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144. Endotoxins

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Preface

An agreement has been signed by the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council of the Netherlands and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). The purpose of the agreement is to write joint scientific criteria documents, which could be used by the national regulatory authorities in both the Netherlands and in the Nordic countries.

The document on endotoxins is an update of a report published by the Dutch Health Council in 1998 and has been reviewed by DECOS as well as by NEG. The members of both committees are listed in Appendix 1. The first draft of this report was prepared by B. van de Ven and G. Speijers from the National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands. The joint document is published separately by DECOS and NEG. The NEG version presented herein has been adapted to the requirements of NEG and the format of Arbete och Hälsa. Editorial work and technical editing has been carried out by Anna-Karin Alexandrie and Jill Järnberg, scientific secretaries of NEG, at the Swedish Work Environment Authority.

NEG is financially supported by the Swedish Work Environment Authority and the Norwegian Ministry of Labour.

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Contents

Abbreviations and acronyms

ASTM	American Society for Testing and Materials
BAL	bronchoalveolar lavage
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
DECOS	Dutch Expert Committee on Occupational Safety
ECP	eosinophilic cationic protein
EN	Committee Européen de Normalisation (European Committee on Standardisation)
EU	endotoxin unit/s
$FEF_{25–75}$	forced expiratory flow at 25–75 % of FVC (same as MMEF)
FEV_1	forced expiratory volume in 1 second (1^{st} second after full aspiration)
ΔFEV_1	(across-shift) change in FEV_1 over an exposure period of several hours
FVC	forced vital capacity
GM	geometric mean
GSD	geometric standard deviation
Ig	immunoglobulin
IHD	ischaemic heart disease
IL	interleukin
LAL	*Limulus* amebocyte lysate
LPS	lipopolysaccharide/s
MEF_x	maximal expiratory flow rate at x % of FVC
MMEF	maximal midexpiratory flow (average expiratory flow over middle half of FVC, same as $FEF_{25–75}$)
MPO	myeloperoxidase
NAL	nasal lavage
NEN	Nederlands Normalisatie Instituut
NF-κB	nuclear factor kappa B
ODTS	organic dust toxic syndrome
OEL	occupational exposure limit
OR	odds ratio
PC	provocative concentration
PEF	peak expiratory flow
PM_{10}	particulate matter < 10 µm in aerodynamic diameter
PMN	polymorphonuclear leukocyte, i.e. neutrophil
RR	relative risk (risk ratio)
SE	standard error
SMR	standard mortality ratio
Th	T-helper cell
TLR	Toll-like receptor
TNFα	tumour necrosis factor alpha

1. Introduction

Endotoxins are components of the outer membrane of Gram-negative bacteria and have been recognised as an important biologically active component in most organic dusts. Such bacteria-containing dust particles originate mainly from animal faeces and contaminated plant materials. Occupational exposure to endotoxins therefore occurs primarily in the agriculture industry and related sectors. To our knowledge, no occupational exposure limits for airborne endotoxins have yet been established.

The present document is a co-production between the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG) and the Dutch Expert Committee on Occupational Safety (DECOS), hereafter called the committees. The document is an update of a previous endotoxin report of the Health Council of the Netherlands published in 1998 (41). The joint document is published separately, and according to different formats, by DECOS (42) and NEG.

It was concluded in the previous Health Council's advice (41) that the most sensitive health effect appeared to be acute and chronic bronchial obstruction, which can be measured by a decrease in FEV_1. Such changes, at low or moderate exposure levels, are at focus in this document, whereas other effects, e.g. respiratory symptoms, are mentioned but not covered in full.

2. Identity, properties and monitoring

If not stated otherwise, information in this chapter is a summary of data from the previous endotoxin report of the Health Council of the Netherlands (41).

2.1 Chemical identity and physical and chemical properties

Endotoxins are components of the external membrane of most Gram-negative bacteria. Bacteria naturally release small quantities of endotoxins as they replicate, and the whole membrane content is released upon death and subsequent cell lysis. "Endotoxin" describes the molecule *in situ*, when still associated with proteins and other molecules of the bacterial membrane. The endotoxin molecules can be obtained by purification and are referred to as lipopolysaccharides (LPS). For further details, see the Health Council's report from 1998 (41).

