Estimation of endotoxin inhalation from shower and humidifier exposure reveals potential risk to human health

William B. Anderson, D. George Dixon and Colin I. Mayfield

ABSTRACT

This paper investigates potential exposure to endotoxin in drinking water through the inhalation of aerosols generated by showers and humidifiers. Adverse health effects attributable to the inhalation of airborne endotoxin in various occupational settings are summarized, as are controlled laboratory inhalation studies. Data from investigations estimating aerosolization of particulate matter by showers and humidifiers provide a basis for similar analyses with endotoxin, which like minerals in water, is nonvolatile. A theoretical assessment of the inhalation of aerosolized endotoxin showed that while the likelihood of an acute response while showering is minimal, the same is not true for humidifiers. Ultrasonic and impeller (cool mist) humidifiers efficiently produce large numbers of respirable particles. It is predicted that airway inflammation can occur if humidifier reservoirs are filled with tap water, sometimes even at typical drinking-water distribution-system endotoxin concentrations. Higher endotoxin levels occasionally found in drinking water ($>1,000$ EU/ml) are very likely to induce symptoms such as chills and fever if used as humidifier feed water. While it is unlikely that treated drinking water would contain extremely high endotoxin levels occasionally observed in cyanobacterial blooms ($>35,000$ EU/ml), the potential for serious acute health consequences exist if used in humidifiers.

Key words | drinking water, endotoxin, impeller humidifier, inhalation, shower, ultrasonic humidifier

William B. **Anderson** (corresponding author)
NSERC Industrial Research Chair in Water
   Treatment,
Dept. of Civil and Environmental Engineering,
200 University Ave. West, University of Waterloo,
Waterloo, Ontario, N2L 3G1,
Canada
Tel.: 519-888-4567, ext. 33265
Fax: 519-746-7499
E-mail: wbanderson@uwaterloo.ca

D. George Dixon
Colin I. **Mayfield**
Department of Biology,
University of Waterloo,
Waterloo, Ontario N2L 3G1,
Canada

INTRODUCTION

Endotoxins are a component of the lipopolysaccharide (LPS) complexes which make up a part of the outer layer of the cell walls of most Gram-negative bacteria (Prescott et al. 2002) and some cyanobacteria (Buttke & Ingram 1975; Sykora & Keleti 1981). LPS complexes are macromolecules composed of three main regions: lipid A, core polysaccharide, and "O" antigens (Braude 1982). The lipid A component is critical for all biological responses to endotoxin (Morrison et al. 1994; Prescott et al. 2002). Potential pathways of water-associated endotoxin exposure in humans include direct introduction into the blood stream by passage through semi-permeable dialysis membranes (in the case of haemodialysis patients) and by intravenous injection

solutions contaminated with endotoxin. The significance of endotoxin in the bloodstream is well understood and measures are in place in the medical and pharmaceutical communities to limit the risk of such occurrences (British Pharmacopeia 1994; United States Pharmacopeia 1995). Although solutions for injection are made with drinking water, it is always pretreated, usually culminating in membrane filtration to remove endotoxin.

Endotoxin contact from drinking water through dermal abrasions is not well documented, although it is well known that bacterial penetration into wounds can lead to infection with associated complications. It is unlikely that endotoxin can penetrate intact human skin. Even if it were assumed

that it could, the surface area of the alveolar region of the lungs ($300\,m^3$; Weibel 1983) and the lung's efficiency with regard to transfer into blood far exceeds that of the dermis ($2\,m^2$; Rao & Brown 1993). While the most obvious route of endotoxin exposure from drinking water is ingestion, very little information concerning occurrence exists. Endotoxin has been detected in the plasma of otherwise healthy patients with irritable bowel disease but it is unclear if this originates from endotoxin uptake from water, food, or bacteria in the gut (indigenous or otherwise) (Caradonna *et al.* 2000). Virtually all patients suffering from cirrhosis of the liver also suffer, to some degree, from systemic endotoxemia (Bauer *et al.* 2002). The cause has been identified as overgrowth of bacteria in the small intestine, characterized by 10^5 or more total colony forming units per milliliter of jejunal secretions (Bauer *et al.* 2002). In this case it would appear that endotoxin originates from localized bacterial growth as opposed to direct ingestion of endotoxin in food or water. There have been reports of an increased incidence of diarrhea in sewage treatment plant workers who have inhaled endotoxin but this response is not universal (Thorn & Rylander 1998; Rylander 1999). Rylander (1999) speculates that particles associated with sewage water aerosols are larger than those associated with dry organic dusts, resulting in substantial deposition in the nasopharyngeal area and transport to the gastrointestinal tract with subsequent inflammatory response in the gut mucosa.

This paper summarizes events associated with inhalation of endotoxin from drinking water, occupational airborne endotoxin exposure, data from recent studies involving controlled endotoxin inhalation experiments in humans, and endotoxin levels in water. A brief discussion of issues related to the formation and inhalation of aerosolized water droplets is followed by several theoretical assessments of the potential for adverse impacts to human health as a result of endotoxin inhalation while showering and in humidifier treated environments.

Inhaled endotoxin events associated with water

Rylander *et al.* (1978) were among the first to theorize that endotoxin was the causal agent in unexplained cases of fever after exposure to contaminated water in humidifiers and in a sauna (Kohler *et al.* 1976; Metzger *et al.* 1976; Pickering *et al.* 1976). This was accomplished by measuring endotoxin O antigen antibody titers in three subjects who developed 'humidifier disease' from a humidifier contaminated with two unidentified species of *Flavobacterium*. Endotoxin exposure-related symptoms such as breathing difficulties, cough, and fever were accompanied by increased white blood cell counts, increased proportions of segmented leukocytes, and increased levels of IgG immunoglobulins and antibodies (Rylander *et al.* 1978).

The earliest confirmed human exposures related to the inhalation of endotoxin from drinking water appear to have occurred in association with a 'bathing fever' epidemic of initially unknown etiology among 100 of the 1,000 inhabitants of Tampere, Finland in 1978 (Aro *et al.* 1980). Symptoms included cough, breathing difficulties, chills, fever, muscle pain, and aching of the joints. Muittari *et al.* (1980*a*, *b*) calculated that Tampere inhabitants who had experienced fever received a total inhaled endotoxin dose of 1.0 to $4.0\,\mu g$, or assuming a 70 kg human, 0.01 to $0.03\,\mu g$ endotoxin/kg body weight. The endotoxin concentration in the contaminated water system ranged from 0.2 to $1.0\,\mu g/ml$. This converts to 200 to 1,000 ng/ml or about 2,000 to 10,000 endotoxin units (EU)/ml assuming 1 ng = 10 EU based on actual factors ranging from about 4 to 17 EU/ng (e.g. Burger *et al.* 1989; Chang *et al.* 2001). This factor (1 ng = 10 EU) has been applied to convert ng to EU throughout this paper, when actual conversions were not provided, because a uniform factor must be utilized in order to roughly standardize data collected prior to the implementation of EU. Following the outbreak, four of the previously affected subjects were given $2 \times 2\,ml$ (inhalation challenge) of contaminated tap water; within 4 h each developed a fall in single breath lung diffusion capacity as well as a fever with coughing and shortness of breath. The calculated doses of 0.01 to $0.03\,\mu g/kg$ (10 to 30 ng/kg) are consistent with intravenous bacterial endotoxin doses which will induce fever (1 to 10 ng/kg weight, Anderson *et al.* 2002).

Humidifier disease has been documented in an occupational setting where airborne endotoxin levels ranged from 130 to $390\,ng/m^3$ (Rylander & Haglind 1984). It was characterized by fever, chills, and chest tightness in affected

employees when a humidifier contaminated with *Pseudomonas* was operating.

A hypersensitivity pneumonitis event attributable to endotoxin inhalation at a community recreation complex in Westminster, Colorado is described by McGregor *et al.* (1993). Lifeguards reported symptoms including dry cough, chest tightening, shortness of breath, and sluggishness. The problem was traced to a deep pool with features including a water levitator, fan sprays, and wall spouts, some or all of which could have contributed to the aerosolization of endotoxin. After an ozone treatment system was installed, pool water endotoxin concentrations dropped from 95–120 ng/ml to less than 1 ng/ml; the symptoms in lifeguards subsequently disappeared. It is not clear whether the ozone controlled biological growth, removed the endotoxin directly, or involved some combination of the two.

Overall, while there are few studies describing outbreaks of adverse health effects associated with aerosolized endotoxin, they clearly occur on occasion. It is likely that given the generic symptoms at low doses (impaired breathing, cough, and quickly resolving fever) most incidents go unreported. All reported incidents have been associated with substantial bacterial contamination and/or the presence of high levels of aqueous endotoxin, along with an effective delivery vector.

OCCUPATIONAL EXPOSURE TO INHALED ENDOTOXIN

In order to put the risk associated with waterborne aerosolized endotoxin into perspective it is useful to survey other airborne endotoxin exposure sources. Potential settings for endotoxin exposure are diverse and include: sawmills, paper recycling (repulping and deinking), fiberglass manufacturing, animal handling, grain handling, manure handling, cotton/textile milling, hemp processing, potato sorting, and cigar and cigarette manufacturing, among others (Rylander *et al.* 1985; Castellan *et al.* 1987; Dahlqvist *et al.* 1992; Christiani *et al.* 1993; Walters *et al.* 1994; Zejda *et al.* 1994; Milton *et al.* 1996; Rix & Lynge 1996; Louhelainen *et al.* 1997; Zock *et al.* 1998; Douwes *et al.* 2000; Reiman & Uitti 2000; Chang *et al.* 2001; Fishwick *et al.* 2001; Melbostad & Eduard 2001; Su *et al.* 2002). Aerosolized

endotoxin concentrations vary widely both between and within occupational settings. For example, within swine enclosures, airborne endotoxin concentrations have been found to range from 438 to 41,307 EU/m^3 (Zejda *et al.* 1994). The highest recorded concentration observed in the above referenced studies was 59,801 EU/m^3 in a hemp processing plant (Fishwick *et al.* 2001). It should be noted though that some measurements were made in areas where there is limited human activity or at levels above or below breathing zones.

