Industrial Health 2013, **51**, 237–255 **Review Article**

Indoor Exposure to Airborne Endotoxin: A Review of the Literature on Sampling and Analysis Methods

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Abstract: Assessment of exposure to airborne endotoxins has been studied for several years, especially in occupational environments, but a large number of procedures are used for sampling and analysis. This lack of standardization makes it very difficult to compare results and set internationally accepted threshold limit values (TLVs) or occupational exposure limits (OELs) for endotoxin exposure. This paper reviews the methods reported, using advanced bibliographical search techniques: 82 papers published from 2004 to the present were selected to analyze methods for the assessment of human exposure to airborne endotoxins, with particular reference to occupational settings, and to examine their performance and critical points. Only few studies have focused on the standardization of sampling and analysis methods. The European Committee for Standardization Guidelines coincide with the procedures most frequently applied, but this does not guarantee the best results in terms of recovery and reproducibility. The factor that mainly affects endotoxin measurements is the extraction method, the main concern being the presence in the samples of a fraction insoluble in aqueous media. If substantial differences in the proportions of this fraction in different environments are confirmed in the future, the contribution of insoluble endotoxins cannot be neglected.

Key words: LAL test, Airborne endotoxin exposure, Air sampling, Analytical methods, Insoluble endotoxins, LPS marker

Introduction

Endotoxin, also known as pyrogen or fever-causing toxin, is an outer membrane component of Gram-negative bacteria made up of lipopolysaccharide (LPS). LPS comprises three components or regions: Lipid A, an R polysaccharide and an O polysaccharide. Lipid A consists of a phosphorylated N-acetylglucosamine (NAG) dimer with six or seven 3-hydroxy fatty acids (FA) attached, all saturated. Some are attached directly to the NAG dimer and others are esterified to the 3-hydroxy fatty acids that are characteristically present. The structure of the lipid A portion is fairly well conserved, but the nature (length and chemical composition) of the polysaccharide side chain varies between genera, species, and even strains of Gram-negative bacteria. The endotoxic principle of LPS resides in the lipid A domain, since polysaccharide-deprived free lipid A appears to exhibit similar endotoxic activities as intact LPS[1]. Chemical differences in the structural make up of Lipid A are reflected by biological

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differences. For example, it has been shown that *P. aeruginosa* LPS is significantly less toxic than enterobacterial preparations[2]. By analyzing synthetic *E. coli* lipid A and partial structures biologically, it has been shown that full endotoxicity is expressed only in hexaacyl preparations, whereas partial structures harbouring a smaller number of fatty acids including pentaacyl lipid A are less active[3]: the fact that the major species of the lipid A structure is a pentaacyl component may, therefore, account for the low endotoxic activity of *P. aeruginosa* LPS. Lipid A from different gram-negative bacteria displays heterogeneity due to the presence and nature of the phosphoryl substituens attached to the lipid A backbone, the type and chain length of fatty acids and the degree of O-acylation of the hydroxy fatty acids. Some authors have shown that the removal of the ester-linked fatty acids significantly reduces the LPS toxicity[4] suggesting that the ester-linked fatty acids are important factors in determining the biological activity of bacterial LPS and of lipid A.

The R polysaccharide or core antigen (R) is attached to the 6-position of one NAG and consists of a short chain of two unusual sugars, heptose and 2-keto-3-deoxyoctanoic acid (KDO).

The O polysaccharide or somatic antigen (O) is attached to the R polysaccharide. The composition of the sugars in the O side chain varies widely between species and even strains of Gram-negative bacteria. The O polysaccharide is much longer than the R polysaccharide, and maintains the hydrophilic domain of the LPS molecule.

The lipid A is a powerful biological response modifier that can stimulate the mammalian immune system; since lipid A is embedded in the outer membrane of bacterial cells, it probably only exerts toxic effects when released from multiplying cells in a soluble form, or when the bacteria are lysed[5]. Release also occurs when intact bacterial cells are phagocytized by macrophages, in which case the liberated endotoxins contain increased toxicity[6].

The most common route of exposure to airborne endotoxin is inhalation. In humans, endotoxins have been recognized as the causal agent of a variety of pathologies. Many occupational studies have reported positive associations between endotoxin exposure and respiratory disorders including infectious diseases, acute toxic effects, allergies, and asthma-like syndromes[7]. Research has shown some major clinical effects of endotoxins including chronic bronchitis and organic dust toxic syndrome (ODTS), up to lethal effects such as septic shock, organ failure and death[8]. However, in contrast, numerous studies have described seemingly protective effects of environmental endotoxin exposure on atopic asthma risk and the development of allergy in early childhood, and atopy also in adults with high occupational endotoxin exposure[9–11]. There is very consistent epidemiologic evidence of a dose-relation between endotoxin and risk reductions for lung cancer[12]. The aerodynamic particles size distribution for airborne endotoxin is an also an important element in determining endotoxin toxicity and its health effects. The European Agency for Safety and Health at Work classifies occupational exposure to endotoxins among the "top ten emerging biological risks"[13]. Endotoxin can be found in all occupational settings where there is organic dust containing particles of plant, animal or microbial origin (farming, cotton production, grain dust, swine confinement buildings, poultry houses). What was initially considered to be a problem in only a few activities has turned out to affect workers in the livestock industry, in waste and sewage treatment, scientists handling rodents, and even office workers. Air humidifiers in buildings and recycled-industrial process waters are also important sources of airborne endotoxins[14]. These components have been found in house dust, too[15].

The problem of assessing exposure to airborne endotoxins has been studied for years, but sampling and analysis procedures are still not standardized and shared. The large number of different procedures used for sampling, sample transport, storage and extraction, and analysis, makes it very difficult to compare results and to choose the best procedure. Guidelines for assessing occupational exposure, like those published by the European Committee for Standardization[16], have been criticized for leaving room for individual interpretation and non-uniform methodology. The different protocols mean there is broad inter-laboratory variability in the results of endotoxin analyses. Standardization for quantitative endotoxin measurements is therefore needed, to reach acceptable inter-laboratory precision and accuracy[17].

The need for validation of methods for these biological agents was already stressed by Douwes *et al.* in 2003, in a review article regarding bioaerosol health effects and exposure assessment[18]. Omland[19] published a review of the literature regarding exposure and respiratory health in farming, mainly from a clinical point of view, in which endotoxins were one of the risk agents considered, together with dust, bacteria, molds and ammonia; however, exposure data were reported without any description of the sampling and analysis methods. A complete review of collection and analysis methods for biological agents was published in 2004 by Martinez *et al.*[20], but endotoxins

were treated separately from other biological agents only for the analysis. Lane *et al.*[21] focused on endotoxin levels and respiratory diseases in the cotton industry and highlighted the fact that there is no standard sample collection and extraction procedure and that protocol differences influence the reproducibility of endotoxin levels measured using the Limulus Amebocyte Lysate enzyme assay (LAL test). They concluded that a uniform protocol would have a significant impact on assessment of endotoxins in the environment.

