For the output of the MPPD model, the human lung was divided into three major regions: the head, tracheobronchial (TB), and pulmonary (P) regions. The summation value of the deposition fractions in the head, TB, and P regions is presented as the total. Parameters of lung morphometry, and breathing parameters and times were input into the MPPD model; otherwise we used default values (CIIT Centers for Health Research, 2006). The model was set for the human scenario, and the default value of the number of segments of the human lung was 24. The other default parameters for lung morphometry such as total lung capacity (TLC), functional residual capacity (FRC), and upper respiratory tract (URT) volume were set to 5564, 3300, and 50 ml, respectively. The breathing frequency was 12 min^{-1}, and the

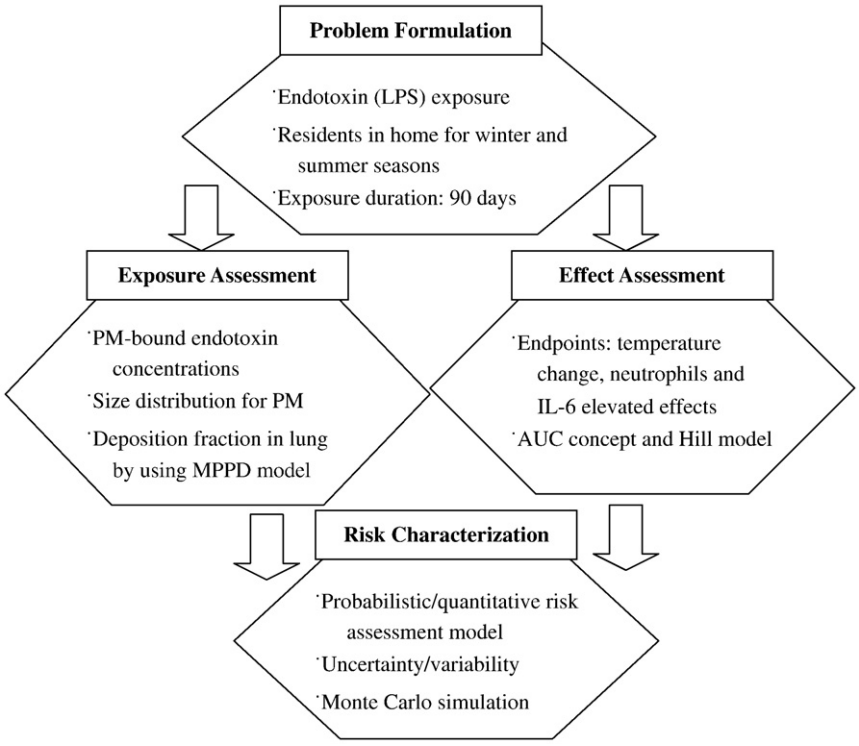

Fig. 1. Proposed probabilistic risk assessment framework to assess airborne endotoxin health risk. Modified from USEPA, 1992.

tidal volume and nasopharyngeal dead space were 625 and 50 ml, respectively (Table 2). Second, we reconstructed the mass-basis dosimetric exposure model using the following equation:

$$D = \left(\sum_{i=1}^{m} C_i \times d_{F,i}\right) \times AB_d \times ET, \quad (1)$$

where D is the mass-based cumulative dose of inhaled endotoxin (EU), m is the total stage number of the size distribution, C_i is the i-stage mass concentration of PM-bound endotoxin ($EU\,m^{-3}$), AB_d is the daily time spent-specific amount of air breathed ($m^3 d^{-1}$), $d_{F,\,i}$ is the i-stage deposition fraction deposited in different human lung regions according to the MPPD model, and ET is the exposure time (d). We treated the PM-bound endotoxin concentration, C, and AB_d ($13.80 \pm 1.71\,m^3\,d^{-1}$) (Chio et al., 2007) probabilistically, and they both had log-normal (LN) distributions. Hence, the internal cumulative dose D could be considered as log-normal distribution. We considered each winter or summer season to consist of 90 days; thus, the deterministic parameter of ET in the model was set to 90 days (Table 2). Parameters used in the MPPD and exposure models are summarized in Table 2.