Several studies have shown that human health effects can be linked to aerosolized endotoxin in the workplace (Rylander *et al.* 1985; Castellan *et al.* 1987; NIOSH 1994; Smid *et al.* 1994; Milton *et al.* 1996; Keman *et al.* 1998; Vogelzang *et al.* 1998; Zock *et al.* 1998; Mandryk *et al.* 1999, 2000). An analysis of these studies suggests that as test methods and statistical analyses become more rigorous, endotoxin levels at which effects are observed are decreasing. The lowest dose resulting in a statistically significant health effect (FEV_1- forced expiratory volume or volume of air exhaled in 1.0 s-the first second) is 0.1 to 2 ng/m^3 (Mandryk *et al.* 1999, 2000). Assuming that 1 ng = 10 EU, then this would correspond to 1 to 20 EU/m^3 which is consistent with other studies showing effects at 40 to 53 EU/m^3 (Milton *et al.* 1996; Zock *et al.* 1998).

In addition to changes in lung function measures, Mandryk *et al.* (2000) found that sawmill workers suffered from specific adverse health related symptoms when compared to a control population. The increased occurrence of chronic symptoms/conditions in green mill (fresh undried wood) workers was statistically significant in all cases and included cough, chronic bronchitis, regular blocked nose, sinus problems, flu-like symptoms, eye irritation, and throat irritation. Workers in both green and dry mills experienced statistically higher frequencies of regular runny nose, regular sneezing, and frequent headaches. Melbostad & Eduard (2001) surveyed Norwegian farmers for a variety of symptoms related to the eyes, nose, throat, and lower airway. They found a higher prevalence of at least one symptom in farmers that carried out tasks such as grain handling (34–55%), and handling of hay and tending of swine and poultry (26–35%). The prevalence of work-related symptoms in allergic farmers was 87% compared to 61% in those without allergies and 71% in

male farmers versus 56% in female farmers. Observations in these studies are confounded by the fact that there are other constituents in dusts and aerosols found in certain occupational environments. For example, symptoms associated with endotoxin exposure are similar to those associated with fungal mycotoxicosis. Mycotoxins are not detected in the endotoxin assays but fungal-derived glucans do interfere. A test is available for glucans.

At least two studies have shown that outdoor air endotoxin concentrations can be substantially lower than those found in some indoor occupational settings. Hartung & Seedorf (1999) found that the median endotoxin concentration in outside air in twenty-two rural and eight urban settings was $0.36 \, \text{ng/m}^3$, with the highest maximum concentration of $1.80 \, \text{ng/m}^3$ occurring in the summer. No significant differences were found between rural and residential outdoor areas. Similarly, Su *et al.* (2002), while investigating airborne endotoxin concentrations in textile mills in Taiwan, measured endotoxin concentrations in a courtyard just outside the mills. They found outdoor concentrations of 1.6 and $3.4 \, \text{ng/m}^3$ ($16-34 \, \text{EU/m}^3$) in Year 1 of the study and 0.4 and $41 \, \text{ng/m}^3$ (4 and $410 \, \text{EU/m}^3$) in Years 2 and 3, respectively. It is clear that indoor endotoxin levels may be orders of magnitude higher than would normally occur outdoors. Not surprisingly, there can also be substantial variability between buildings. Teeuw *et al.* (1994) showed endotoxin levels in buildings associated with 'sick building syndrome' to be six to seven times higher than those in control buildings (254 vs. $46 \, \text{ng/m}^3$).

Laboratory endotoxin inhalation studies in humans

Endotoxin response can vary widely between individuals. There is a disconnect between the levels of endotoxins some people can tolerate without reporting symptoms and those which can clearly be measured or characterized in human studies. This could be attributable to the species of bacteria generating the endotoxin, the amount of endotoxin actually respired, the size of particles with which the endotoxin is associated, and/or individual response variability (e.g. Laude-Sharp *et al.* 1990; Hansen *et al.* 1999; Koyama *et al.* 2000). Table 1 summarizes some controlled laboratory studies involving human volunteers. All studies used *E. coli* endotoxin with 4 of 5 using that from strain O26:B6 while

the fifth used O11:B4. Although indicators of immunological activity and mild symptoms such as shortness of breath occur at inhaled doses around $5 \, \mu\text{g}$, a dose of about $50 \, \mu\text{g}$ is required to elicit symptoms such as chills and fever (at least for *E. coli* O26:B6 endotoxin). For comparative purposes these doses have been assumed to correspond to roughly 50,000 and 500,000 EU, respectively.

Symptoms that were observed significantly more frequently 24 h following exposure to $40 \, \mu\text{g}$ (roughly 400,000 EU) of endotoxin than before, were breathlessness (29% of subjects), irritation in the throat (38%), dry cough (33%), headache (62%), heaviness in the head (71%) and unusual tiredness (57%) (Thorn & Rylander 1998). On the other hand, Michel (1998) indicates that the no-response threshold to acute inhalation of endotoxin is less than $0.5 \, \mu\text{g}$, corresponding to $50 \, \text{ng/m}^3$ (roughly $500 \, \text{EU/m}^3$) of airborne endotoxin. The threshold may be lower for allergy and asthma sufferers. Eldridge & Peden (2000) demonstrated that an interaction between allergens and endotoxin in allergy sufferers produces a significant increase in total and differential inflammatory cell counts and in IL-6 and ECP concentrations. *E. coli* O26:B6 endotoxin was administered to volunteers at a dosage of 1,000 ng (roughly 10,000 EU). The allergen used was dust mite allergen (*D. farinae* at a dose of 100 AU).

PROPOSED STANDARDS FOR INHALED ENDOTOXIN

A few jurisdictions have proposed standards for inhaled dust borne endotoxins. The Health Council of the Netherlands has proposed an occupational exposure limit of $50 \, \text{EU/m}^3$ (roughly $5 \, \text{ng/m}^3$), based on inhalable dust exposure, measured as an eight hour time-weighted average (Health Council of the Netherlands 1998). This recommendation was based on observed decreases in FEV_1 (forced expiratory volume or volume of air exhaled in 1.0 s). Palchak *et al.* (1988) are quoted by Laitinen *et al.* (1992) as stating that a proposed occupational exposure limit for endotoxin is $30 \, \text{ng/m}^3$ as an 8 h time-weighted average (no further details provided).

A 1993 endotoxin criteria document, prepared by the International Commission on Occupational Health

Table 1 | Survey of responses to inhaled endotoxin under laboratory conditions. All responses were statistically significant. Endotoxin was administered in 5% hypertonic saline water

Dose (μg)	Delivery system	Endotoxin origin	Response	Reference
0.5	Jet nebulizer[1]	*E. coli* O26:B6	Decrease in sputum PMN[2]	Michel *et al.* 1997
5	"	"	Increase in blood CRP[3], blood PMN, sputum PMN, monocytes, MPO[4]	"
50	"	"	Increase in temperature[5], blood PMN, blood & urine CRP, sputum PMN, monocytes, lymphocytes, MPO, TNF-α[6], ECP[7]	"
40	Nebulizer[8]	*E. coli* O26:B6	Decrease in FEV_1[9] and FVC[10]; increase in MPO[4] and ECP[7]	Thorn & Rylander 1998
$\leq$6.5	Nebulizer[11]	*E. coli* O11:B4	$\geq$20% decline in FEV_1	Kline *et al.* 1999
5	Closed-circuit ultrasonic nebulizer[12]	*E. coli* O26:B6	Induced neutrophil influx to airway, decreased phagocytosis in both airway and circulating cells, modified CD11b expression-no fever, myalgia, malaise, shortness of breath, chest tightness or hypotension observed	Alexis *et al.* 2003
5	Jet nebulizer[1]	*E. coli* O26:B6	Airway inflammation that correlated with CD14 expression and eosinophil numbers-asthmatics more sensitive	Alexis *et al.* 2001

[1]Generated heterodisperse droplets with a mass median aerodynamic diameter of 1 to 4 μm, 9 μl per dose (Mefar MB3 dosimeter [Mefar, Brescia, Italy]).
[2]Polymorphonuclear neutrophil luminol-enhanced chemiluminescence (could reflect a process of margination and/or extravascular sequestration of activated PMN); also referred to as airway neutrophilic inflammation (Alexis et al. 2001).
[3]CRP = C-reactive protein.
[4]MPO = myeloperoxidase.
[5]Temperature increase was 0.7°C.
[6]TNF = tumor necrosis factor.
[7]ECP = eosinophil cationic protein.
[8]'Pari Boy' nebulizer (20 puffs [4 μl per dose] of endotoxin [500 μg/ml] up to a total of 40 μg).
[9]Forced expiratory volume in one second (the first second).
[10]Forced vital capacity.
[11]DeVilbiss 646 powered by compressed air at 30 psi.
[12]DeVilbiss 99 (total fluid volume = 10 ml).

(Milan, Italy) suggests guidelines for no observed effect levels for environmental endotoxin (Rylander & Peterson 1993 as quoted by Rylander 1997; ATS 1998). The no observed effect level for organic dust toxic syndrome (ODTS) or toxic pneumonitis is $200\,ng/m^3$ ($\sim 2{,}000\,EU/m^3$); for systemic effects it is $100\,ng/m^3$ ($\sim 1{,}000\,EU/m^3$); and for airway inflammation the no observed effect level is $10\,ng/m^3$ ($\sim 100\,EU/m^3$) (Rylander & Peterson 1993 as quoted by Rylander 1997; ATS 1998).