The present paper reviews the scientific literature using advanced bibliographical search techniques, reporting the different sampling and analysis methods used for the assessment of human exposure to airborne endotoxin, particularly workers, examining their performances and critical points, in order to understand what is collected and how and what is actually measured, and to identify future research needs.

Methods

Systematic review of the literature

A bibliographic search was done on the "Scopus" database from 2004 to the present, to identify potentially eligible peer-reviewed publications reporting collection and analysis methods for airborne endotoxins. The search criteria were based on the following keyword combinations: "*Airborne Endotoxin*", "*Endotoxin Airborne Analysis*", "*Airborne Endotoxin Exposure*", "*Endotoxin Air Sampling*", "*Endotoxin Analysis*" and "*Endotoxin Analytical Method*".

Data extraction and compilation

A total of 315 papers concerning exposure to endotoxin in occupational and residential settings were retrieved and examined to identify them as relevant or not to our review on the basis of the following criteria:

Inclusion criteria

- observational and experimental studies
- studies that considered airborne endotoxins
- studies of exposure in indoor/industrial environments (occupational and home)
- studies describing the sampling and/or analysis methods or giving a reference for their description.

Exclusion criteria

- reviews retrieved and examined but not included in the study
- endotoxins detected only in matrices other than air (food, drugs, biological fluids, settled dust and dust deposits).
- only outdoor studies
- articles not in English.

All articles were read carefully and the information was entered on an electronic spreadsheet with the following columns in a row for each paper: environment, sampler type, filter type and/or liquid used, sampling time, sampling rate, number of samples, storage of samples, matrix (extraction solution), extraction procedure, analytical method, and reference number. When information was not available, cells were left empty.

Results and Discussion

From the review of the literature, applying the inclusion and exclusion criteria, 82 papers were examined (Table 1). Only a quarter of these papers matched the inclusion criteria (26%), indicating that many authors do not focus closely on the method used for sampling and detection. The content of each column of the table is described below.

Environment

The first column describes the environment where the study was carried out, using a definition permitting the paper to be grouped in certain categories. The largest groups are those studying the endotoxin contamination of animal housing (24.4% of the total papers), homes (22%), agricultural environments (13.4%) and textile industry (7.3%). Some papers refer both to indoor and open-air environments, and therefore report meteorological data (air temperature, wind speed and direction, relative humidity and solar radiation)[22–28].

Madsen[27] focuses in particular on the background levels of endotoxins, that are rarely mentioned in papers, and reports endotoxin levels of different life and work environments, mainly outdoor, suggesting that these values could be used for reference by public health practitioners, epidemiologists and industrial hygienists; however, it must borne in mind that most workplaces are indoor environments. Another paper from the same author[25] is aimed to characterize the distribution of endotoxin on particles of different sizes (inhalable, thoracic and respirable) in offices and outdoor air; previous studies on agricultural environments and outdoor air showed that airborne endotoxins were associated with the airborne particulate matter > 1 µm, while in homes they were associated with smaller aerodynamic diameters, <1 µm[29].

Table 1. Sampling and analysis methods for the assessment of exposure to airborne endotoxins

Environment	Sampler type/ commercial name	Filters type (porosity)/ liquid	Sampling time	Sampling rate (l/min)	N. samples (type)	Storage of samples	Matrix	Extraction procedure	Analytical method	Ref. n.
Agricultural − Farms and l industry	Inhalable aerosol sampler/ GSP	GF (1.6 µm)		3.5	249 (filter)		PFW-Tween 20		Kinetic chromo-genic LAL	11)
Different environments (office, hospital, train station, student room, subway and commercial street) indoor and outdoor	Impinger/ BioSampler; Inhalable aerosol sampler/Buttom; vacuum cleaner	Deionized water (impinger); Gelatin (Buttom)	Impinger and Buttom: 30 min; vacuum cleaner: 5 min	12.5 (impinger) 4 (buttom)		4 °C (impinger solution and dust); −20 °C (gelatin dissolution)	Deionized water (impinger and gelatin); deionized water-tween 20 (dust)	Dust: shaking for 2 h at RT	Kinetic chromo-genic LAL	19)
Animal house − Milking cow dairy	Inhalable aerosol sampler/ Open face filters holder, Delrin	PC (1 µm)	90 min	2	81 (filter)	−20 °C	PFW-Tween 20	Sonication for 15, 30, 60 and 120 min; centrifugation	Kinetic chromo-genic LAL	22)
Homes after hurricane and outdoor	Total dust / Cassette	Teflon (2 µm)	6 h	10	14 (filter)		Triethylamine phosphate buffer	Sonication (1 h)	Kinetic chromo-genic LAL	23)
Biofuel plants, offices and outdoor	Three-stage impactor/Respicon; Triplex cyclone; closed-face cassette	Teflon (Respicon and cassette), polycarbonate (Triplex cyclone)	Respicon: 6 h 7 min (average time/day); tryplex cyclone: 6 h 58 min (average time/day) cassette: 6 h 9 min (average time/day)	3.1 (respicon) 3.5 (triplex cyclone) 1.9 (cassette)	15 (filter)	−80 °C	Teflon: PFW-Tween 20; polycarbonate: Tween 80–0.85% NaCl	Teflon: orbital shaking (300 rpm) 60 min at RT; polycarbonate: shaking (300 rpm) 15 min at RT; teflon and polycarbonate: centrifugation at 1,000 g 15 min	Kinetic chromo-genic LAL	25)
Homes, offices, outdoor	Impinger/BioSampler	Deionized water	30 min	12.5	- (liquid)	4 °C	Deionized water	Direct analysis	Kinetic chromo-genic LAL	26)
Different environments and different seasons, outdoor and indoor	Inhalable aerosol sampler/ GSP	Teflon (1 µm)	4–6 h		169 (filter)		PFW-Tween 20	Orbital shaking (300 rpm) at RT for 1 h and centrifuged (1,000· g) for 15 min	Kinetic chromo-genic LAL	27)
Biofuel plants − indoor and outdoor	Inhalable aerosol sampler/ GSP	Teflon (1 µm)	6 h	3.5	- (filter)		PFW-Tween 20	Orbital shaking (300 rpm) at RT for 1 h and centrifuged (1,000· g) for 15 min	Kinetic chromo-genic LAL	28)
Sawmills	Respirable aerosol sampler/ Casella; settled dust				10 filters and 10 settled dust	−20 °C	PFW-Tween 20	Centrifugation and supernatant heated at 75 °C for 20 min to avoid any possible interference.	Endpoint chromo-genic LAL	32)
Animal houses	Inhalable and respirable aerosol samplers	GF	day: 1 h night: 6 h	3.5 (respirable fraction); 2.0 (inhalable fraction)	140 (filter)		PFW	Rapid shaking	Kinetic chromo-genic LAL	33)