2.3. Effect assessment

The administration of reference endotoxin to humans is an important means to study inflammation *in vivo*. Endotoxin sensitivity in healthy subjects can be defined in two ways: the systemic response to temperature increase and the local response to change in airway responsiveness. Both types of effects are associated with inflammation in their respective compartments (Michel et al., 2001). *In vivo*

Table 1
Size range-specific percentage of particles and mass concentrations for particulate matter-bound endotoxin.

Particle size (μm)	Endotoxin concentration ($EU\,m^{-3}$)[a]		Particle size fraction[b]	
	Winter	Summer	Winter	Summer
0.056–0.1	1.14 (1.02)	0.48 (0.37)	0.14	0.11
0.1–0.18	1.65 (0.78)	1.17 (1.73)	0.20	0.26
0.18–0.32	1.65 (2.38)	0.34 (0.38)	0.20	0.07
0.32–0.56	0.92 (0.86)	0.23 (0.31)	0.11	0.05
0.56–1	0.62 (0.56)	0.35 (0.31)	0.07	0.08
1–1.8	1.60 (1.32)	1.01 (0.78)	0.19	0.23
1.8–3.2	0.85 (0.94)	0.87 (0.76)	0.10	0.20
Total	8.43 (2.23)	4.45 (3.32)	1.00	1.00

[a] Data acquired from Figs. 5 and 6 and Table 5 in Kujundzic et al (2006). Data performed as mean (standard deviation) values. EU, endotoxin units.
[b] Particle size fraction was estimated by the mean concentration in each size range divided by the corresponding "Total" value.

Table 2
Parameters used in the multiple-path particle dosimetry (MPPD) and exposure models.

Model parameter	Value
MPPD model	
1. Lung morphometry	
Number of segments	24 (default)
Total lung capacity (TLC)	5564 ml (default)
Functional residual capacity (FRC)	3300 ml (default)
Upper respiratory tract (URT) volume	50 ml (default)
2. Breathing parameters and times	
Breathing frequency	12 min^{-1} (default)
Tidal volume	625 ml (default)
Nasopharyngeal dead space	50 ml (default)
3. Particle size range	0.01–10 μm
Exposure model	
Daily time spent-specific air breathing rate (AB_d)	$13.80 \pm 1.71\,m^3\,d^{-1}$[a]
Exposure time (ET)	90 days[b]

[a] We only take into account the exposure group indoors with residents in a home setting (Chio et al., 2007).
[b] We considered that each winter or summer season consisted of 90 days.

studies based on dose–response data are used to describe relationships of the clinical effects and inflammatory responses to different doses of endotoxin in healthy human subjects (Suffredini et al., 1999). These dose–response relationships would provide significant references for mathematical modeling in an attempt to assess human health risk from endotoxin exposure. Studies showed that the intravenous administration of endotoxin resulted in dose-related increases in symptoms, temperature, and acute-phase reactants including neutrophils and the cytokine, interleukin (IL)-6 (Suffredini et al., 1999). Therefore, in the present study, the clinical response (body temperature) and the inflammatory responses (neutrophil and IL-6 levels) were selected as endpoints for the endotoxin responses because (i) fever was a very common response when endotoxin stimulated host cells to produce endogenous pyrogens that can affect the hypothalamus (Burrell, 1994); (ii) inhaled endotoxin was shown to mainly cause a neutrophil-dominated inflammatory response (Thorn, 2001); and (iii) IL-6 was also identified as a key cytokine affected by endotoxin exposure (Husain et al., 2003).

Dose–response data from Suffredini et al. (1999) allowed us to examine the relationships among endotoxin cumulative dose, clinical response (body temperature) and the inflammatory responses (neutrophil and IL-6 levels). Yet there were several steps to reconstruct the dose–response profiles for endotoxin exposures. First, we selected a mass-based dose metric for endotoxin exposure and adjusted the dose unit as endotoxin unit (EU). Second, we incorporated the concept of area under curve (AUC) into the selected responses (temperature change, neutrophil count, and IL-6 level) because they showed a time-dependent fashion (Suffredini et al., 1999). The transformation algorithms of body temperature change were described in the followings as example, and responses for neutrophil count and IL-6 level were also transformed. Here, the area under temperature change induced by endotoxin exposure ($T_{\text{Endotoxin}}$) versus time curve was calculated as

$$\int_0^t T_{\text{Endotoxin}}(t)dt = AUC_{\text{Endotoxin}}. \tag{2}$$

The time-dependent area under temperature change curve for placebo scenario (T_{Placebo}) was written as

$$\int_0^t T_{\text{Placebo}}(t)dt = AUC_{\text{Placebo}}. \tag{3}$$

Then, the relative elevated times based on AUC concept would be obtained as

$$\frac{AUC_{\text{Endotoxin}} - AUC_{\text{Placebo}}}{AUC_{\text{Placebo}}} = AUC_{\text{Foldchange}}. \tag{4}$$