DROPLETS AND AEROSOLS

Understanding exposure to endotoxins via inhalation of aerosolized water can be further complicated (relative to dust) by the nature of aerosols. Inhalation exposure for a nonvolatile compound such as endotoxin is a function of the volume of aerosol produced, the size distribution of the aerosols, growth, or shrinkage of aerosols, aerosol transport within the exposure area and the concentration of endotoxin in the water (Weisel *et al.* 1999).

Aerosols can be present in liquid form and can be formed when water droplets completely evaporate, leaving only particles that were contained in the droplet. Within the context of this study, aerosols are defined as airborne particles sufficiently small (diameter $D_p \leq 10\,\mu m$) that they do not rapidly settle out of air (as opposed to water droplets which are $> 10\,\mu m$) (Pandis & Davidson 1999). Droplets are rapidly removed from inhalation environments by gravity (settling velocity of a $50\,\mu m$ droplet is $\sim 11\,cm/s$ vs. $\sim 0.3\,cm/s$ for a $10\,\mu m$ aerosol; Hinds 1982), while aerosols ($< 10\,\mu m$) can persist in respirable indoor environments for hours (Pandis & Davidson 1999). This delineation is important because droplets are too large to be respirable; the aerosol concentration needs to be estimated. Aerosols produced when droplets evaporate are expected to be liquid only at relative humidities exceeding 60% at ambient temperatures (Seinfeld & Pandis 1997). Therefore, droplets larger than $10\,\mu m$ are only important in the context of being aerosol precursors. In addition to evaporation, however, droplets can produce aerosols by impacting other solid or liquid surfaces (e.g. the human body, sink or shower surfaces etc.) (Seinfeld & Pandis 1997). Gunderson & Witham (1988, as quoted in Pandis & Davidson 1999)

indicate that the effect of the collision of water droplets with human bodies is likely to be significant and probably cannot be neglected in estimating exposure.

If particles $\leq 10\,\mu m$ are considered to be aerosols but water droplets $> 10\,\mu m$ can evaporate, how then is aerosol production quantified? Owen *et al.* (1992) indicated that the diameter of an aerosol particle (d_a) resulting from the complete evaporation of a droplet tap water with a diameter (d_{dr}) will be given by $d_a = 0.05\,d_{dr}$. The authors assumed that the tap water contained $300\,mg/L$ of total dissolved solids, and that the dissolved material had a density of $2.5\,g/cm^2$. Given the calculation, droplets with a diameter $> 200\,\mu m$ can be ignored when quantifying aerosol exposure since those droplets will yield aerosol particles with a diameter $> 10\,\mu m$ i.e. particles which are not respirable. As such, these authors assume, for the purposes of estimating inhalation exposure to aerosols, droplets with diameters larger than $200\,\mu m$ can be ignored (i.e. larger droplets will produce nonrespirable, $d_a > 10\,\mu m$, dry particles even if they completely evaporate).

Furthermore, from a settling perspective, water droplets in high humidity environments (e.g. in a shower) larger than $50\,\mu m$ can be neglected for aerosol exposure estimates and those larger than $100\,\mu m$ can be neglected in most other indoor environments (Pandis & Davidson 1999). Droplets in excess of these values settle too rapidly to form substantial quantities of aerosols. During water evaporation, the mass concentration of nonvolatile aerosol components remains constant (as opposed to volatile components, which are transferred to the gas phase during volatilization). In some respects this simplifies the determination of inhaled endotoxin.

FACTORS INFLUENCING ENDOTOXIN EXPOSURE AND UPTAKE

Several factors should be considered when estimating human exposure to endotoxins: body weight, sources of inhalation, endotoxin concentrations in those sources, inhalation rates, as well as both exposure frequency and duration. Potential household water-related exposures include: bathing, dish washing (manual or automatic), clothes washing, beverage and food preparation, personal

washing, toilet use, household cleaning, cooking, and outdoor use. While all of these may contribute to airborne contaminant loading (particularly in the case of volatile compounds), most would not be important from the point of view of aerosolization of a nonvolatile compound such as endotoxin under conditions of typical usage. In high use environments such as stadium or theme park settings the risk to the occupationally-exposed from activities such as toilet flushing may be somewhat increased. Additional water-related exposures which would be more likely to include endotoxin aerosolization include showers, humidifiers, hot tubs, saunas, and pools in which fountains, showers, or aeration are present.

AEROSOLS IN SHOWERS

Although there is very little information in the literature related to aerosol inhalation exposure associated with most of the sources noted above, some information is available for showers and humidifiers. If inhalation exposure to aerosols is to be estimated one must know the rates of droplet/aerosol production (mass of droplets per unit of time per mass of water), other activity-related parameters (flux of water, showerhead type, etc.), and the size distribution of the droplets (Pandis & Davidson 1999). The characterization of shower generated aerosols is discussed in more detail by Keatin & McKone (1992; in Mercer 1999).

There are several sources of information for exposure assumptions associated with showering, and respiration rates and tidal volumes for humans of different ages at various levels of activity (James & Knuiman 1987; Hofmann *et al.* 1989; USEPA 1989; USEPA 1992; AIHC 1994; Page 1994; USEPA 1996; Mills *et al.* 1998) These are not discussed here but a summary can be found in Anderson (2004).

Aerosols produced by humidifiers

In a study investigating aerosol emissions in humidifiers, Highsmith *et al.* (1988, 1992) observed that PM_{10} concentrations (the concentration of particles with an aerodynamic diameter $<10\,\mu m$) exceeding 400 and 7,000 $\mu g/m^3$ were emitted when an ultrasonic humidifier was operated

under whole-house and single room conditions, respectively. Humidifier water had a total dissolved solids content of 300 mg/L and hardness of 145 mg/L. It was noted that more than 90% of the particles generated were in the respirable range, and virtually all had diameters $<1\,\mu m$). The authors also examined impeller type (cool mist) humidifiers and traditional steam models. The impeller units generated less than half of the aerosol mass generated by the ultrasonic units and a steam unit generated no increase in PM_{10} (Highsmith *et al.* 1988). Approximately 60 to 75% of the particles generated by impeller humidifiers were in the respirable range. The particle constituents were primarily calcium, silica, and sulfur with sodium, magnesium, and copper present in minor amounts. Calcium carbonate and aluminosilicate particles were observed. All of these are characteristic of what would be expected if tap water were to be aerosolized. The authors expressed concern that occupational standards for respirable nuisance particulates were being exceeded. They did not consider aerosolization of microorganisms or microbial constituents such as LPS/endotoxin. As endotoxin is nonvolatile and extremely environmentally resilient, it would be reasonable to assume that it would behave similarly with respect to aerosolization as the inorganic constituents found in tap water.

Respiratory uptake

As discussed above, only particles/aerosols with a diameter $<10\,\mu m$ are respirable. The majority of particles with a diameter of 5 to 10 μm are removed in the upper respiratory tract. Of the remaining particles with a diameter of $>5\,\mu m$, only approximately 20% are in fact deposited throughout the entire respiratory tract (Schlesinger 1985). The alveolar-capillary barrier (gas exchange region) of the human lung consists of a 0.2 μm thick epithelial cell layer and lumen through which gases and particles are exchanged into red blood cells. Larger particles are removed in the upper respiratory tract and conducting airways by deposition and subsequent clearance by ciliated cells in a mucus layer (Mercer *et al.* 1991). The cells move particles and inhaled bacteria upward to the glottis, where mucous and particles are removed by swallowing. This process does not occur in the alveolar gas exchange region but it is known that, on

average, each alveolus in the lung contains an average of 12 macrophages which are thought to process all particles making it to that point (Stone *et al.* 1992). It is these macrophages that are responsible for initial pulmonary responses to the inhalation of endotoxin (Davis *et al.* 1980; Rylander 1989).

THEORETICAL ASSESSMENT OF ENDOTOXIN EXPOSURE FROM SHOWER AND HUMIDIFIER AEROSOLS

For this and the following assessments only those conditions resulting in acute health outcomes are considered. Chronic effects in occupational settings have been documented but will not be investigated here. Endotoxin exposure from shower aerosols and two types of humidifiers (ultrasonic and impeller) are assessed.

Shower

This assessment uses a previously established formula for estimating concentrations of a target compound in air when the concentration in water is known. Gunderson & Witham (1988, as quoted in Pandis & Davidson 1999), working with mannequins, found that the expected respirable particulate concentration for a nonvolatile species, C_a, at a concentration C_w in showers can be approximated by:

$$C_a(\mu g\, m^{-3}) = AC_w(mg\, L^{-1}) \tag{1}$$

where A is an empirical constant with values varying from 0.03 to 0.2.

Using this equation, two worst-case scenarios with respect to endotoxin in treated drinking water were evaluated. Previous work has shown that following shutdown of biological drinking water filters, endotoxin concentrations can be as high as 750 EU/ml (Peppler *et al.* 1995; Anderson 2004). This value was rounded up to 1,000 EU/ml as a benchmark for the calculations below. In a study by Rapala *et al.* (2002) it was observed that the highest endotoxin concentration found in untreated surface water was 38,000 EU/ml at the site of a cyanobacterial bloom. These two concentrations will be used in this assessment and those to follow.

Table 2 provides airborne endotoxin concentrations (C_a) obtained by solving Equation 1 for aqueous endotoxin concentrations of 1,000 and 38,000 EU/ml (using the low and high constant [A] values provided).