Environment	Sampler type/ commercial name	Filters type (porosity)/ liquid	Sampling time	Sampling rate (l/min)	N. samples (type)	Storage of samples	Matrix	Extraction procedure	Analytical method	Ref. n.
Animal houses − Piggeries	Respirable aerosol sampler/ Cyclone		>4h	2	191 (filter)		PFW		Kinetic chromo-genic LAL	34)
Animal houses − Piggeries	Respirable aerosol sampler/ Cyclone		8h	1.9					Kinetic chromo-genic LAL	35)
Agricultural − Grape and cytrus farm fields	High volume air sampler	PC (0.4 µm)	90 min	30	2 samples daily (filter)		PFW	Extraction for 1 h and centrifuga-tion for 10 min at 1,000 g	Kinetic chromo-genic LAL	36)
Textile industry − Jute mill	High volume air sampler / Staplex TFIA	GF			8 (filter)				Gel clot LAL	37)
Sawmills	Aerosol sampler/Cas-sette; impinger/ AGI-30	PC (0.4 µm); pyrogen free saline	4 h (aerosol sampler) − 15 min (impinger)	1.5; 12.5	25 (poly-carbonate filters and liquid)	−20 °C for 1–3 months	PFW (filter), Pyrogen free saline 0.09% (impinger)	Shaking at RT for 1 h; vigorously vortexed (filter). Vortexed for 15 min after thawing (liquid)	Kinetic chromo-genic LAL	39)
Textile industry − cotton	Vertical Elutria-tor/GMW-4000	PVC (5µm); GF (1µm)		7.4	- (filter)	RT, in the dark			Kinetic chromo-genic LAL	40)
Food industry − Gin house, offices	Vertical Elutria-tor/GMW-4000	GF	2h	7.4	15 (filter)		PFW	Shaking at RT	Kinetic chromo-genic LAL	41)
Textile industry − cotton	Vertical Elutria-tor			7.4	346 (filter)				Kinetic chromo-genic LAL	42)
Homes	Impactor/Har-vard impactor	Teflon (2.0-µm)	6–8 d	10	96–128 (filter)	−20 °C	PFW-Tween 20	Shaking for 1 h, centrifugation at 1,000 g	Kinetic chromo-genic LAL	43)
Metal Working Fluids	Low Pressure Impactor/ELPI − Impinger/ BioSampler	PC (0.2 µm); PFW	2h	30 (ELPI); 12.5 (Im-pinger)	- (filter and liquid)	4 °C	PFW	Centrifugation at 2,200 rpm at 4°C for 10 min (filters). Liquid analyzed directly.	Kinetic chromo-genic LAL	44)
Homes	Inhalable aerosol sampler/ Aerosol monitor (cassette filter)	GF (0.5 µm)	24h	3.5	140 (filter)	4 °C	PFW	Shaking at RT for 1h	Kinetic chromo-genic LAL	45)
Wastewater treatment plant	Total dust /Cas-sette; impinger/ Midget Im-pinger	GF (1 µm)/ PC (0.4 µm); PFW	30 min	1.60–1.64 (Cas-sette); 2.06–2.10 (Im-pinger)	30 (filter and liq-uid)		PFW	Sonication at 10 min intervals for 1 h (GF and PC filters). Im-pinger liquid: direct analysis	Kinetic chromo-genic LAL	46)
Homes − Urban and rural	Inhalable aerosol sampler/ IOM − Vacuum cleaner AEG Vampyr 5030	GF	18–24 h	2 − vacuum cleaner 2 min/m^2	23 (filter)	dust stored at +6° for 2 weeks	PFW	Shaking for 1 h, centrifugation at 1,000 g	Kinetic chromo-genic LAL	47)
Animal houses	Inhalable aero-sol sampler	GF	24h	3	32 (filter)				Kinetic chromo-genic LAL	48)

Environment	Sampler type/ commercial name	Filters type (porosity)/ liquid	Sampling time	Sampling rate (l/min)	N. samples (type)	Storage of samples	Matrix	Extraction procedure	Analytical method	Ref. n.
Animal houses – Duck, pig, rat and mouse stables, office archive	Inhalable aerosol sampler/ PAS-6	GF	from 30 min to 3 h	2	120 (filter)	Immediately processed or stored at −70 °C for 4 to 12 wk	PFW, PFW-Tween 20	Orbital shaking (300 rpm) at RT for 1 h and centrifuged (1,000· g) for 15 min	Kinetic chromogenic LAL	49)
Homes	Total dust / Cassette	GF	8 h	2	300 (filter)	−80 °C	PBS	Shaking at RT for 1 h; centrifugation for 10 min at 1,000 g	Kinetic chromogenic LAL	50)
Animal house – Pig farm, grass seed processing	Inhalable aerosol sampler/ PAS-6	GF (1.6 µm) and Teflon	1, 2, 3, 5, 6 h	2	386 (filter)	From 12 to 14 days: either 4 °C or 20 °C, depending on the assigned treatment	PFW, PFW -Tween 20	Horizontal shaking for 1 h at RT, centrifugation	Kinetic chromogenic LAL	51)
Dental hygienist	Impinger/ AGI-30	Saline solution (0.85%)	16 min	12.5	12 (liquid)	−20 °C (within 6 month)	Saline solution 0.85%		Gel clot LAL	52)
Homes	Total dust / Cassette	PVC (5 µm)	5–5.30	2	16 (filter)		Peptone water-Tween 80	Soaking for 10 min; vortexing for 2 min and shaking for 15 min	Glucatell assay	53)
Agricultural – Grass seed production, pig farm, household-waste composting, potato processing, sewage treatment	Inhalable aerosol sampler/ PAS-6	GF (1.6 µm)	1–8 h		250 (filter)	from 56 to 90 days at −20 °C	PFW, PFW-Tween 20, PFW-Tris, PFW-triethyl-amine-phosphate [TAP]	Horizontal shaking for 10 min and 1 h; centrifugation at 1,000 g for 15 min	Kinetic chromogenic LAL	54)
Animal houses, hay storage barns	Single-unit aspirator/AP-2A	GF (1.0 µm)	30 min	2	25 (filter)	−15 °C	PFW	Extraction 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Endpoint chromogenic LAL and GC-MS/MS	57)
Animal house – Industrial poultry hatchery	Stationary sampler/AS-50	Polypropylene		50	10 before biofilter, 25 after (filter)		PFW	Extraction 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Gel clot LAL	58)
Agricultural – medicinal herbs	Single-unit aspirator/AP-2A; stationary sampler/AS-50	GF (1 µm) and PVC	30 min	2 (AP-2A); −50 (AS-50)	13 sites (filter)		PFW	Extraction 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Gel clot LAL	59)
Textile industry – flax farms	Stationary sampler/AS-50	PVC			10 (filter)		PFW	Extraction 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Gel clot LAL	60)