In this study, the AUC_{Placebo} was used as a reference value to calculate the AUC's fold change of body temperature. Subsequently, we used the Hill model (described below) to fit and reconstruct a dose–response curve based on the $AUC_{\text{Foldchange}}$ of body temperature. Furthermore, we selected a change in body temperature of 0.8 °C as the critical endotoxin concentration with a potential risk of endotoxin exposure as it is regarded as having a fever for adults. Hence we transformed the temperature change effect as the difference of temperature (°C) instead of fold change (dimensionless) by using inverse algorithm. For the relative effect change of neutrophil and IL-6 levels, we transformed them as following equations,

$$E = \frac{E(D) - E(0)}{E(0)}, \tag{5}$$

where $E(D)$ and $E(0)$ represent the effect at dose D and zero dose of endotoxin exposure, respectively. Here the E is the fold change of effect compared to the control group.

To obtain dose–response curves, a three-parameter Hill equation model (Hill, 1910) was used to fit the published data (Suffredini et al., 1999) to reconstruct dose–response profiles by taking into account the effects of endotoxin, including ED50 and $E_{\max}$ estimations. The Hill equation model could be written as

$$E = \frac{E_{\max}}{(1 + (ED50/D)^n)}, \tag{6}$$

where D is the cumulative endotoxin dose (EU), $E_{\max}$ is the maximum dose effect, ED50 is the specific dose that causes an equal effect of half that of the $E_{\max}$, and n is a slope factor referred to as the Hill coefficient which determines the overall shape of the curve. The Hill coefficient is a measure of cooperativity of the ligand binding to the enzyme or receptor. A coefficient of 1 indicates completely independent binding, regardless of how many additional ligands are already bound. Numbers >1 indicate positive cooperativity, while numbers <1 indicate negative cooperativity.

2.4. Risk characterization

Risk characterization is the phase of risk assessment where the results of the exposure and quantitative effect assessments are integrated to provide an estimate that quantifies the magnitude of individual risks. In the present study, it entailed combining the exposures, measured as the endotoxin dose in the human lung pulmonary region with the quantitative dose–response relationship between endotoxin doses and associated clinical and inflammatory mediator responses determined from experimental studies. This results in a joint probability function (JPF) or an exceedance risk (ER) profile, which describes the probability of exceeding the concentration associated with a particular degree of effect. A graphical display of the JPF also provides a means of assessing how alterations in ambient concentrations of endotoxin affect the risk assessment. This can be expressed mathematically as a probabilistic risk profile as:

$$R(D) = P(D) \times P(E|D), \tag{7}$$

where $R(D)$ is the risk at a specific dose, D, $P(D)$ is the probability of having an internal tissue dose, D, and $P(E|D)$ is the conditional probability of an adverse effect, given the internal dose, D, in a specific target tissue. Here, $P(E|D)$ can be considered as Hill-based or sigmoid dose–response functions, and $R(D)$ is 1-CDF distributions, where CDF is cumulative distribution function.

2.5. Uncertainty analysis

Uncertainty is a key component in risk assessment. Uncertainty arises from estimations of both the exposure and effects. We recognized the inherent problem of uncertainty and variability of the data sources. Airborne endotoxin levels in homes are compounded by numerous variables, such as the season of sampling, number of inhabitants, presence of pets, humidity, and temperature (Braun-Fahrländer et al., 2002; Dassonville et al., 2008). Moreover, discrepancies in extraction and analysis of endotoxin samples also contributed to the measured endotoxin concentration. There is also considerable inter-individual variability in the amplitude of both the clinical and inflammatory responses to purified LPS by inhalation (Castellan et al., 1987) or by intravenous administration (Michie et al., 1988). Additionally, the LPS-induced lung function response may be modulated by environmental factors such as virus, ozone, and smoking (Wardlaw, 1993).