At the lower aqueous endotoxin concentration (1,000 EU/ml) the estimated aerosol concentrations range from 30 to 200 EU/m^3 while at 38,000 EU/ml the aerosol concentrations range from 1,140 to 7,600 EU/m^3. Three of the four calculated concentrations are above the Health Council of the Netherlands (1998) proposed occupational exposure limit of 50 EU/m^3 based on personal inhalable dust exposure, measured as an eight hour time-weighted average. It should be noted that much shorter times are spent in the shower. One way of 'normalizing' these concentrations is to determine how much would be inhaled during a shower and compare this to the amount inhaled at an 8-h exposure to 50 EU/m^3. This does not take into account the amount of endotoxin that actually deposits in the respiratory system and assumes that endotoxin accumulates over the 8-h period without significant clearance. It also assumes that some threshold value for shorter periods of time is not exceeded.

The 50th and 90th percentile residence times while showering were selected (corresponding to 7.2 min [0.12 h] and 12 min [0.20]) based on those provided by the USEPA (1989). Sedentary and maximal activity respiration rates for a 20-year old were assumed to be 7 L/min and 122 L/min, respectively (Hofmann *et al.* 1989). These correspond to 0.42 m^3/h and 7.32 m^3/h based on the 0.12 and 0.20 h shower residence times.

Table 2 | Calculated endotoxin concentrations in shower air from Equation 1 (C_a)

Constant value used	Airborne endotoxin concentration assuming aqueous concentrations below		Units
	1,000 EU/ml (100 ng/ml)	38,000 EU/ml (3,800 ng/ml)	
A = 0.20	200	7,600	EU/m^3
	20	760	ng/m^3
A = 0.03	30	1,140	EU/m^3
	3	140	ng/m^3

Table 3 | Endotoxin inhaled while showering based on airborne concentrations of 30, 200, 1,140, and 7,600 EU/m³ (from Table 2) for shower times of 0.12 and 0.20 h (EU)

Airborne endotoxin (EU/m³)	30			200			1,140			7,600		
Inhalation rate (m³/h)[a]	0.42	3.45	7.32	0.42	3.45	7.32	0.42	3.45	7.32	0.42	3.45	7.32
0.12 h in shower	2[b]	12	26	10	83	176	57	472	1,001	383	**3,146**	**6,676**
0.20 h in shower	3	21	44	17	138	293	96	787	**1,669**	638	**5,244**	**11,126**

Values in bold exceed the 5,000 EU required to induce symptoms (Table 1) or the 1,380 EU value shown in Table 4.
[a]0.42, 3.45 and 7.32 m³/h are sedentary, midpoint, and maximal respiration rates.
[b]Example: 0.12 h × 30 EU/m³ × 0.42 m³/h = 2 EU (rounded up).

Using data provided in Table 2, the total amount of endotoxin inhaled in 0.12- and 0.20-h showers at sedentary, midpoint and maximal breathing rates can be calculated (Table 3). Table 4 summarizes total endotoxin inhalation over a period of 8 h at a background concentration of 50 EU/m³ (roughly 5 ng/m³). This roughly normalizes brief exposures while showering to the 8-h proposed occupation exposure limit.

The total volume of endotoxin inhaled over 8 h would range from 168 to 2,928 EU depending upon respiration rate, at a constant airborne endotoxin concentration of 50 EU/m³. It is probably reasonable to assume that respiration rates would be on the higher side in occupational settings. If it were assumed that the actual respiration rate was the midpoint of the sedentary and maximal rates (3.45 m³/h) then the total amount of endotoxin inhaled in 8 h would be 1,380 EU (Table 4).

Examination of Table 3 shows that at airborne endotoxin concentrations of 30 and 200 EU/m³ (which correspond to an aqueous concentration of 1,000 EU/ml) none of the inhaled endotoxin concentrations exceed 1,380 EU. This is reassuring in that drinking water distribution system endotoxins are typically below 1,000 EU/ml. If, however, water was pumped into a system without treatment, or solely with chlorination, and this occurred during a period of high cyanobacterial activity near the intake (i.e. 38,000 EU/ml), endotoxin levels could be high enough to induce low-level endotoxicosis. This is demonstrated by the fact that the calculated total endotoxin inhaled midpoint concentrations, 3,156 and 5,244 EU, exceed the calculated 1,380 EU 8-h total exposure at the occupational limit of 50 EU/m³ (Table 3). The upper values

also approach or slightly exceed 0.5 μg, which has been demonstrated to initiate inflammatory responses in laboratory studies (Table 1). Nonetheless, even the highest predicted endotoxin concentration should not result in health outcomes that would cause more than temporary discomfort.

Ultrasonic humidifier

Highsmith *et al.* (1988) proposed the following steady-state Equation (2) to determine humidifier fine particle formation based on the mass balance approach of Alzona *et al.* (1979):

$$C_{air} = \frac{C_w \times WCR \times F \times 1,000 + C_b}{V \times M \times (AER + K_L)} \qquad (2)$$

This equation was solved using inputs both listed and defined in Table 5, assuming that endotoxin and minerals in water behave similarly. As with showers, aqueous endotoxin concentrations of 1,000 EU/ml (0.1 mg/L) and 38,000 EU/ml (3.8 mg/L) were used in this scenario. In

Table 4 | Endotoxin inhaled over 8 h at an airborne endotoxin concentration of 50 EU/m³

Activity level	Respiration rate (m³/h)	Total air inhaled in 8 h (m³)	Total endotoxin inhaled over 8 h period assuming concentration is 50 EU/m³ (EU)
Sedentary	0.42	3.36	168
Midpoint	3.45	27.6	**1,380**
Maximum	7.32	58.6	2,928

addition, a more typical drinking water distribution level of 25 EU/ml (0.0025 mg/L) was assessed. Whole-house endotoxin concentrations were calculated for an ultrasonic humidifier (Table 6) and separate assessments using two different values for M (degree of mixing factor) for a single bedroom are shown in Table 7.

The data (Table 6) show that at high aqueous endotoxin levels respirable endotoxin concentrations exceed those known to cause moderate to severe health impacts. Even at typical distribution system endotoxin concentrations, the 0.5 μg threshold (for those without allergies and asthma) can be exceeded in 8 hours. At an endotoxin concentration of 1,000 EU/ml, which occasionally occurs in distribution systems, it is possible to inhale between 1.25 and 22 μg of endotoxin in 8 hours (12,528 to 218,347 EU). At extremely high endotoxin concentrations, which would only rarely be seen, up to 104 μg (1,037,150 EU) could be respired in 1 hour and anywhere from about 48 to 830 μg (476,069 to 8,297,203 EU) could be respired in 8 hours. As discussed previously, in human endotoxin inhalation challenges, symptoms such as breathlessness, throat irritation, dry cough, headache, and heaviness in the head are observed at around 40 μg and chills and fever are observed above 50 μg.

The above was a whole-house assessment; if the humidifier were located in a small room, airborne endotoxin levels could arguably be higher. The calculations were repeated for a smaller room (27 m^3), such as a bedroom, at two 'degree of mixing factors' (M = 0.53 and 1.0) (Table 7). The concentrations were approximately 30 times higher in the small room vs. the whole house when M = 1.0. At typical distribution system levels, approximately 1 to 15 μg (~8,600 to 150,000 EU) of endotoxin could be inhaled over an 8-h period. If the background aqueous endotoxin concentration were to increase to 1,000 EU/ml, 1-h inhalation exposure would range from 4 to 75 μg (43,000 to 749,568 EU), while 8-h exposure would range from 34 to 600 μg (344,064 to 5,996,544 EU). At an aqueous endotoxin concentration of 38,000 EU/ml inhaled endotoxin concentration levels at both 1 and 8 h of exposure exceeded fever inducing endotoxin exposure concentrations of around 50 μg (160 to 23,000 μg; 1,634,304 to 227,868,672 EU).

Impeller humidifier

A similar theoretical exercise was carried out for impeller (cool-mist) humidifiers, which generate droplets and aerosols by using a spinning impeller to draw water from a reservoir and force it at high speeds against a ring of staggered baffles. The same assumptions provided in Table 5 were used except for the proportion of particles generated

Table 5 | Inputs for equation 2[a]

Factor	Abbreviation	Value(s) assumed	Units
Airborne endotoxin concentration	C_{air}	solve for	μg/m^3
Endotoxin content of water	C_w	0.0025; 0.1; 3.8	mg/L
Humidifier water consumption rate	WCR	0.48	L/h
Proportion of particles generated in the collected size fraction	F	0.9	–
Background airborne endotoxin	C_b	ignored	μg/m^3
Volume of whole house/room	V	393; 27	m^3
Unitless factor for degree of mixing	M	0.53; 1.0	–
Room/house air exchange rate	AER	0.5	h^{-1}
Loss rate of particles due to settling (fine particles)	K_L	0.14	h^{-1}

[a]Input values obtained from Highsmith et al. (1988), except for endotoxin concentrations.