Environment	Sampler type/ commercial name	Filters type (porosity)/ liquid	Sampling time	Sampling rate (l/min)	N. samples (type)	Storage of samples	Matrix	Extraction procedure	Analytical method	Ref. n.
Agricultural facilities (farms, food, cotton, grain)	Single-unit aspirator/AP-2A	GF (1 µm)		2	14 sites (filter)		PFW	Extraction 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Gel clot LAL	61)
Agricultural environments, homes	Settled dust / Omega HEPA vacuum					−20 °C	PFW-Tween 20	Vortex for 1 min followed by 1 h at 100 rpm on a rotary mixer	Recombinant factor C assay (rFC)	62)
Animal houses − pig, chicken, turkey, cows, horses	Inhalable aerosol sampler/ IOM and Buttom; closed-face cassette; Respirable aerosol sampler/ Cyclone	Polyvinyl chloride (5 µm)	From 8 to 12 h	2 (IOM and cassette); 4 (Buttom); 2.5 (Cyclone)	804	4 °C	PFW-Tween 20	Shaking for 2 h at RT; centrifugation at 600 × g for 15 min	Kinetic chromogenic LAL and rFC assay	63)
Homes − Student rooms		Teflon (0.5 µm)	5 h	18	14 (filter)	−20 °C	Heptane (GC-MS/MS); 0.01% (v/v) triethylamine in distilled water (LAL assay)	Sonication for 30 min: the extract was neutralised by the addition of 200 ml of 1 M Tris-HCl, pH 7.5	Gel clot LAL and GC-MS/ MS	65)
Animal house − Chicken and swine barns; corn processing	Total dust / Cassette	GF	from 10 to 50 min	2	14 (filter)	Cassettes containing silica gel dessiccant at 4 °C			Interlaboratory comparison: endpoint chromogenic LAL, kinetic chromogenic LAL and GC/ MS of fatty acids	66)
Biotech − Plant of BSCP (bacterial single cell protein)	Inhalable aerosol sampler/ PAS-6	GF	Whole work shift	2	42 (filter)		Plasma of 8 workers in parallel with environmental monitoring		Kinetic chromogenic LAL	67)
Different environments (animal houses and workers)	Total dust / Cassette	PTFE		0.2–3.5	- (filter)		PFW-Tween 20 and human whole blood (environmental and biological monitoring in parallel)	Orbital shaking for 1 h at RT, centrifugation at 1,000 g for 10 min	Kinetic chromogenic LAL − Endosafe IPT (Blood Elisa for IL-1β) Comparison between methods	68)
Homes	Impinger/Bio-Sampler − Surface sampler/ HVS3	Deionized water	30 min; − 12.5 (Impinger); 5 min (HVS3)	12.5	- (liquid)	4 °C (1 d)	Deionized water-0.05% Tween 20 (impinger and HVS3)	Direct analysis (impinger); Shaking for 2 h (1,100 rpm) at RT-vortex for 1 min	Kinetic chromogenic LAL	70)

Environment	Sampler type/ commercial name	Filters type (porosity)/ liquid	Sampling time	Sampling rate (l/min)	N. samples (type)	Storage of samples	Matrix	Extraction procedure	Analytical method	Ref. n.
Homes	Passive dust sampler/ECD		4 wk		120	RT; −20 °C	PFW; PFW-Tween 20; PBS-Tween 20	Shaking for 1 h at RT; centrifugation for 15 min	Kinetic chromo-genic LAL	71)
Single case – SPA centres	Aerosol sampler (personal and stationary)	PC	25 min	2					Endpoint chromo-genic LAL	72)
Animal houses – Livestock and poultry	Impinger	PFW	20 min	12.5	- (filter)		PFW		Gel clot LAL	73)
Wastewater treatment plant	Impinger/Bio-Sampler	PBS		12.5	- (liquid)		PBS	Direct analysis	Endpoint chromo-genic LAL	74)
Homes	Inhalable aero-sol sampler	PC (0.4 µm)	average of 1.5 d	2	360 (main study); 82 (valida-tion study) (filter)				Kinetic chromo-genic LAL	75)
Animal house – Poultry	Inhalable aero-sol sampler	PC (0.8 µm)	30–60 min	2	82 (filter)	RT, in dark and dry con-ditions	PFW	Shaking for 15 min	Kinetic chromo-genic LAL	76)
Single case – Solid waste treatment plant	Inhalable aero-sol sampler	GF	2		33 (filter)				Kinetic chromo-genic LAL	77)
Wastewater treatment plant	Inhalable aerosol sampler (stationary point and personal sampling)/End-ofree cassette	PC (0.4 µm)	Stationary point: 4h; personal sampling: 22–170 min	1.5	- (filter)	−20° C for 1–3 months	PFW	Shaking at RT for 1 h; extraction solution vortexed vigorously	Kinetic chromo-genic LAL	78)
Homes	Inhalable aerosol sampler/ Buttom Settled dust /vacuum cleaner Imping-er/BioSampler	PC (2 µm); HEPA filter	24 h (but-tom) 5 min (vacuum cleaner); 15 min (impinger)	4 (but-tom) 12.5 (im-pinger)		−20 °C	PFW (dust) PFW-Tween 20 (filter)	Sonication for 1h; centrifugation at 7,000 rpm for 1 min (Dust)	Kinetic chromo-genic LAL	79)
Homes	Total dust / Cassette	Teflon (2 µm)	20 min	2.5	- (filter)		PFW-Tween 20	Shaking for 1 h at 25 °C; vortex	Kinetic chromo-genic LAL	80)
Animal house – Equine stable	Total dust / Cassette	PC (0.4 µm)	4 h	2	- (filter)		PFW		Kinetic turbidimet-ric LAL	81)
Homes	Total dust / Cassette	PC	5–7 d	2	75 (filter)	RT under dry con-ditions	PFW-Tween 20	Shaking for 2.5 h on a shaker set at 220 rpm	Endpoint chromo-genic LAL	82)
Food industry – Microwave popcorn pack-aging plant	Total dust / Cassette	PVC		3	55 (filter)				Kinetic chromo-genic LAL	83)
Wastewater treatment plant	Total dust / Cassette	GF	4–5 h	2	104 (filter)		PFW-Tween 20		Kinetic chromo-genic LAL	84)