In order to quantify the uncertainty and its impact on the risk estimates, a Monte Carlo (MC) simulation that includes input distributions for the parameters of the derived dose–response function as well as for estimated exposure parameters was performed. Ten thousand MC simulations were performed, and the 95% confidence interval (CI) for

the expected risk was determined on the basis of the 2.5th and 97.5th quantiles of the simulation results. A risk curve was generated from the cumulative distribution of the simulation outcomes. The statistical analyses and simulations were implemented using Crystal Ball software (Vers. 2000.2, Professional Edition, Decisionerring, Denver, CO, USA).

3. Results

3.1. Exposure assessment

Box plots of the interquartile and median value predictions associated with whisker plots indicating the 2.5th- and 97.5th percentile predictions of endotoxin concentrations in homes during the summer and winter are shown in Fig. 2. The results showed that the median airborne endotoxin level in winter (8.6 EU m^{-3}, 95% CI: 3.3–14.0 EU m^{-3}) appeared to be slightly higher than that in summer (6.7 EU m^{-3}, 95% CI: 0.9–12.5 EU m^{-3}). Differences in the endotoxin level may have been due to environmental factors such as temperature and humidity.

Establishing an approximate aerodynamic particle size distribution for airborne endotoxin is an important factor in determining endotoxin toxicity and its health effects; in the present study, Fig. 3 shows the cumulative fraction of the PM-bound endotoxin size distribution in the range 0.056–3.2 μm measured in homes during the winter and summer seasons (Kujundzic et al., 2006). This indicates that a particle diameter of <1 μm had a greater deposition fraction in the alveolar region of the lung (Asgharian et al., 2001). Fig. 3 shows that the cumulative probabilities of PM-bound endotoxin of <1 μm diameter were 0.71 and 0.58 for winter and summer, respectively.

Predicting the amount of particles deposited in the human lung following exposure to airborne particulate matter is the first step toward evaluating the risks associated with exposure to airborne pollutants. The major route of entry into the body of airborne endotoxin is inhalation, causing deposition and accumulation in the human respiratory tract (HRT). To estimate the lung deposition of particulate endotoxin, the MPPD (CIIT Centers for Health Research, 2006) and mass-basis dosimetric exposure models were employed. We first reanalyzed published data of airborne endotoxin measurements in homes (Kujundzic et al., 2006) and then incorporated the MPPD model to estimate the endotoxin doses in the pulmonary region. Fig. 4 shows the probability profiles for the predicted endotoxin doses in the pulmonary region in homes during the winter and summer seasons. The results indicated a higher endotoxin dose distribution of LN (1309.74 EU, 1.47) during the winter season in homes, whereas during summer season, an endotoxin dose distribution of LN (724.96 EU, 1.62) was estimated.

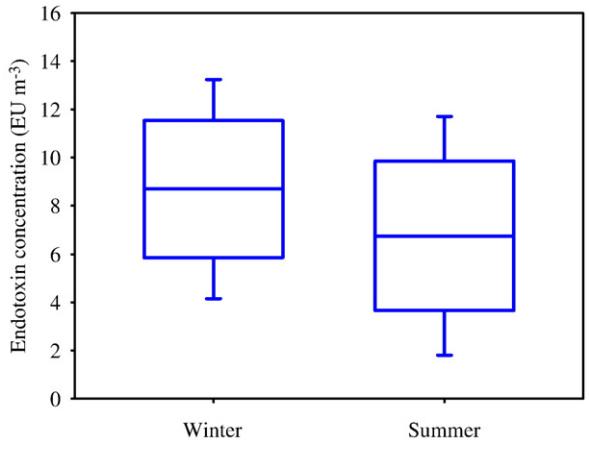

Fig. 2. Box and whisker diagrams of endotoxin exposure concentrations measured in home settings during the winter and summer seasons. The lower and upper boundaries of each box indicate the 25th and 75th percentiles, respectively. The line within the box indicates the median, and whiskers above and below the box indicate the 95th and 5th percentiles, respectively.