Table 6 | Whole house endotoxin loading (ultrasonic humidifier)

Fixed Parameters						
Endotoxin in water (EU/ml)	25	25	1,000	1,000	38,000	38,000
Inhalation rate (m^3/h)^a	0.42	7.32	0.42	7.32	0.42	7.32
Calculated Endotoxin in Air and Total Endotoxin Inhaled; Whole House (393 m^3)^b						
Endotoxin in air (EU/m^3)	93	93	3,729	3,729	141,687	141,687
Total endotoxin inhaled in 1 h (EU & [µg])	39 [0.004]	682 [0.07]	1,566 [0.16]	27,293 [**2.7**]	59,509 [**6**]	1,037,150 [**104**]
Total endotoxin inhaled in 8 h (EU & [µg])	313 [0.03]	5,459 [**0.55**]	12,528 [**1.25**]	218,347 [**22**]	476,069 [**48**]	8,297,203 [**830**]

Values in bold exceed 0.5 µg threshold for symptoms related to endotoxin inhalation.
^{a}For a 20 year old; 0.43 m^3/h = sedentary, 7.32 m^3/h = maximal.
^{b}Degree of mixing factor (M) = 0.53.

in the fine (respirable) size fraction (F): 0.9 was used for ultrasonic humidifiers while values of 0.6 and 0.75 were used for impeller humidifiers. This represents the range of formation of fine/respirable particles measured by High-smith *et al.* (1992). The total estimated endotoxin inhaled was 15 to 30% less than that calculated for an ultrasonic humidifier at corresponding conditions (Tables 8 and 9). In 1 hour, at typical aqueous endotoxin concentrations (~25 EU/ml), it is possible to inhale in excess of 0.5 µg and with an 8-h exposure the 5 µg symptom level is exceeded at both midpoint and maximal respiration levels.

To help visualize the significance of the calculated inhaled endotoxin concentrations, Table 10 shows the conditions under which total inhaled endotoxin exceeds 0.5 µg (the no observed effects level-NOEL), 50 µg (the level at which body aches and fever are induced), and 500 µg, a level for which human trials have not been conducted. The degree of mixing factor is 1.0 for all data reported. This table also includes midpoint respiration rate categories, data not shown in previous tables.

DISCUSSION

A number of uncertainties plague researchers trying to accurately assess risks associated with endotoxin inhalation. Although there are numerous studies that look

at airborne endotoxin in a variety of environments, absolute comparisons are difficult. Studies report results in different units (ng or µg vs. EU). Many studies, including recent ones, report results in terms of chemical concentration as opposed to units of activity. It would be useful if all studies reported in EU relative to a standard endotoxin *and* provided the conversion factor to calculate the actual concentration in ng/m^3. Equal concentrations of endotoxin from different species can produce differing endotoxic responses; a switch to endotoxin units would at least partially overcome this problem. For example, based on the most common method used to measure endotoxin concentration, the *Limulus* amoebocyte lysate (LAL) assay, Hansen *et al.* (1999) found that LPS from *Escherichia coli* and *Salmonella enteritis* was about four times more potent than that from *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Laude-Sharp *et al.* (1990) found that *E. coli* LPS is about 100 times more potent than *Shigella flexneri* LPS and *Pseudomonas testosteroni* LPS is about 60% more active than *E. coli* LPS. Even within species there can be wide variations in LAL potency with a 625-fold range in some *E. coli* serotypes (Koyama *et al.* 2000).

Not surprisingly, structural differences in LPS also play a major role in the degree of cytokine production in human monocytes. This is significant in terms of the severity of an immune response in that unlike pathogens, which set up

Table 7 | Small bedroom endotoxin loading (ultrasonic humidifier)

Fixed Parameters						
Endotoxin in water (EU/ml)	25	25	1,000	1,000	38,000	38,000
Inhalation rate (m^3/h)[a]	0.42	7.32	0.42	7.32	0.42	7.32
Calculated Endotoxin in Air and Total Endotoxin Inhaled; Bedroom (27 m^3)[b]						
Endotoxin in air (EU/m^3)	1,357	1,357	54,272	54,272	2,062,336	2,062,336
Total endotoxin inhaled in 1 h (EU & [μg])	570 [0.06]	9,932 [**1.0**]	22,794 [**2.3**]	397,271 [**40**]	866,181 [**87**]	15,096,300 [**1,510**]
Total endotoxin inhaled in 8 h (EU & [μg])	4,559 [**0.5**]	79,454 [**7.9**]	182,354 [**18**]	3,178,168 [**318**]	6,929,449 [**693**]	120,770,396 [**12,077**]
Calculated Endotoxin in Air and Total Endotoxin Inhaled; Bedroom (27 m^3)[c]						
Endotoxin in air (EU/m^3)	2,560	2,560	102,400	102,400	3,891,200	3,891,200
Total endotoxin inhaled in 1 h (EU & [μg])	1,075 [0.1]	18,739 [**1.9**]	43,008 [**4.3**]	749,568 [**75**]	1,634,304 [**163**]	28,483,584 [**2,848**]
Total endotoxin inhaled in 8 h (EU & [μg])	8,602 [**0.9**]	149,914 [**15**]	344,064 [**34**]	5,996,544 [**600**]	13,074,432 [**1,307**]	227,868,672 [**22,787**]

Values in bold exceed 0.5 μg threshold for symptoms related to endotoxin inhalation.
[a]For a 20 year old; 0.43 m^3/h = sedentary, 7.32 m^3/h = maximal.
[b]Degree of mixing factor (M) = 0.53.
[c]Degree of mixing factor (M) = 1.0.

Table 8 | Small bedroom endotoxin loading (impeller humidifier, F = 0.75)

Fixed Parameters						
Endotoxin in water (EU/ml)	25	25	1,000	1,000	38,000	38,000
Inhalation rate (m^3/h)[a]	0.42	7.32	0.42	7.32	0.42	7.32
Calculated Endotoxin in Air and Total Endotoxin Inhaled; Bedroom (27 m^3)[b]						
Endotoxin in air (EU/m^3)	1,131	1,131	45,227	45,227	1,718,613	1,718,613
Total endotoxin inhaled in 1 h (EU & [μg])	475 [0.05]	8,276 [**0.8**]	18,995 [**1.9**]	331,059 [**33**]	721,818 [**72**]	12,580,250 [**1,258**]
Total endotoxin inhaled in 8 h (EU & [μg])	3,799 [0.4]	66,212 [**6.6**]	151,962 [**15**]	2,648,474 [**265**]	5,774,541 [**577**]	100,641,997 [**10,064**]
Calculated Endotoxin in Air and Total Endotoxin Inhaled; Bedroom (27 m^3)[c]						
Endotoxin in air (EU/m^3)	2,133	2,133	85,333	85,333	3,242,667	3,242,667
Total endotoxin inhaled in 1 h (EU & [μg])	896 [0.1]	15,616 [**1.6**]	35,840 [**3.6**]	624,640 [**62**]	1,361,920 [**136**]	23,736,320 [**2,374**]
Total endotoxin inhaled in 8 h (EU & [μg])	7,168 [**0.7**]	124,928 [**12**]	286,720 [**29**]	4,997,120 [**500**]	10,895,360 [**1,090**]	189,890,560 [**18,989**]

Values in bold exceed 0.5 μg threshold for symptoms related to endotoxin inhalation.
[a]For a 20 year old; 0.43 m^3/h = sedentary, 7.32 m^3/h = maximal.
[b]Degree of mixing factor (M) = 0.53, F = 0.75.
[c]Degree of mixing factor (M) = 1.0, F = 0.75.

Table 9 | Small bedroom endotoxin loading (impeller humidifier, F = 0.6)

Fixed Parameters						
Endotoxin in water (EU/ml)	25	25	1,000	1,000	38,000	38,000
Inhalation rate (m^3/h)[a]	0.42	7.32	0.42	7.32	0.42	7.32
Calculated Endotoxin in Air and Total Endotoxin Inhaled; Bedroom (27 m^3)[b]						
Endotoxin in air (EU/m^3)	905	905	36,181	36,181	1,374,891	1,374,891
Total endotoxin inhaled in 1 h (EU & [μg])	380 [0.04]	6,621 [**0.7**]	15,196 [**1.5**]	264,847 [**26**]	577,454 [**58**]	10,064,200 [**1,006**]
Total endotoxin inhaled in 8 h (EU & [μg])	3,039 [0.3]	52,969 [**5.3**]	121,569 [**12**]	2,118,779 [**212**]	4,619,633 [**462**]	80,513,597 [**8,051**]
Calculated Endotoxin in Air and Total Endotoxin Inhaled; Bedroom (27 m^3)[c]						
Endotoxin in air (EU/m^3)	1,707	1,707	68,267	68,267	2,594,133	2,594,133
Total endotoxin inhaled in 1 h (EU & [μg])	717 [0.07]	12,493 [**1.3**]	28,672 [**2.9**]	499,712 [**50**]	1,089,536 [**109**]	18,989,056 [**1,899**]
Total endotoxin inhaled in 8 h (EU & [μg])	5,734 [**0.6**]	99,942 [**10**]	229,376 [**23**]	3,997,696 [**400**]	8,716,288 [**872**]	151,912,448 [**15,191**]

Values in bold exceed 0.5 μg threshold for symptoms related to endotoxin inhalation.
[a]For a 20 year old; 0.43 m^3/h = sedentary, 7.32 m^3/h = maximal.
[b]Degree of mixing factor (M) = 0.53, F = 0.6.
[c]Degree of mixing factor (M) = 1.0, F = 0.6.