LPS are stable water-soluble molecules composed of lipids and polysaccharides. In water, LPS usually convert into insoluble aggregates. The lipid moiety of LPS, a phosphoglycolipid, is termed "lipid A" and is a major contributor to the toxic properties of LPS. The hydrophilic polysaccharide moiety is composed of O-specific side chains (O-antigens) and core sugars. The composition of the core is relatively constant and usually contains 2-keto-3-deoxy-D-manno-octulosonic acid (KDO). The O-specific side chain is a heteropolysaccharide consisting of repeating units of up to eight sugar monomers. Among various bacterial species, the composition of lipid A is remarkably constant, whereas the O-specific side chains

vary considerably. For further details, see the Health Council's report from 1998 (41).

Endotoxins are relatively heat stable. The temperature reported to inactivate LPS is 177 °C (40), and LPS are stable at 121 °C for at least 1 hour (2). It is estimated that a single cell of *Salmonella* contains 10 femtograms (10×10^{-15} g) of LPS (2), which is 4 % of the total bacterial weight. Aggregations of endotoxins in aqueous solution can have a mass of 1 000 000 Dalton. Upon the application of surfactants such as sodium dodecyl sulphate, individual monomers may form with molecular weights of approximately 2 500–25 000 Dalton (136).

2.2 European Union classification and labelling

Endotoxins are naturally occurring substances and have not been classified and labelled by the European Union.

2.3 Analytical methods

Endotoxin levels in air can be measured either in absolute terms or as functional (bioactive) units per cubic metre of air. Endotoxin weight/m^3 can be measured by use of gas chromatography combined with mass spectrometry (GC-MS). Functional endotoxin levels can be measured by use of the *Limulus* amebocyte lysate (LAL)-assay and are expressed as endotoxin units (EU)/m^3. In the LAL-assay, the reaction between endotoxins in the sample and a pro-enzyme purified from horseshoe crab (*Limulus*) amebocytes (blood cells) is measured. Subsequent coagulation can be evaluated by an increase in optical density measured spectrophotometrically. Test values are read from a standard endotoxin calibration curve. A range of LAL-assay reagents is now available as kits, and the two main test types are endpoint and kinetic tests (65, 136). An additional feature of the most widely used chromogenic test variant is that it improves detection in more highly diluted samples, hence avoiding the disadvantage of dose-dependent inhibition by interfering agents. Table 1 shows a comparison between the older and more recent protocols for measuring endotoxin levels in air.

As endotoxin air levels measured in a functional assay correlate better with toxic effects than air levels measured in weight/m^3 (64), the chromogenic LAL-test is the most accepted assay for endotoxin exposure measurements. The detection limit of measurement of airborne environmental endotoxin is at present approximately 0.05 EU/m^3 (43).

In 2003, a NEN-EN (Nederlands Normalisatie Instituut-Committee Européen de Normalisation (European Committee on Standardisation)) 14031 protocol was published concerning a standardised method for the extraction and analyses of endotoxin concentrations in the environment (55).

Despite the specificity of the LAL-method, significant differences in calculated levels of exposure have been reported by different laboratories analysing the same samples, as demonstrated by several round robin studies. A round robin study allows an evaluation of a test method by examining two parameters critical to any

Table 1. Comparison between analytical methods used for determining occupational endotoxin levels.[1]

	Method described by Spaan *et al*, 2007 (125)	Method used by Castellan *et al*, 1987 (13)	Method used by Smid *et al*, 1992 (120), Post *et al*, 1998 (98)
Sample			
Dust fraction	Inhalable dust	Inhalable dust	Inhalable dust
Filter type	Glass fibre	Teflon	Glass fibre
Extraction and storage			
Storage temp. filter	-18 ºC	4 ºC	4 ºC
Extraction solution	Pyrogenic water with 0.05 % Tween	Pyrogenic and sterile water	Pyrogenic and sterile water
Storage temp.	-18 ºC	No storage	No storage
Defrost yes/no	No	Not stated	Not stated
Analyses			
Material	Not stated	Plastic	Not stated
Analyses	In pyrogenic water without Tween	In pyrogenic water without Tween	In pyrogenic water without Tween
LAL-test type	Not stated	Pyrostat test	Kabi Vitrum test

LAL: *Limulus* amebocyte lysate.