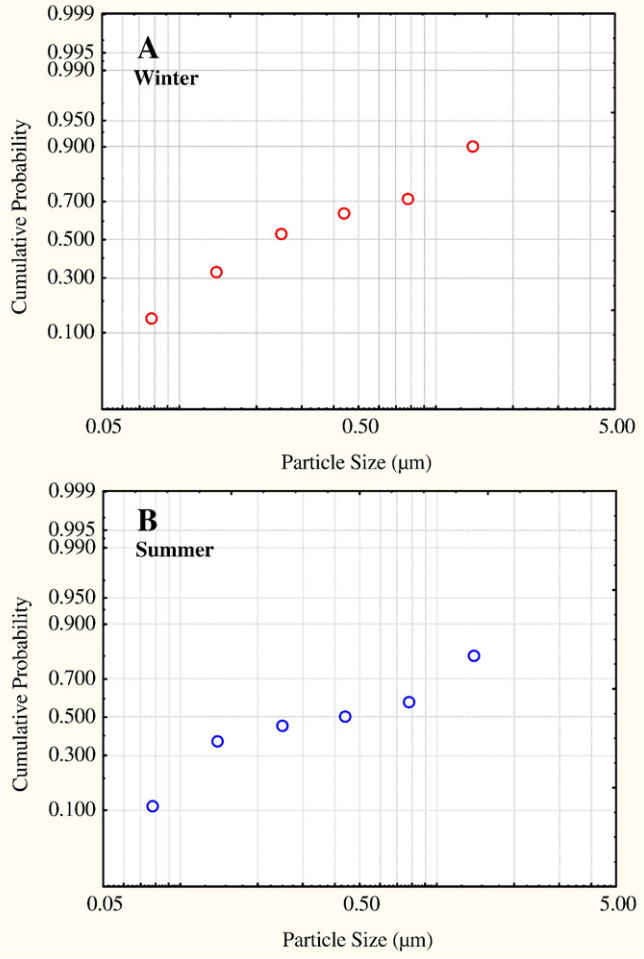

Fig. 3. Cumulative fraction of particulate matter-bound endotoxin size distribution measured in homes in (A) winter and (B) summer.

3.2. Effect assessment

The Hill model was employed to describe the dose–response profile based on data of the inflammatory effects of intravenous endotoxin in humans (Suffredini et al., 1999). The reconstructed dose–response profiles were implemented using the TableCurve 2D package for endotoxin on changes in body temperature ($r^2 = 0.89$) (Fig. 5A), absolute number of neutrophils ($r^2 = 0.98$) (Fig. 5B), and concentrations of IL-6 ($r^2 = 0.85$) (Fig. 5C). The median effective endotoxin doses (ED50) were estimated to be 707.9, 481.8, and 1174.5 EU for the elevated effect of body temperature changes (95% CI: 308.8–1287.0 EU), neutrophils (95% CI: 333.2–630.3 EU), and IL-6 (95% CI: 816.0–1532.9 EU), respectively. The Hill coefficients (n) were estimated to be 0.68 for the elevated effect for body temperature change, 1.23 for the elevated effect for neutrophils, and 1.87 for the elevated effect for IL-6.

3.3. Risk characterization for pulmonary deposition

Risk curves for endotoxin-induced clinical and inflammatory responses (body temperature change, absolute number of neutrophils, and concentration of IL-6) were generated to reveal the expected risk in