Table 10 | Conditions under which inhaled endotoxin is calculated to be ≥0.5[•] μg[a]; ≥50[••] μg[b]; and ≥500[•••] μg[c]

	Humidifier type								
	Ultrasonic			Impeller (F = 0.75[d])			Impeller (F = 0.6[d])		
Aqueous endotoxin (EU/ml)	25	1,000	38,000	25	1,000	38,000	25	1,000	38,000
1 h exposure									
Sedentary[e]		•	••		•	••		•	••
Midpoint	•	•	•••	•	•	•••	•	•	•••
Maximal	•	••	•••	•	••	•••	•	••	•••
8 h exposure									
Sedentary	•	•	•••	•	••	•••	•	•	•••
Midpoint	•	••	•••	••	••	•••	•	••	•••
Maximal	•	•••	•••	••	•••	•••	•	••	•••

[a]No observed effect limit for specified humidifier types.
[b]Fever-inducing inhaled endotoxin dose for specified humidifier types.
[c]10 times fever-inducing inhaled endotoxin dose for specified humidifier types.
[d]Proportion of respirable particulate (<2.5 μm).
[e]Respiration rates: sedentary = 0.42 m³/h, midpoint = 3.45 m³/h, maximal = 7.32 m³/h.

infection, a dramatic response to endotoxin can be an over reaction in terms of the actual threat posed by the endotoxin. Caroff et al. 2002 (summarizing several studies) placed the cytokine-inducing capacity (CIC) of bacterial species into three categories: weak, enterobacterial-strength, and strong, with some species falling into more than one category. This arises since some LPS can have different CIC on different cytokines. Even if the issue of CIC were not so complex, information on CIC is not complete, especially when it comes to environmental strains. Information on the species origin of endotoxin being measured in air is often not available. To complicate matters further, LPS also stimulates other cells such as dendritic cells to undergo a maturation process that involves the expression of co-stimulatory molecules, HLA molecules, and production of cytokines and chemokines (Caroff et al. 2002). It would, therefore, be useful for future research to specify from which species the endotoxin being studied originated. Realistically this may be difficult to do.

A related problem is variable response in test subjects. Asthmatics and those with other breathing-related con-ditions are often more susceptible to endotoxin. Endotoxin exposure can also make these hypersensitive individuals more susceptible to allergens (Boehlecke et al. 2003); Michel (1998) notes that exposure to domestic allergen provokes extravasation of LPS-binding protein and sCD-14. The presence of these compounds in the bronchoalveolar compartment may greatly enhance the capacity of inhaled endotoxin to activate the inflammatory cascade, leading to air way disease. This suggests that in certain allergy sufferers, the no-response threshold value of endotoxin exposure is probably substantially less than the 0.5 μg inhaled no-response threshold to acute inhalation of LPS which has been calculated for non-allergic individuals (Michel 1998). This could, to some extent, be overcome by selecting highly susceptible individuals for testing and setting guidelines or regulations to protect the most easily affected individuals.

The fact that there are two mechanisms leading to human endotoxicosis is often understated in the occupational literature. The primary and most widely invoked mechanism results in toxicity when endotoxin encounters

CD14 and lipopolysaccharide-binding-protein (LBP) in plasma. The second mechanism (DeLucca *et al.* 1988) involves the binding of LPS to pulmonary surfactants. This can result in altered surface tension in the lung and changes in pulmonary function. Overall, additional work on the second mechanism, and the relative importance of the two, is warranted.

The final major area of uncertainty in airborne endotoxin risk assessment involves variability in the quantification of exposure by the currently available sampling and analytical methods. Sampling, which often involves elution of airborne endotoxin from filters, may suffer from relatively unquantified losses at each point in the process (sampling, elution, adsorption, and storage). Douwes *et al.* (1995) showed a seven-fold higher endotoxin yield when 0.05% v/v of Tween-80 was added to the elution medium (endotoxin-free water). If this observation is generally applicable, endotoxin concentrations in many studies conducted to-date could be substantially underestimated.

The evaluation performed above shows that ultrasonic and impeller type humidifiers can theoretically produce sufficient levels of endotoxin to facilitate the inhalation of endotoxin above the NOEL of 0.5 μg. This can occur even at typical distribution system endotoxin levels (25 EU/ml). Calculations also show that, for scenarios where higher endotoxin levels in water are present, the inhaled doses are well beyond what would elicit chills and fever. While it could be argued that definitive conclusions cannot be drawn on the basis of uncertainties in the above discussion it would be prudent to minimize exposure to high levels of aerosolized endotoxin. This could be accomplished by introducing procedures or treatments to ensure that levels in drinking water are low. However, it will not be possible to completely keep endotoxin out of treated drinking water as biofilms are present in the distribution system. Another alternative would be to use deionized water for humidifiers but even deionized water can contain endotoxin.

The total amount of endotoxin inhaled is dependent on inhalation rate, which would normally be lower in a bedroom (assuming that the primary activity is sleep). However, humidifiers are often used when someone is suffering from illnesses such as colds or influenza. These subjects are already compromised and may be breathing more heavily if they are experiencing discomfort. It should also be recalled that these

calculations were done assuming breathing rates of a healthy 20 year old. This was done to compare with findings from controlled inhalation studies. A necessary follow-up from this work will be to demonstrate that predicted respirable endotoxin concentrations can be verified in controlled laboratory tests. This was beyond the scope of the present study due to the requirement for a test environment in which air flow can be monitored and controlled.

CONCLUSIONS

Airborne endotoxin originating in water has been previously linked to adverse human health effects. Identified episodes are typically linked to secondary use of drinking water (e.g. in air around swimming pools, baths, and saunas). Saunas, humidifiers, and to some extent showers, can produce sufficient quantities of aerosolized products to be of concern, particularly with respect to volatile organic compounds. Endotoxin is, however, nonvolatile and becomes airborne only in association with dust particles and water droplets, either in small aggregates/membrane vesicles, or in association with bacteria.

On the basis of this theoretical exercise the following conclusions can be drawn:

- Ultrasonic humidifiers are potentially capable of discharging large amounts of respirable endotoxin in short periods of time, even without accounting for potential growth of bacteria in tanks or reservoirs.
- While not as efficient as ultrasonic humidifiers, impeller-type humidifiers are nonetheless capable of significantly increasing respirable endotoxin concentrations in short periods of time.
- Even at typical distribution system endotoxin concentrations (25 EU/ml) the potential for adverse health effects associated with humidifier use exists.
- At endotoxin concentrations that can occur following shutdown and subsequent restart of biological drinking water filters, and those associated with cyanobacterial blooms, it is theoretically possible to inhale sufficient quantities to cause chills and fever, and potentially more severe symptoms.
- It would appear that the quantities of endotoxin that are aerosolized in showers are not substantial enough to be

of human health concern at typical distribution system endotoxin concentrations.

- If drinking water was drawn in the vicinity of a cyanobacterial bloom containing high levels of endotoxin, short-term health consequences such as those associated with airway inflammation could occur from showering. Chills and fever would not be expected.

- In order to keep endotoxin levels in finished drinking water low it is suggested that drinking water biofilters be operated in such a manner as to preclude activities that will allow endotoxin to accumulate in biological processes. One of the most important mechanisms by which endotoxin can accumulate is the shutdown of biologically active filters, followed by subsequent restarting without immediately backwashing or sending the first 5 pore volumes to waste (Anderson 2004).

- If controlled laboratory studies show that the risks of endotoxin exposure associated with ultrasonic and impeller type humidifiers are as substantial as predicted, humidifier operation and potential design should be re-examined.

- This study did not consider aerosolization of bacteria. Given the efficiency of humidifiers to produce respirable particles this may be worth investigating.

REFERENCES

Alexis, N. E., Eldridge, M., Reed, W., Bromberg, P. & Peden, D. B. 2001 CD14-dependent airway neutrophil response to inhaled LPS: role of atopy. *J. Allergy Clin. Immunol.* **107**, 31–35.

Alexis, N. E., Eldridge, M. W. & Peden, D. B. 2003 Effect of inhaled endotoxin on airway and circulating inflammatory cell phagocytosis and CD11b expression in atopic asthmatic subjects. *J. Allergy Clin. Immunol.* **112**, 353–361.

Alzona, J., Cohen, B. L., Rudolph, H., Jow, H. N. & Frohlinger, J. O. 1979 Indoor-outdoor relationships for airborne particulate matter of outdoor origin. *Atmos. Environ.* **13**(1), 55–60.

American Industrial Health Council (AIHC) 1994 Exposure Factors Sourcebook, Washington, DC.

American Thoracic Society (ATS) 1998 Respiratory health hazards in agriculture. *Am. J. Respir. Crit. Care Med.* **158**(5) Part 2, S1–S76 (Supplement).

Anderson, W. B. 2004 *Endotoxin in Drinking Water: Mechanisms of Release from Biofilms and Response to Treatment Technologies*. Ph.D. Thesis. Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.

Anderson, W. B., Slawson, R. M. & Mayfield, C. I. 2002 A review of drinking-water-associated endotoxin, including potential routes of human exposure. *Can. J. Microbiol.* **48**(7), 567–587.

Aro, S., Muittari, A. & Virtanen, P. 1980 Bathing fever epidemic of unknown aetiology in Finland. *Intern. J. Epidemiol.* **9**, 215–218.

Bauer, T. M., Schwacha, H., Steinbrückner, B., Brinkmann, F. E., Ditzen, A. K., Aponte, J. J., Pelz, K., Berger, D., Kist, M. & Blum, H. E. 2002 Small intestinal bacterial overgrowth in human cirrhosis is associated with systemic endotoxemia. *Am. J. Gastroenterology* **97**(9), 2364–2370.

Boehlecke, B., Hazucha, M., Alexis, N. E., Jacobs, R., Reist, P., Bromberg, P. A. & Peden, D. B. 2003 Low-dose airborne endotoxin exposure enhances responsiveness to inhaled allergen in atopic asthmatics. *J. Allergy Clin. Immunol.* **112**(6), 1241–1242.

Braude, A. I. 1982 Bacterial Endotoxins, Chapter 6. In: *Microbiology* (ed. A. I. Braude, C. E. Davis & J. Fierer), W.B. Saunders Company, Toronto, Ontario, pp. 63–75.

British Pharmacopeia 1994 International Edition, Crown Copyright.

Burger, J. S., Grabow, W. O. K. & Kfir, R. 1989 Detection of endotoxins in reclaimed and conventionally treated drinking water. *Wat. Res.* **23**, 733–738.