test method: inter-laboratory and intra-laboratory variation. In one study, the performance of six laboratories using their own laboratory specific protocol for older endpoints and newer kinetic versions of the *Limulus*-based assays for analysis of organic dusts from three agricultural environments (chicken, swine and corn) was compared. This comparison revealed 10-fold differences in measured endotoxin concentrations between laboratories. Precision of assays performed within laboratories was very good, with pooled coefficients of variation for replicate samples ranging from 1 to 11 % over all laboratories and all dust types (105). In another round robin study, 13 laboratories measured endotoxin concentrations that also varied up to a factor 10, despite the use of a common procedure for extraction of endotoxins from cotton dust (18). The variation in endotoxin concentration in 20 samples measured by three laboratories, all using the NEN-EN 14031 protocol for extraction and analyses, was smaller, maximally a factor 4.2 between the different laboratories (55). Therefore, it was concluded in several studies that a reliable assessment of exposure to endotoxin activity is only possible when standard operation procedures for sampling and determination are established (70, 126). On the other hand, Spaan *et al,* 2008, also showed using an endotoxin exposure database (with a fairly similar protocol for exposure measurement) that the analytical error for endotoxins is generally less than 20 %. In addition, the authors concluded that most of the variability in endotoxin exposure is an inherent part of the true exposure. This is presumably caused by the fact that endotoxins originate from Gram-negative bacteria, which grow and amplify (126).

According to Rylander, 2002, the results of the LAL-test also depend on the physical state of the endotoxins in the sample. If they are present in a water solution, the values represent all of the endotoxins present in the sample. If the analysis

[1] Adapted from data provided by Industox as comment on the DECOS public draft.

is made on a dust sample where endotoxins are still part of fragments of an intact bacterial cell wall, the results of the LAL-test may underestimate the total amount of bioactive material. Some attempts have been made to calculate the relation between the amounts detected in the analysis of dust and the bioactive amount, suggesting a ratio of 1:10 (107).

In conclusion, the present NEN-EN procedure still leaves some aspects of the protocol open for interpretation by individual laboratories. Nevertheless, the committees emphasise that airborne endotoxin exposure should be assessed using standardised methods. Two extensive studies (funded by the Dutch Government under supervision of the Ministry of Social Affairs and Employment) investigated the gaps in the NEN-EN protocol and presented several adjustments to the guideline protocol for further standardisation. One specific development on these topics requires consideration. Optimally, extraction should be performed using a diluted detergent (e.g. Tween), while analysis should be undertaken in pyrogen free water to decrease potential interference with a diluted detergent. For some sectors of industry, systematic differences might be observed depending on procedures, probably because of matrix effects. This should be established on a case by case basis (70, 124, 125). The committees recommend to adapt these adjustments of Spaan *et al,* 2007 (125), in the NEN-EN protocol.

Finally, in the earlier versions of the LAL-assays, the relation between EU and the amount of endotoxins in weight is that 1 EU is usually considered equivalent to 0.1 ng, although this is dependent on the potency of the specific species of endotoxins used to create the standard curve (65).

Moreover, the committees are aware that the more recent versions of the LAL-assay (NEN-EN protocol and Spaan *et al,* 2007 (125)) are more sensitive in measuring the endotoxin exposure. Compared to these recent protocols, the older assays most likely underestimated the endotoxin exposure levels (104, 105, 124, 125).

2.4 Environmental and occupational monitoring

Workplace monitoring of endotoxins is usually performed by sampling airborne inhalable dust with a subsequent aqueous extraction. Dust is sampled on filters using pumps to draw air through the filters. Repeated freeze-thaw cycles might reduce the detectable endotoxin level. Furthermore, the committees are of the opinion that since endotoxins are components of growing microorganisms, the variation in exposure is expected to be higher for endotoxins than for other compounds at the workplace. Therefore, in order to determine the exposure, the committees recommend to monitor endotoxin air levels more frequently than normally applied for workplace control measurements (124).

The American Society for Testing and Materials (ASTM) has recently approved an endotoxin standard, i.e. “Standard test method for determination of endotoxin concentration”. While this standard provides an improved consensus method to measure endotoxin concentrations in bulk metal working fluid samples, it does

not address the issue of airborne endotoxin aerosolised from metal working fluid. Presently, there are no data concerning the relationship between endotoxin in bulk metal fluids and endotoxin concentrations in metal working fluid aerosols generated during machine operations. In addition, the ASTM approved a "Standard practice for personal sampling and analysis of endotoxin in metal working fluid aerosols in workplace atmospheres" (131).