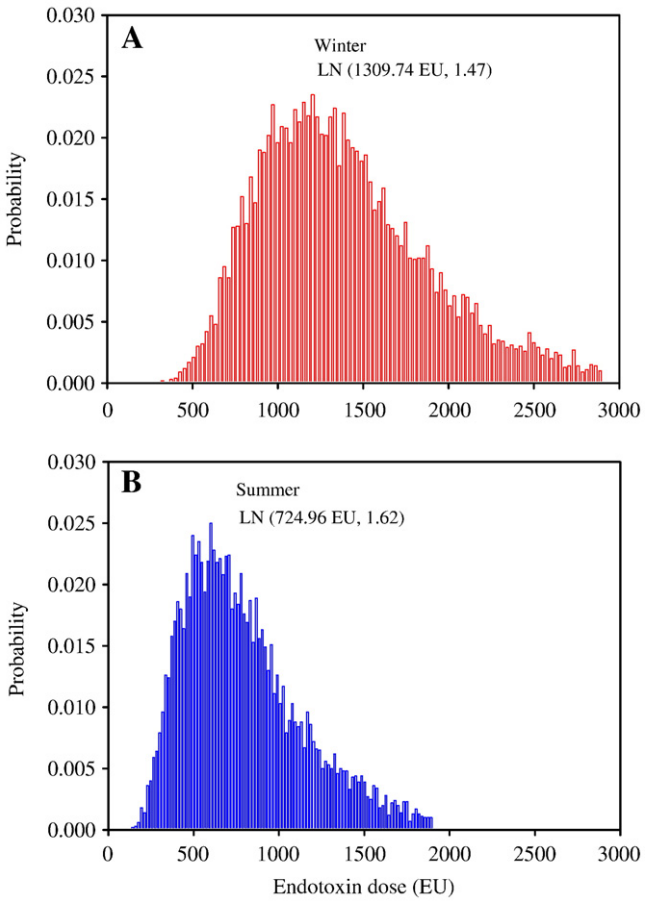

Fig. 4. Estimated endotoxin dose of the human pulmonary region in a home setting during (A) winter and (B) summer seasons. LN (gm, gsd) denotes a log-normal distribution with the geometric mean and standard deviation.

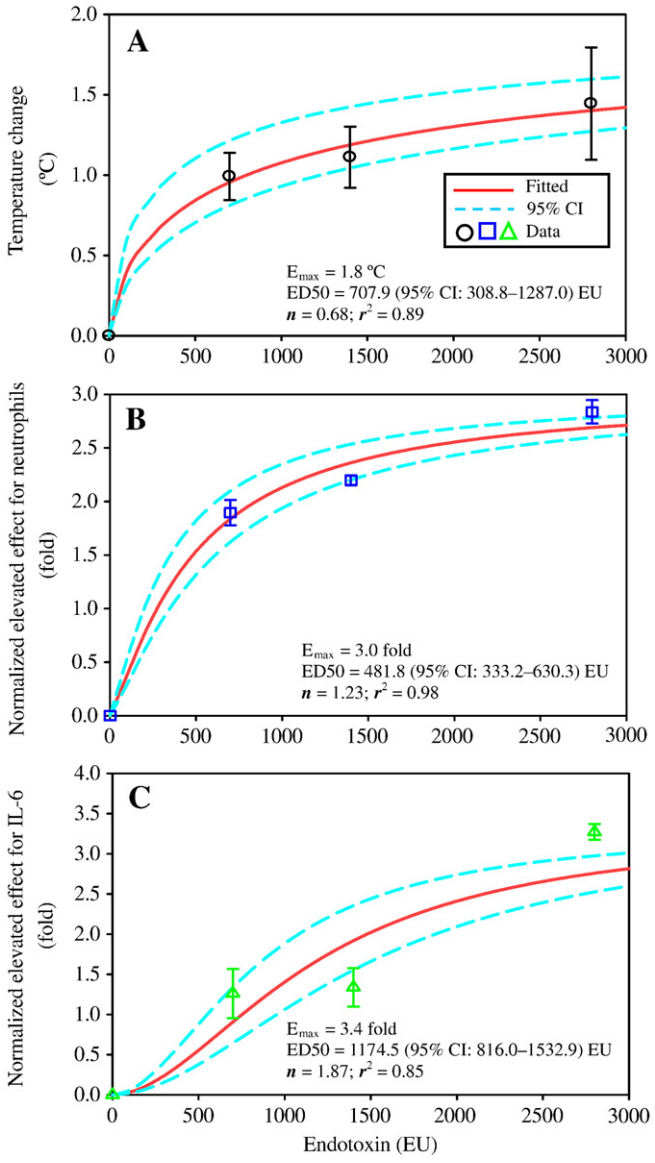

Fig. 5. Reconstructed dose–response profiles between the (A) temperature change, normalized elevated effects of (B) number of neutrophils, and (C) IL-6 levels after exposure to various airborne endotoxin levels, respectively.

the lung pulmonary region during the winter and summer seasons in homes (Fig. 6). The plotted probabilities, calculated from the outcome of the MC simulation followed a JPF shown by Eq. (7) describing the exceedance cumulative distribution functions (CDFs) associated with a dose–response relationship (Fig. 5), by taking into the account the uncertainty in estimating the risk (Fig. 6). Fig. 6A and B indicates that the human body temperature change was estimated to be 1.09 (95% CI: 0.94–1.34) °C compared to a normal body temperature in the winter season and 0.91 (95% CI: 0.76–1.18) °C in the summer season in homes at a 50% probability (exceedance risk (ER) = 50). Fig. 6C and D shows that neutrophil cell elevations were estimated to be 2.32 (95% CI: 2.16–2.50)- and 1.87 (95% CI: 1.65–2.13)-fold compared to the control in the winter and summer seasons, respectively, in homes at an ER50, whereas the ER50 values for IL-6 elevation effects were estimated to be 1.88 (95% CI: 1.50–2.34) and 0.98 (95% CI: 0.70–1.43) for the winter and summer seasons in homes, respectively (Fig. 6E, F).