Buttke, T. M. & Ingram, L. O. 1975 Comparison of lipopolysaccharides from *Agmenellum quadruplicatum* to *Escherichia coli* and *Salmonella typhimurium* by using thin layer chromatography. *J. Bacteriol.* **124**, 1566–1573.

Caradonna, L., Amati, L., Magrone, T., Pellegrino, N.M., Jirillo, E., and Caccavo, C. (2000). Lagda, N.L.(ed.). **6**, pp. 205–214.

Caroff, M., Karibian, D., Cavaillon, J.-M. & Haeffner-Cavaillon, N. 2002 Structural and functional analyses of bacterial lipopolysaccharides. *Microbes and Infection* **4**, 915–926.

Castellan, R. M., Olenchock, S. A., Kinsley, B. S. & Hankinson, J. L. 1987 Inhaled endotoxin and decreased spirometric values. *New Engl. J. Med.* **317**(10), 605–610.

Chang, C.-W., Chung, H., Huang, C.-F. & Su, H.-J. J. 2001 Exposure assessment to airborne endotoxin, dust, ammonia, hydrogen sulfide, and carbon dioxide in open style swine houses. *Ann. Occup. Hyg.* **45**(6), 457–465.

Christiani, D. C., Velazquez, A., Wilcox, M. & Olenchock, S. A. 1993 Airborne endotoxin concentrations in various work areas within a cotton mill in Central America. *Environ. Res.* **60**, 187–192.

Dahlqvist, M., Johard, U., Alexandersson, R., Bergstrom, B., Ekholm, U., Eklund, A., Milosevich, B., Tornling, G. & Ulfvarson, U. 1992 Lung function and precipitating antibodies in low exposed wood trimmers in Sweden. *Am. J. Ind. Med.* **21**(4), 549–559.

Davis, W. B., Barsoum, I. S., Ramwell, P. W. & Yeager, H. Jr. 1980 Human alveolar macrophages: effects of endotoxin *in vitro*. *Infect. Immun.* **30**, 753–758.

DeLucca, A. J. II, Brogden, K. A. & Engren, R. 1988 *Enterobacter agglomerans* lipopolysaccharide-induced changes in pulmonary surfactant as a factor in the pathogenesis of byssinosis. *J. Clin. Microbiol.* **26**, 778–780.

Douwes, J., Versloot, P., Hollander, A., Heederik, D. & Doekes, G. 1995 The influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl. Environ. Microbiol.* **61**, 1763–1769.

Douwes, J., McLean, D., van der Maarl, E., Heederik, D. & Pearce, N. 2000 Worker exposures to airborne dust, endotoxin and β(1,3)-glucan in two New Zealand sawmills. *Am. J. Indust. Med.* **38**, 426–430.

Eldridge, M. W. & Peden, D. B. 2000 Allergy provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma. *J. Allergy Clin. Immunol.* **105**(3), 475–481.

Fishwick, D., Allan, L. J., Wright, A. & Curran, A. D. 2001 Assessment of exposure to organic dust in a hemp processing plant. *Ann. Occup. Hyg.* **45**(7), 577–583.

Gunderson, E. C. & Witham, C. L. 1988 *Determination of Inhalable Nonvolatile Salts in a Shower*. SRI Project No. PYC-6631. SRI International, Menlo Park, California.

Hansen, L. A., Poulsen, O. M. & Wurtz, H. 1999 Endotoxin potency in the A549 lung epithelial cell bioassay and the *Limulus* amebocyte lysate assay. *J. Immunol. Methods* **226**, 49–58.

Hartung, J. & Seedorf, J. 1999 Orienting endotoxin measurement in the atmosphere. *Dtsch. Tierarztl. Wochenschr.* **106**(12), 552–555.

Health Council of the Netherlands: Dutch Expert Committee on Occupational Standards (DECOS) 1998 *Endotoxins*. Publication No. 1998/03WGD. Health Council of the Netherlands, Rijswijk.

Highsmith, V. R., Rodes, C. E. & Hardy, R. J. 1988 Indoor particle concentrations associated with the use of tap water in portable home humidifiers. *Environ. Sci. Technol.* **22**(9), 1109–1112.

Highsmith, V. R., Hardy, R. J., Costa, D. L. & Germani, M. S. 1992 Physical and chemical characterization of indoor aerosols resulting from the use of tap water in portable home humidifiers. *Environ. Sci. Technol.* **26**(4), 673–680.

Hinds, W. C. 1982 *Aerosol Technology*. John Wiley & Sons, New York.

Hofmann, W., Martonen, T. B. & Menache, M. G. 1989 Age-dependent lung dosimetry of radon progeny. In: *Extrapolation of Dosimetric Relationships for Inhaled Particles and Gases*. (ed. J. D. Crapo, F. J. Miller, E. D. Smolko, J. A. Graham & A. W. Hayes), pp. 317–327. Academic Press, New York.

James, I. R. & Knuiman, M. W. 1987 An application of Bayes methodology to the analysis of diary records from a water use study. *J. Am. Stat. Assoc.* **82**(399), 705–711.

Keatin, G. A. & McKone, T. E. 1992 Measurements and evaluation of the water-to-air transfer and air concentration for trichloroethylene in a shower chamber. In: *Symposium on modeling of indoor air quality and exposure, ASTM STP 1205* (ed. N. L. Lagda). American Society for Testing and Materials, Pittsburgh, PA, NTIS/DE93007498, pp. 14–24.

Keman, S., Jetten, M., Douwes, J. & Borm, P. J. A. 1998 Longitudinal changes in inflammatory markers in nasal lavage of cotton workers: relation to endotoxin exposure and lung

function changes. *Int. Arch. Occup. Environ. Health* **71**(2), 131–137.

Kline, J. N., Cowden, D., Hunninghake, G. W., Schutte, B., Watt, J. L., Wohlford-Lenane, C. L., Powers, L. S., Jones, M. P. & Schwartz, D. A. 1999 Viable airway responsiveness to inhaled endotoxin. *Am. J. Respir. Crit. Care Med.* **160**, 297–303.

Kohler, P. F., Gross, G., Salvaggio, J. & Hawkins, J. 1976 Humidifier lung-hypersensitivity pneumonitis related to thermotolerant bacterial aerosols. *Chest* **69**, 294.

Koyama, S., Sato, E., Nomura, H., Kubo, K., Miura, M., Yamashita, T., Nagai, S. & Izumi, T. 2000 The potential of various lipopolysaccharides to release IL-8 and G-CSF. *Am. J. Physiol. Lung Cell. Mol. Physiol* **278**, L658–L666.

Laitinen, S., Nevalainen, A., Kotimaa, M., Liesivuori, J. & Martikainen, P. J. 1992 Relationship between bacterial counts and endotoxin concentrations in the air of wastewater treatment plants. *Appl. Environ. Microbiol.* **58**, 3774–3776.

Laude-Sharp, M., Haeffner-Cavaillon, N., Caroff, M., Lantreibecq, F., Pusineri, C. & Kazatchine, M. D. 1990 Dissociation between the interleukin 1-inducing capacity and *Limulus* reactivity of lipopolysaccharides from gram-negative bacteria. *Cytokine* **2**, 253–258.

Louhelainen, K., Kallunki, H., Kangas, J. & Kallionpää, M. 1997 FARM 2001 – Chemical health hazards in modern Finnish cowhouses. Kuopio Regional Institute of Occupational Health, FIN-70701, Kuopio, Finland, web site (viewed on Jan. 14, 2002): www.occuphealth.fi/ttl/osasto/kattl/tutkimus/maametsa/chh_cow.htm.

Mandryk, J., Alwis, K. U. & Hocking, A. D. 1999 Work-related symptoms and dose-response relationships for personal exposures and pulmonary function among woodworkers. *Am. J. Ind. Med.* **35**, 481–490.

Mandryk, J., Alwis, K. U. & Hocking, A. D. 2000 Effects of personal exposures on pulmonary function and work-related symptoms among sawmill workers. *Ann. Occup. Hyg.* **44**(4), 281–289.

McGregor, F. R., Walenczak, W. D., Rogers, R. & Magnetti, L. 1993 Case study: ozone-based water treatment for high quality air and water in a municipal swimming center. In: *Ozone in Water and Wastewater Treatment*. Proceedings, International Ozone Association's 11th Ozone World Congress, San Francisco International Ozone Association Pan American Committee, Stanford, CT, pp. S-12A-1-8.

Melbostad, E. & Eduard, E. 2001 Organic dust-related respiratory and eye irritation in Norwegian farmers. *Am. J. Indust. Med.* **39**, 209–217.

Mercer, R. R. 1999 Respiratory uptake. In: *Exposure to Contaminants in Drinking Water* (ed. S. S. Olin), CRC Press LLC, Boca Raton, Florida, pp. 125–135.

Mercer, R. R., Anjilvel, S., Miller, F. J. & Crapo, J. D. 1991 Inhomogeneity of ventilatory unit volume and its effects on reactive gas uptake. *J. Appl. Physiol.* **70**, 2193.

Metzger, J. W., Patterson, R., Fink, J., Smerdijan, R. & Roberts, M. 1976 Sauna-takers' disease: hypersensitivity pneumonitis due to contaminated water in a home sauna. *Journ. Amer. Med. Assoc.* **236**, 2209.

Michel, O. 1998 The role of endotoxin exposure in asthma. *Res. Immunol.* **149**, 27–228.

Michel, O., Nagy, A.-M., Schroeven, M., Duchateau, J., Neve, J., Fondu, P. & Sergysels, R. 1997 Dose-response relationship to inhaled endotoxin in normal subjects. *Am. J. Respir. Crit. Care Med* **156**(4), 1157–1164.