Environment	Sampler type/ commercial name	Filters type (porosity)/ liquid	Sampling time	Sampling rate (l/min)	N. samples (type)	Storage of samples	Matrix	Extraction procedure	Analytical method	Ref. n.
Homes – Urban and rural	Total dust / Cassette	PC (0.45 µm)	7-d	2	20 homes (filter)	Plastic bags at ambient temperature under dry conditions	PFW-Tween 20		Endpoint chromogenic LAL	85)
Animal house – Mouse facilities, research laboratories	Total dust / Cassette	PVC (5 µm)	30–189 min	3	52 (filter)		PFW-Tween 20	Extraction at RT for 1h	Endpoint chromogenic LAL	86)
Homes	Total dust / Cassette	PC (0.4 µm)	36–144 min	3	31 (filter)	−20 °C	PFW-Tween 20	Platform shaking at RT for 1 h	Kinetic chromogenic LAL	87)
Homes after hurricane	Total dust / Cassette	PC (0.4 µm)		3	20 (filter)		PFW- Tween 20		Kinetic chromogenic LAL	88)
Animal house – Racing stable	Total dust / Cassette	PC (0.4 µm)	4 h	2	- (filter)		PFW		Kinetic turbidimetric LAL	89)
Homes	Total dust / Cassette	PC (0.45 µm)	45 min – 1 h		2 homes (filter)				Kinetic chromogenic LAL	90)
Dental hygienist	Total dust / Cassette	PC (0.4 µm)	150 min	2.5	413 (filter)	+4 °C one week	PFW- Tween 20	Gentle agitation 3 h at RT, 0.45 filtration, freeze drying and reconstitution with 1 ml PFW	Kinetic chromogenic LAL	91)
Metal Working Fluids	Total dust / Cassette	GF	2h	2	346 (filter)	−20 °C	0.9% NaCl-Tween 20	Vortex for 1 h centrifugation at 500 g for 10 min	Endpoint chromogenic LAL	92)
Biofuel plants – indoor	Inhalable aerosol sampler/ GSP	Teflon (1 µm)	5–7 h	3.5	32 (filter)		PFW-Tween-20	Orbital shaking (300 rpm) at RT for 1 h and centrifuged (1,000 g) for 15 min	Kinetic chromogenic LAL	93)
Textile industry – cotton	Inhalable aerosol sampler/ GSP	GF	2 h	3.5	22 (filter)		PFW	Extraction for 1 h and centrifugation for 10 min at 1,000 g	Kinetic chromogenic LAL	94)
Wastewater treatment plant	Inhalable aerosol sampler/ GSP	GF	23 min – 3.5 h	3.5	81 (filter)	−20 °C	PFW-Tween 20	Shaking for 1 h, centrifugation at 1,000 g for 15 min	Kinetic chromogenic LAL	95)
Agricultural – Tomato greenhouse	Inhalable aerosol sampler/ GSP; CIS Total dust/cassette	Teflon (1µm)	6 h	3.5	34 (filter)		PFW-Tween 20	Orbital shaking for 60 min at RT; centrifugation at 1,000 g for 15 min	Kinetic chromogenic LAL	96)
Animal house – Swine farms	Inhalable aerosol sampler/ IOM	GF	6–8 h	2	47 (filter)				Endpoint chromogenic LAL	97)
Metal Working Fluids	Inhalable aerosol sampler/ IOM	GF	2 h	2	42 (filter)				Endpoint chromogenic LAL	98)
Animal houses	Inhalable aerosol sampler/ IOM	GF	4h	2	70 (filter)		PFW-Tween 20	Shaking (22 °C) for 1h	Kinetic chromogenic LAL	99)

Environment	Sampler type/ commercial name	Filters type (porosity)/ liquid	Sampling time	Sampling rate (l/min)	N. samples (type)	Storage of samples	Matrix	Extraction procedure	Analytical method	Ref. n.
Single case − Glass bottle recycling	Inhalable aerosol sampler/ IPM		average time: 5.7 h	2	270 (filter)				Kinetic turbidimetric LAL	100)
Agricultural − Grain farming	Inhalable aerosol sampler/ PAS-6	GF (1.6 µm)	10–60 min	2	106 (filter)	−20 °C	PFW, PFW-Tween 20	Rocked either vigorously (level 8) either quietly (level 4) for 1 h at RT or 68 °C, centrifugation at 1,000 g for 15 min	Kinetic chromogenic LAL	101)
Biotech − Plant of BSCP (bacterial single cell protein)	Inhalable aerosol sampler/ PAS-6	GF	whole work-shift	2	16 (filter)				Kinetic chromogenic LAL	102)
Animal house − Horse stables	Inhalable aerosol sampler/ PAS-6	GF	Stationary point: 6.2 h; personal sampling: 6.5 h	2	91 (filter)	−20 °C	PFW-Tween 20	Horizontal shaking for 60'; centrifugation at 1,000 g for 15 min	Kinetic chromogenic LAL	103)
Single case − Animal hospital	Inhalable aerosol sampler/ PAS-6; electrostatic dust fall collector	GF				−20 °C	PFW-Tween 20	Shaking for 1h, centrifugation for 15 min at 1,000 g	LAL assay	104)
Metal Working Fluids	Inhalation chamber/Sibata, SIS-20RG	GF	2 h	2	- (filter)		PFW	Centrifugation 150 rpm for 1h	LAL assay	105)
Animal house − Swine barn	Personal aerosol sampler/Dupont Air sampler, P4l	GF (0.8 µm)	5h	2	- (filter)		PFW		Kinetic chromogenic LAL	106)
Agricultural − lupulus	Single-unit aspirator/AP-2A	GF (1 µm)		2	19 (filter)		PFW	Extraction 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Gel clot LAL	107)
Agricultural − medicinal herbs	Single-unit aspirator/AP-2A; stationary sampler/AS-50	GF (1 µm) and PVC	30 min	2 (AP-2A); −50 (AS-50)	15 (filter)		PFW	Extraction 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Gel clot LAL	108)
Agricultural − Plant processing industries	Stationary sampler/AS-50	GF (1µm)	50	50	10 facilities (filter)	−20 °C	PFW	Extraction for 1 h at RT, heated to 100 °C (Koch apparatus) for 15 min and cooling	Gel clot LAL	109)
Textile industry − cotton		GF		2	19 workplaces (filter)		PFW	Extraction for 1 h and centrifugation for 10 min at 1,000 g	Kinetic chromogenic LAL	110)