Table 3 summarizes the exposure exceeding thresholds for the probabilities of clinical and inflammatory responses with selected endpoints (elevated temperature change, number of neutrophils, and IL-6 concentration) at risk levels of 0.1 and 0.5 for adults exposed to airborne endotoxin in homes during the winter and summer seasons. As the normal oral temperature for adults is approximately 36.7 (range, 36.4–37.2) °C, an increase in temperature to 37.5 °C is regarded as having a fever in adults. Therefore, we took a change in temperature of 0.8 °C as the critical endotoxin concentration (441 EU) with a potential risk of endotoxin exposure. Our analysis thus indirectly indicated that airborne endotoxin in homes may pose potential risks, whereas a higher risk for elevation of neutrophils and cytokine IL-6 appeared in the winter season.

4. Discussion

Endotoxin is a pro-inflammatory agent that induces local and systemic inflammatory responses in normal subjects that can contribute to the risk of developing asthma and chronic obstructive lung diseases. Thus, there is a need to control endotoxin exposure to prevent the development of such diseases. Additionally, information on the dose–response relationship is a prerequisite for defining the no-response threshold of exposure that should be considered a safe concentration of endotoxin contamination in airborne dust, but available data on the dose–response relationship are less extensive. Since the majority of the population spends more than 80% of their time indoors including at home (Lange, 2002), endotoxin control in homes must be considered a possible route for reducing respiratory diseases such as asthma. Moreover, research on seasonal patterns of indoor airborne endotoxin levels may be useful in understanding seasonal patterns of respiratory diseases (Dales et al., 1996; Johnston et al., 1996). To the best of our knowledge, there are no published risk assessment reports about seasonal variations in airborne endotoxin exposure in homes. Therefore, given the potential health risks posed

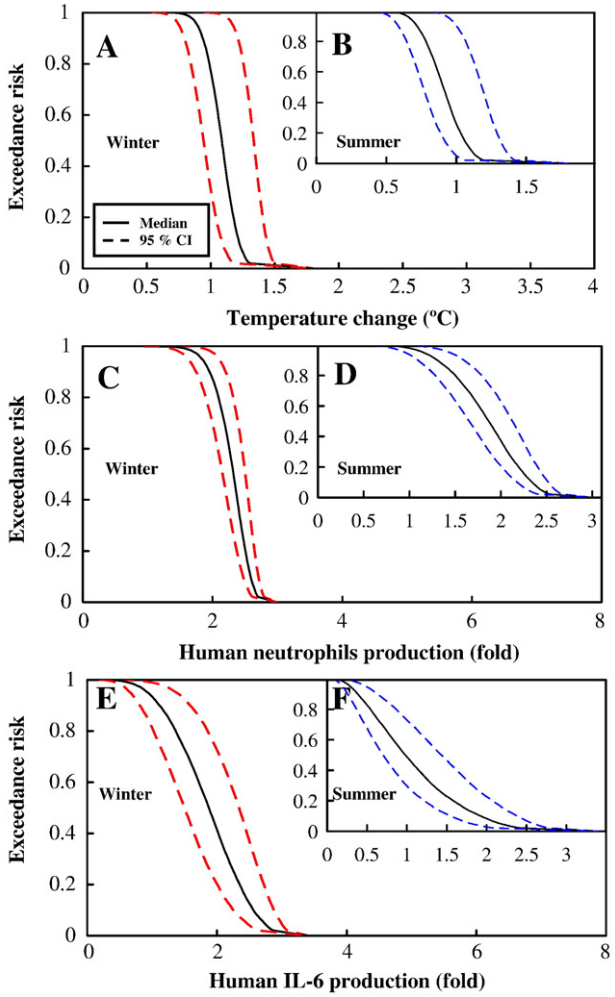

Fig. 6. Risk profiles of (A, B) an inflammatory symptom (temperature change), and two inflammation mediators (C, D) neutrophils elevated effect, and (E, F) IL-6 elevated effect for residents in homes during winter and summer seasons.

by airborne endotoxin in homes, the purpose of this study was to use a probabilistic approach to quantitatively assess the potentially inhalation risk of airborne endotoxin in homes during the winter and summer seasons.