Mills, W. B., Lew, C. S. & Loh, J. Y. 1998 Predictions of potential health and ecological risks from power plant discharges of total residual chlorine and chloroform into rivers. *Environ. Sci. Technol.* **32**(14), 2162–2171.

Milton, D. K., Wypij, D., Kriebel, D., Walters, M. D., Hammond, S. K. & Evans, J. S. 1996 Endotoxin exposure-response in a fiberglass manufacturing facility. *Am. J. Indust. Med.* **29**(1), 3–13.

Morrison, D. C., Danner, R. L., Dinarello, C. A., Munford, R. S., Natanson, C., Pollack, M., Spitzer, J. J., Ulevitch, R. J., Vogel, S. N. & McSweegan, E. 1994 Bacterial endotoxins and pathogenesis of gram-negative infections: current status and future direction. *J. Endotoxin Res.* **1**(2), 71–83.

Muittari, A., Rylander, R. & Salkinoja-Salonen, M. 1980a Endotoxin and bath-water fever. *Lancet II* **8185**, 89.

Muittari, A., Kuusisto, P., Virtanen, P., Sovijärvi, A., Grönroos, P., Harmoinen, A., Antila, P. & Kellomäki, L. 1980b An epidemic of intrinsic allergic alveolitus caused by tap water. *Clin. Allergy* **10**, 77–90.

NIOSH (National Institute of Occupational Safety and Health) 1994 *Request for Assistance in Preventing Toxic Organic Dust Toxic Syndrome.* Publication No. 94–102.

Owen, M. K., Ensor, D. S. & Sparks, L. E. 1992 Airborne particle sizes and sources found in indoor air. *Atmos. Environ.* **26A**, 2149–2162.

Page, N. P. 1994 Human health risk assessment. In: *Basic Environmental Toxicology* (ed. L. G. Cockerham & B. S. Shane). CRC Press, Boca Raton, Florida. pp. 561–581.

Palchak, R. B., Cohen, R., Ainslie, M. & Lax Hoerner, C. 1988 Airborne endotoxin associated with industrial-scale production of protein products in Gram-negative bacteria. *Am. Ind. Hyg. Assoc. J.* **49**, 420–421.

Pandis, S. N. & Davidson, C. 1999 Aerosols and water droplets. *Exposure to Contaminants in Drinking Water* (ed. S. S. Olin). CRC Press LLC, Boca Raton, Florida, pp. 101–110.

Peppler, M. S., Huck, P. M., Flowerdew, G. & Anderson, W. B. 1995 *Production of Endotoxin in a Biological Drinking Water Treatment Process.* In: Proceedings, American Water Works Association 1994 Water Quality Technology Conference (San Francisco). American Water Works Association, Denver, Colorado, pp. 1365–1396.

Pickering, C. A. C., Moore, W. K. S., Lacey, J., Holford-Srevans, V. C. & Pepys, J. 1976 Investigation of a respiratory disease associated with an air-conditioning system. *Clinical Allergy* **6**, 109.

Prescott, L. M., Harley, J. P. & Klein, D. A. 2002 Chapter 34.3 Pathogenesis of Bacterial Diseases. In: *Microbiology* Fifth Edition. McGraw-Hill Companies, Inc., New York, NY, pp. 799–801.

Rao, H. V. & Brown, D. R. 1993 A physiologically based pharmaco-kinetic assessment of tetrachloroethylene in groundwater for a bathing and showering determination. *Risk Anal.* **13**, 37.

Rapala, J., Lahti, K., Räsänen, L. A., Esala, A.-L., Niemelä, S. I. & Sivonen, K. 2002 Endotoxins associated with cyanobacteria and their removal during drinking water treatment. *Wat. Res.* **36**(10), 2627–2635.

Reiman, M. & Uitti, J. 2000 Exposure to microbes, endotoxins, and total dust in cigarette and cigar manufacturing: an evaluation of health hazards. *Ann. Occup. Hyg.* **44**(6), 467–473.

Rix, B. A. & Lynge, E. 1996 Industrial hygiene measurements in a new industry: the repulping and deinking of paper waste. *Am. J. Indust. Med.* **30**(2), 142–147.

Rylander, R. 1987 The role of endotoxin for reactions after exposure to cotton dust. *Am. J. Ind. Med.* **12**, 687–697.

Rylander, R. 1997 The endotoxin criteria document: the risk evaluation. In: *Proceedings of the 21st Cotton and Organic Dusts Research Conference-Beltwise Cotton Conferences* (ed. P. J. Wakelyn, R. R. Jacobs & R. Rylander), Natural Cotton Council, Memphis, Tenn, pp. 153–156.

Rylander, R. 1999 Health effects among workers in sewage treatment plants. *Occup. Environ. Med.* **56**, 354–357.

Rylander, R., Haglind, P. L., Lundholm, M., Mattsby, I. & Stenqvist, K. 1978 Humidifier fever and endotoxin exposure. *Clinical Allergy* **8**, 511–516.

Rylander, R. & Haglind, P. 1984 Airborne endotoxins and humidifier disease. *Clinical Allergy* **14**, 109–112.

Rylander, R., Haglind, P. L. & Lundholm, M. 1985 Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. *Am. Rev. Resp. Dis.* **131**(2), 209–213.

Rylander, R. & Peterson, Y. 1993 *Second Glucan Inhalation Toxicity Workshop. ICOH Committee on Organic Dusts report.* Health Council of the Netherlands, Rijswijk.

Schlesinger, R. B. 1985 Comparative deposition of inhaled aerosols in experimental animals and humans. *J. Toxicol. Environ. Health* **15**, 197.

Seinfeld, J. H. & Pandis, S. N. 1997 *Atmospheric Chemistry and Physics: from Air Pollution to Global Change.* John Wiley & Sons, New York.

Smid, T., Heederik, D., Houba, R. & Quanjer, P. H. 1994 Dust- and endotoxin-related acute lung function changes and work-related symptoms in workers in the animal feed industry. *Am. J. Crit. Care Med.* **149**, 1407–1412.

Stone, K. C., Mercer, R. R., Gehr, P., Stockstill, D. & Crapo, J. D. 1992 Allometric relationships of cell numbers and size in the mammalian lung. *Am. J. Respir. Cell Mol. Biol.* **6**, 235.

Su, H.-J. J., Chen, H.-L., Huang, C.-F., Lin, C.-Y., Li, F.-C. & Milton, D. K. 2002 Airborne fungi and endotoxin concentrations in different areas within textile plants in Taiwan: A 3-year study. *Environ. Res. Section A* **89**, 58–65.

Sykora, J. L. & Keleti, G. 1981 Cyanobacteria and endotoxins in drinking water supplies. In: *The Water Environment, Algal Toxins and Health* (ed. W. W. Carmichael), pp. 285–301. Plenum Press, New York.

Teeuw, K. B., Vandenbroucke-Grauls, C. M. & Verhoef, J. 1994 Airborne gram-negative bacteria and endotoxin in sick

building syndrome. A study in Dutch governmental buildings. *Arch. Intern. Med.* **154**(20), 2339–2345.

Thorn, J. & Rylander, R. 1998 Inflammatory response after inhalation of bacterial endotoxin assessed by the induced sputum technique. *Thorax* **53**(12), 1047–1052.

United States Pharmacopeia 1995 USP 23. Rand McNally. pp. 1184.

USEPA (United States Environmental Protection Agency) 1989 *Exposure Factors Handbook. Office of Health and Environmental Assessment*. U.S. Environmental Protection Agency, Washington, DC.

USEPA (United States Environmental Protection Agency) 1992 *Dermal Exposure Assessment: Principles and Applications*. Report No. EPA/600/891/011B. Office of Health and Environmental Assessment. U.S. Environmental Protection Agency, Washington, DC.

USEPA (United States Environmental Protection Agency) 1996 Reference residence. In: *Office of Research and Development, National Center for Environmental Assessment, Exposure Factors Handbook*. EPA/600/P-95-002Bc, Vol. 3 of 3. U.S. EPA, Washington, D.C. pp. 16–33 (www.epa.gov/ORD/WebPubs/exposure).

Vogelzang, P. F. J., van der Gulden, J. W. J., Folgering, H., Kolk, J. J., Heederik, D., Preller, L. M. J. M., Tielen, M. J. M. & van Schayck, C. P. 1998 Endotoxin exposure as a major determinant of lung function decline in pig farmers. *Am. J. Respir. Crit. Care Med.* **157**(1), 15–18.

Walters, M., Milton, D., Larsson, L. & Ford, T. 1994 Airborne environmental endotoxin: A cross-validation of sampling and analysis techniques. *Appl. Environ. Microbiol.* **60**, 996–1005.

Weibel, E. R. 1983 Is the lung built reasonably? *Am. J. Respir. Dis.* **128**, 752.

Weisel, C. P., Little, J. C., Chiu, N., Pandis, S. N., Davidson, C. & Wilkes, C. R. 1999 Developing exposure estimates. In: *Exposure to Contaminants in Drinking Water* (ed. S. S. Olin). CRC Press LLC, Boca Raton, Florida, pp. 86–89.

Zejda, J. E., Barber, E., Dosman, J. A., Olenchock, S. A., McDuffie, H. H., Rhodes, C. & Hurst, T. 1994 Respiratory health status in swine producers relates to endotoxin exposure in the presence of low dust levels. *J. Occup. Environ. Med.* **36**(1), 49–56.

Zock, J.-P., Hollander, A., Heederik, D. & Douwes, J. 1998 Acute lung function changes and low endotoxin exposures in the potato processing industry. *Amer. J. Ind. Med.* **33**, 384–391.

Available online May 2007