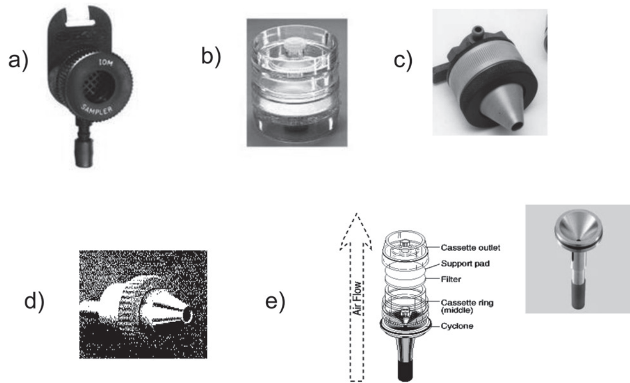

Fig. 1. Aerosol samplers.
a) IOM b) Cassette filters c) GSP d) PAS-6 e) Cyclone

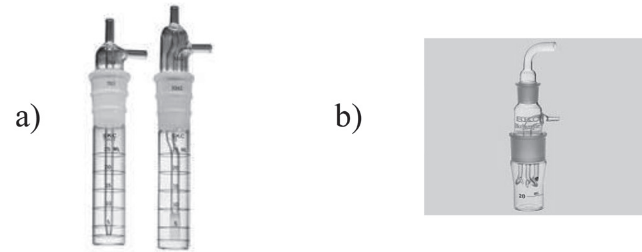

Fig. 2. Impingers.
a) Midget Impinger b) BioSampler

Madsen concludes that the endotoxin concentration was higher in airborne thoracic dust than in airborne total dust, and that a high variation in endotoxin concentration and in fractions of respirable or thoracic size is found in different working areas[25].

Collection systems

Information on the sampling methods is given in columns 2 to 6 of Table 1.

The sampler types are based on two main principles: 1 − filtration or impact on a porous membrane; 2 − collection into a liquid (impinger). Combinations of different collection systems are often used in the same study to permit comparisons.

Filters are designed to collect particles mainly through impaction and interception mechanisms. However, diffusion, electrical and gravitational forces can also affect the filter's collection system[30]. Total and inhalable aerosol samplers (IOM, GSP, PAS-6, cassette filter) are the most widely used samplers for airborne endotoxins (Fig. 1a–d), cassette filters being the most common. These disposable personal sampling heads contain a membrane on which the particles collect. Particles pass through one or more orifices and are collected on a filter in a holder. Closed face cassette filters are the most common because of their simplicity and low cost, despite it is recognized low sampling efficiency for particles > 30 μm and other limitations (inner wall losses, non-uniform deposition on collection filter, under-sampling when inlet orifice is oriented downward). Cassette inclination, thus its aspiration direction, with respect to a horizontal position is a prime parameter in relation to this device's sampling efficiency[31].

Aerosol is aspirated at various flow rates, mainly depending on the sampler type (2l min^{-1} for IOM and PAS-6, 3.5 L min^{-1} for GSP, between 2.5 and 10 L min^{-1} for cassette filters).

Samplers for the respirable personal aerosol (the sub fraction of the inhaled particles that penetrates into the alveolar region of the lung, having 50% cut-off point at 4 μm) were used in four studies[32–35]. The last two[34, 35] specify that samplers were based on the cyclone, which separates respirable dust particles on a filter for analysis while larger particles fall into the grid pot and are discarded (Fig. 1e).

Environmental area samplers are also used, mainly impingers. A stationary environmental area sampler produced in Poland (AS-50) was used in five cases and a high-volume sampler in two[36, 37]. The impinger is a collection system in which the pump forces the air flow into a liquid, generally phosphate buffer saline (PBS) or pyrogen-free water (PFW). Impingers (Fig. 2a, b) are designed to collect particles in general but have proved effective and versatile for bioaerosol sampling[38]. They collect microorganisms by inducing airborne particles to collide with the agitated surface of the collection fluid; the impinger suspension is available for analysis of culturable organisms on various media, of non-culturable organisms (by direct count or flow cytometry) and also of endotoxins. All-glass Impingers direct the air stream downward through a single jet, causing the fluid to roll vigorously. The swirling aerosol collector (commercially available as BioSampler by SKC Inc. USA) has three jets that set up a swirling motion of the collection liquid[26].

Generally a higher endotoxin level is measured with impingers than by filter sampling: as it is highly unlikely that particle sampling efficiency of impingers is higher than that of filters (as impingers generally do not collect particles below 1 μm while filters collect practically all particles), this is attributable mainly to the poor recovery of filter extraction or to differences in sampling time[39] but it could be also explained by the fact that the impinger

may fragment bacterial cells which may liberate more soluble endotoxins.

Single papers report the use of particular samplers like a vertical elutriator, an instrument that must be suspended vertically[40–42], a Harvard impactor[43], and an ELPI low-pressure impactor[44].

The column *Filter type/liquid* reports the media for filters or the solution used for impingers, and the next two columns specify sampling time and rate. The preferred filter type is glass fiber (GF), with pore size between 0.5 and 1.6 μm, followed by polycarbonate membranes (PC) with pore size 0.2–1 μm, and polytetrafluoroethylene (PTFE) with porosity 0.5–2 μm. Other filters such as polyvinylchloride (PVC) are rarely used.

When personal sampling is aimed at assessing occupational exposure and at comparing the results to limit values it should cover the whole work-shift (usually 6–8 h) in order to obtain a time weighted average value. In real cases, this can either be shortened or even prolonged up to a few days, on the basis of the real working activity or the amount of material collected in each unit of time. Practical reasons may limit the sampling time, e.g. the sampling time of impingers containing aqueous solutions may not exceed 30 min. In view of the wide variability, repeated measures and integrated samples from long collection periods are a good way of stabilizing the estimates of long-term airborne endotoxin exposure in cohort studies[45].

One study compared the Midget impinger with GF and PC filters[46]. The impinger gave the best recovery, but also the highest variability. Impinger results were correlated with GF filter results, which seemed more suitable than PC filters; these findings highlight the need for more detailed studies.

Some authors focused on settled dust collection: bed dust, floor dust, vacuum cleaner dust bag, and air samples were collected in homes and compared, concluding that the best compromise for assessing endotoxin levels in homes is floor dust sampling, followed by bed dust and settled dust. Endotoxin levels in settled dust correlated moderately well with the air samples[47].