In the present study, we present an approach which links a model of exposure, internal dosimetry, and health effects to estimate the potential risks to human health to airborne endotoxin exposure in homes during the winter and summer seasons. We evaluated the effects of the particle size distribution and phase composition of endotoxin on the exposure hazard. The Hill model was used to reconstruct dose–response profiles based on data of intravenous endotoxin on human body temperature changes, and elevation of neutrophils and IL-6 to respectively correlate clinical and inflammatory responses (Suffredini et al., 1999). MPPD and mass-based dosimetric exposure models were employed to predict the internal doses of inhaled PM-bound endotoxin, further estimating the likelihood of risk characterized by clinical and inflammatory responses. Three major findings are presented in our study: (i) the half-maximum effect of the endotoxin dose (ED50) was estimated to be 707.9 (95% CI: 308.8–1287.0) EU for an increase in body temperature, 481.8 (95% CI: 333.2–630.3) EU for elevation of neutrophils, and 1174.5 (95% CI: 816.0–1532.9) EU for elevation of IL-6, respectively; (ii) airborne endotoxin in the home may pose potential risks, and a higher risk for elevation of neutrophils and cytokine IL-6 appeared in winter than in summer; and (iii) the exposure risk curves are pivotal results for current public policy.

There is no consensus on endotoxin's 'no observable effect levels' (NOELs) for health endpoints that have been described to range from 50 to several hundred EU m^{-3} (Heederk and Douwes, 1997; Rylander, 1997). A health-based exposure limit was proposed in the Netherland by the Dutch Health Council of 50 EU m^{-3} (Heederk and Douwes, 1997). However, the introduced endotoxin exposure safety level is compounded by numerous problems, such as discrepancies in extraction and analysis of endotoxin samples and inter-individual variations in inhalation responses. Hence, there is controversy surrounding the precise exposure limit to endotoxin that should be implemented in order to achieve optimal disease prevention. Our proposed probabilistic approach to quantitatively assess the potential inhalation risk to airborne endotoxin may compensate for the discrepancy in the NOEL by using a scientifically based framework for assessing the risk of airborne endotoxin that may be present in either indoors or outdoors.

We believe that a probabilistic risk-based framework, probability distributions, and risk profiles, as presented in Fig. 6, are effective scientific assessments for human responses to airborne endotoxin exposure in the home. To the best of our knowledge, this risk-based framework for endotoxin exposure has not been addressed until now. We recognized limitations in each of our data sources and model assumptions, particularly the inherent problem of uncertainty and variability of the data sources. Additionally, we used default, or simplifying, assumptions where data were missing or of poor quality in the MPPD and exposure models which may have introduced uncertainty into the final predictions of ambient concentrations, exposure, and risk. Although the suitability and effectiveness of approaches for presenting uncertain results are context dependent, we believe that such probabilistic methods are valuable for communicating an accurate view of current scientific knowledge to those seeking information for decision-making. The probabilistic framework and approaches presented in this study produce general conclusions that are more robust than estimates made with a limited set of scenarios or without probabilistic presentations of outcomes. Therefore, our present study offers a risk-management framework for discussing future establishment of limits for respiratory exposure to airborne endotoxin.

Table 3
Potential median responses with 95% confidence intervals (CIs) for three selected effects at 10% and 50% exceedance risks (ERs) in winter and summer seasons.

	Winter	Summer	*p*-value[a]
ER10			
Temperature change (°C)	1.22 (1.09–1.44)	1.10 (0.95–1.34)	0.198
Neutrophils (fold)	2.59 (2.47–2.71)	2.34 (2.18–2.52)	0.031
IL-6 (fold)	2.56 (2.25–2.88)	1.92 (1.55–2.38)	0.018
ER50			
Temperature change (°C)	1.09 (0.94–1.34)	0.91 (0.76–1.18)	0.119
Neutrophils (fold)	2.32 (2.16–2.50)	1.87 (1.65–2.13)	0.007
IL-6 (fold)	1.88 (1.50–2.34)	0.98 (0.70–1.43)	0.007

[a] *p*-value was calculated based on *t*-test.

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