The number of samples collected varies widely, from a few units to two or three hundred, and the largest are in studies on agricultural facilities, animal housing and homes, or when different environments are compared. Duplicate samplings (two filters at the same time and in the same position) to control for the different pumps, and field blanks (no air pulled through the filters), should be performed to verify that the filters are not contaminated, and also to determine the detection limit (the mean of an appropriate number of repeated field blanks plus three times standard deviation)[48]: measurements results below the detection limit should not be accepted.

Analytical methods

Columns 7–10 describe the procedures followed after sampling: how the loaded filters were stored until analysis, the extraction procedure and the method used for endotoxin qualitative or quantitative determination. Most papers gave no details of the sample storage conditions. It is generally recommended to store loaded filters at −20 °C, but this recommendation is probably made because sample storage at room temperature has not been studied systematically.

In some cases filters were stored frozen at −15/−20 °C for different times, ranging from 12 days to six months. In two cases the filters were stored at lower temperatures: −70 °C[49] and −80 °C[50]. In the remaining studies the loaded filters were stored at room temperature (RT), often in dark and dry conditions, or at 4 °C, and processed as soon as possible (not more than one day). In one case filters were stored at 4 °C for longer, from 12 to 14 d[51]. In general, room temperature or +4 °C is advisable if filters are extracted within 24 h, while −20 °C is advisable for longer storage, in order to avoid bacterial growth. The most common storage temperature for impinger solutions was +4 °C for periods of 24–48 h[26, 42]. The liquids used for sampling airborne bacteria and endotoxins generated during dental cleaning were stored frozen at −20 °C for six months[52].

The *matrix* column lists the medium used for extracting the filters or the liquid of impingers. PFW is one of the most widely used media for extraction but a 0.05% aqueous solution of Tween 20 (the commercial name for polyoxyethylene (20) sorbitan monolaurate), a widely used surfactant, is frequently mentioned. One study[50] used PBS in PFW. Another two[23, 53] used respectively triethylamine phosphate buffer and peptone water plus Tween 80.

The effects of the extraction and assay media on the endotoxin analysis were also studied and besides PFW and PFW-Tween, PFW-Tris HCl (tris (hydroxymethyl) methylamine hydrochloride) and PFW-TAP (triethylamine phosphate) have also been tested: TAP interfered with the assay; Tris had an effect similar to PFW. Application of Tween 20 to extraction appear to increase significantly the detectable endotoxin activity in dust, but it modifies marginally the LAL test, especially in relation to standard curves. Therefore at least a 50-fold dilution before LAL testing is needed in order to avoid a dose-dependent

inhibitory effect (Tween might reduce the availability of LPS by partially capturing it in micelles, or it might affect the molecular interactions with and between the LAL factors)[54]. In addition, different extraction volumes seem influence the results significantly while centrifugation speed, shaking and material of extraction tube (glass and plastic) had no significant influence[49].

Impingers also use PFW or pyrogen-free saline (0.09% NaCl in PFW) or PBS. PFW is confirmed as the simplest and best matrix for extraction, but this was the conclusion of papers that all referred to water-soluble endotoxins. Some authors suggest that endotoxin exposure may be underestimated because part may be non-soluble and therefore is not detectable because it is not extracted in aqueous media.

Eduard *et al.*[55] studied the solubility of the endotoxins from *Escherichia coli* and *Pseudomonas aeruginosa*, determining the amount of 3-OH-FA by GC/FID in the soluble and insoluble fractions separated by centrifugation; it ranged from 9% to 83% and they suggested that the LAL test may underestimate endotoxins in environmental samples because the non-soluble fraction remains undetected.

Rylander[56] suggests that the Limulus method detects only about a third of the biologically active endotoxin and that the remainder is present inside the fragments of dust particles/bacterial cells, but still able to exert effects when deposited in the lung. If substantial differences in the proportion of soluble endotoxins in different work environments is confirmed in future studies, the contribution of non-soluble endotoxins must be taken into consideration[56].

Column 9 of Table 1 refers to the *extraction method*. In many papers this process is not described: however, the most widely used procedure appears to be shaking (horizontal, orbital or not specified) for different times, usually at room temperature, with or without centrifugation. In five studies[57–61] after extraction the supernatant was heated to 100 °C in a Koch apparatus “for better dissolving of endotoxin and inactivation of interfering substances”; however a validation study for this procedure is not cited. Other procedures used are soaking, vortexing, rocking or sonication. However, shaking seems adequate. The impinger liquid is analyzed directly or vortexed after thawing[39].

The most widely used analytical method is the Limulus Amebocyte lysate (LAL) assay, in its kinetic chromogenic version. Almost all papers refer to the LAL test and its several variants: only two did not specify the type used and mention only a “LAL assay”. LAL is a functional assay based on enzymatic coagulation of the blood of a primitive marine arthropod, the horseshoe crab (*Limulus polyphemus*) in the presence of a minimal amount of endotoxin, due to the properties of the biologically active endotoxin constituent LPS. The gel-clot-test detects the presence of endotoxin through the clotting reaction between LPS and the lysate. The concentration can then be determined semi-quantitatively by subsequent dilutions. The method is the simplest and least expensive, but is not very sensitive; it was used in 11 studies.

The kinetic turbidimetric assay measures the time optical density starts to rise, indicating increasing turbidity; if there is no endotoxin there is no “time of onset” and samples are not assigned a numerical value; only three authors described this method.

The endpoint chromogenic LAL, used in ten studies, is a quantitative test for Gram-negative bacterial endotoxin. If the sample contains endotoxin, a yellow color will develop and the absorbance can then be measured spectrophotometrically. The kinetic-chromogenic assay is the one most frequently used in the studies selected. It is a quantitative kinetic assay for the detection of Gram-negative bacterial endotoxin. A sample is mixed with the LAL/substrate reagent, placed in an incubating plate reader, and monitored over time for the appearance of a yellow color. This LAL version is much more accurate, sensitive and reproducible than the gel-clot and turbidimetric assays, and has become the method of choice for endotoxin analysis.

Some papers report alternative methods for endotoxin analysis: the kinetic Glucatell® assay kit is cited only in one paper[53]: this assay is based on a modification of the LAL pathway, specific for (1–3)-β-D-glucan. The Glucatell reagent is processed to eliminate Factor C, and the reagent does not react to other polysaccharides, including beta-glucans with different glycoside linkages. Fairly recently, a new method has become available with similar sensitivity and potentially greater specificity using recombinant Factor C (rFC) from the horseshoe crab *Carcinoscorpius rotundicauda*. rFC is activated by endotoxin binding, and the resulting active moiety then cleaves a synthetic substrate, resulting in the generation of a fluorogenic compound. The rFC assay uses this mechanism as an *in vitro* end-product endotoxin test[62, 63].

While the LAL test detects endotoxins (pyrogens) on the basis of their biological activity, GC/MS or GC-MS/MS can be used for quantitative detection of 3-OH fatty acids in lipid A, which are used as chemical markers of the presence of LPS. Larsson[64] first determined endotoxin

applying acid hydrolysis directly after collecting the airborne material on filters, without dispersing the material in aqueous phase, by quantifying 3-OH-FAs of 10–18 carbon chain lengths.

A significant correlation was found between endotoxin determined with LAL and LPS determined with GC/MS/MS only in some animal facilities. In some cases LAL gave larger results than GC/MS/MS leading to the hypothesis of non-specific LAL reactions[57]. Air concentrations of endotoxins measured by GC/MS of 3-OH fatty acids indicated that cigarette smoke was a further source of endotoxin exposure[65]. An inter-laboratory comparison of LAL findings (endpoint chromogenic and kinetic chromogenic) with GC-MS of 3-hydroxy fatty acids was also presented and discussed, concluding that the factor that mainly affects endotoxin measurements seems to be the extraction method and that GC/MS offers additional information, optimizing sample treatment, and may be useful for comparisons across different environments[66]. Eduard *et al.*[55] found LPS markers in the residues after centrifugation of an endotoxin suspension. As the LAL test detects the water-soluble endotoxin, while the total amount of LPS gives information about the presence of organic dust, it can be hypothesized that with the extraction of the sample in PFW followed by centrifugation probably a fraction of the endotoxins in the environment considered remains undetected. All the sampling and analysis methods are able to detect water-soluble endotoxins and they all collect also the insoluble fraction but it is not extracted if aqueous media are used, like in the case of filters. Samples (filters or centrifugation residues) may be directly treated with suitable media (e.g esterification of 3OH fatty acids in anhydrous methanolic HCL) in order to be analyzed by GC/MS or GC/FID to detect LPS markers.

The determination of LPS markers instead of LAL can also be useful when determining endotoxin in samples rich in gram positive bacteria and moulds, which are known to contain peptidoglycans and glucans who can activate the Limulus reaction[64]. The GC/MS method for analysis of the LPS-characteristic 3-OH fatty acids detects LPS with a low likelihood of interference and also provides some information about the bacteria that are sources of the LPS, because the relative distributions of the individual 3-OH FAs differ among species of gram-negative bacteria.

Besides GC, as well as GC/MS, has contributed greatly to the elucidation of fatty acids contained in LPS[4].

In one case workers were biologically monitored in parallel with the environmental monitoring of airborne endotoxins, and the LAL test was done on plasma from eight workers producing bacterial single-cell protein: endotoxin was detected in serum of these highly exposed workers but no correlation was found between plasma and airborne endotoxin levels[67]. However, the short biological half-life of endotoxins in blood and the full shift endotoxin sampling probably prevented finding as an association.

A completely different-test for pyrogens is also proposed, based on the human whole blood cytokine response; the results consider not just endotoxins but the total inflammatory potential[68].

Current guidelines and limit values

Guidelines for the determination of airborne endotoxins in workplaces have been issued by the European Committee for Standardization[16]. For the environmental monitoring of endotoxins, sampling inhalable aerosols is recommended, using GF filters. The filters must then be transported in dry conditions, preferably with a dehumidifier, at room temperature. If the sample cannot be extracted within a few days of arrival at the laboratory it must be stored at −20°C or lower, but it must never be repeatedly frozen and thawed, as this may affect its detectable endotoxin content. A standard shaker apparatus for extraction of the sample in PFW should be used. After extraction, samples must be centrifuged for 15 min at 1,000 × g and the supernatant is collected. If not used immediately supernatants of extracts must be stored frozen at approximately −20°C or lower.

Kinetic chromogenic LAL-assays are to be used for the detection and quantification of endotoxin levels; the method should be validated at the laboratory with an international endotoxin assay standard. The LAL assay results for duplicate extract analysis should differ by less than 20%. Endotoxin results are expressed in endotoxin units (EU) per m^3.

Nevertheless, there is still a lack of consensus on standardized procedures for analysis of endotoxin, and as a consequence there are no internationally accepted threshold limit values (TLVs) or occupational exposure limits (OELs). Clearly differences in sampling and extraction procedures and in the types of analytical method used can produce significant differences in the amount of endotoxin detected, and often the results are even expressed in different units (nanograms or EU). Studies published before 2010 refer to the recommendations of the Dutch Expert Committee on Occupational Standards (DECOS) of 1997: a health-based occupational exposure limit for airborne endotoxin of 50 EU/m^3(approximately 4.5 ng/m^3) based on personal dust exposure, measured as an eight-hour time-weighted average[69]. Most recent papers[111–113]

refer to a new health-based occupational exposure limit for airborne substances to which people are exposed in the workplace recommended in 2010 by the DECOS, following the request of the Minister of Social Affairs and Employment, as a basis in setting legally binding occupational exposure limits by the Minister. DECOS concludes that a health-based occupational exposure limit (HBROEL) for endotoxins should be based on the avoidance of effects after both acute, short-term and chronic airway exposure: DECOS recommends an HBROEL of 90 EU/m^3 for both chronic and short-term exposure to inhalable endotoxins[114]. In addition, to measure the endotoxin exposure, the committee recommends the NEN-EN 14031 method[16] with the adjustments described by Spaan *et al.*[51].

Conclusions

Our literature search underlines that only few studies focused on the standardization, validation or comparison of sampling and analysis methods but there is substantial agreement that standardization is needed so as to be able to compare results from studies investigating endotoxin exposure, related health effects and compliance with exposure limits.

The indications given by European Committee for Standardization[16] coincide with the procedures most frequently employed. Both personal sampling and impinger should be used for a fuller assessment of exposure risk, in order to avoid false-negative results in cases of low exposure or exposure peaks. Personal exposure measurements should be considered in the context of health-related exposure measurement, as the type of information needed cannot be gathered by stationary sampling. Different extraction volumes influence the results and PFW is confirmed as a very cheap, suitable matrix for extraction. The kinetic-chromogenic LAL assay is the most widely used endotoxin analysis method.

A totally different approach uses GC/MS or GC/MS/MS to detect 3-OH fatty acids: further investigation is still needed to understand the significance of the differences between results with LAL or GC/MS. If substantial differences are found between work environments, the contribution of non-soluble endotoxins cannot be overlooked in evaluating the health risks from endotoxin exposure.

With reference to occupational exposure limits in 2010 DECOS recommended an HBROEL of 90 EU/m^3 for both chronic and short-term exposure to inhalable endotoxins[114].

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