2.5 Recommendations

In the former report of the Health Council (41), recommendations on procedures for collection, storage, extraction and analysis of airborne dust samples for endotoxins were made. In 2003, a NEN-EN 14031 protocol was published. Spaan *et al,* 2007 investigated the gaps in this NEN-EN protocol and presented several adjustments to the guideline protocol for further standardisation (125). The committees recommend to adapt these adjustments in the NEN-EN protocol and recommend further standardisation.

3. Sources

The protection of workers against toxic effects of endotoxins in occupational settings primarily concerns airborne exposure. Endotoxins become airborne during manufacturing or handling of organic materials. Endotoxin exposure is therefore most relevant in agricultural and related industries such as pig, chicken, cow and horse farming, grain elevators, cotton and linen industry, potato processing industry, poultry slaughterhouse, animal feed industry, water sewage treatment and sewage composting plants, garbage handling facilities, organic waste composition facilities, wood chip composting and timber storing facilities. Endotoxin exposure seems mainly associated with organic dust exposure. Although organic dust has a heterogeneous composition, endotoxins have been recognised to be a very important biologically active component in most organic dusts (41).

4. Exposure

4.1 Environmental exposure

Water

In the report of the Health Council from 1998, one publication on endotoxin levels in lake and tap water in Finland was available. More than 100 people in one community in Finland experienced respiratory health problems after inhaling aqueous aerosol from an endotoxin contaminated drinking water source. Analyses of tap and lake water revealed endotoxin concentrations ranging from 200 to 1 000 ng/ml. These concentrations were exceptionally high and situations like that were considered not likely to occur in the Netherlands (41).

Since 1998, new data has become available. In studies reviewed by Anderson *et al,* 2002, it was indicated that endotoxin levels in raw (untreated) water ranged from < 1 to 1 050 ng/ml (< 10–10 500 EU/ml), but were mostly below 50 ng/ml (500 EU/ml). Values approaching 38 000 EU/ml were reported in a cyanobacterial bloom. In distribution systems containing drinking water obtained from surface water, the endotoxin content ranged from 0.8 to 11.4 ng/ml (8–114 EU/ml), whereas it ranged from 1 to 3 ng/ml (10–30 EU/ml) (n = 60) when obtained from groundwater. Water treatment plants can remove up to 97 % of the endotoxin levels by coagulation, flocculation and sedimentation. Ozonation reduce concentrations maximally by only 10 %, whereas chlorination did not reduce endotoxin levels (2).

Food

In the report of the Health Council from 1998, it was stated that actual endotoxin levels in human diet were not known. In a pig study, high dietary doses of endotoxins did not cause clinical symptoms (41). It therefore seemed justified that, although there are no intake data, the oral route is not likely to be a relevant route of exposure. Since 1998, no further data has become available.

Air

In the report of the Health Council from 1998, it was concluded that relevant airborne exposure was mainly limited to occupational environments (41). However, the general population is exposed to endotoxins to a small extent as endotoxins are a component of house dust. The general population may further be exposed to endotoxins when living in the vicinity of agricultural and related industries that emit organic dust to the environment.

Since 1998, new exposure data on the general population has become available. Endotoxin levels in outdoor air were recently measured at 13 different locations in Southern California, United States. The 24-hour levels of PM_{10} (particulate matter below 10 μm in aerodynamic diameter) and the associated endotoxin component were measured once every 6 weeks for one year. The geometric mean ± geometric standard deviation (GM ± GSD) of endotoxin levels was 0.44 ± 3.1 EU/m^3 (range 0.03–5.5 EU/m^3). Endotoxin concentrations differed significantly across regions as shown by the fact that GM levels by sampling site were 0.19–1.85 EU/m^3 (90). A small measurement series was also reported by Schulze *et al,* 2006 (111).

Indoor airborne endotoxin levels were measured in a 14-month study in 20 homes and ranged from 0.02 to 19.8 EU/m^3 (0.002–1.98 ng/m^3) (95). Endotoxins in indoor air are suspected of playing a role in "sick building syndrome". Concentrations were highest in the spring and lowest in the winter and were not well correlated with endotoxin concentrations in settled dust (108). Similar air levels have been observed in Dutch homes (93). Woskie *et al,* 1996, reported an exposure of 1.9 ± 6.4 EU/m^3 (0.19 ± 0.64 ng/m^3, GM ± GSD) in office workers (n = 34) (140). Wan *et al,* 1999, reported a mean endotoxin concentration of 0.065 ng/m^3 (0.65 EU/m^3) in houses (133).

Hasday *et al,* 1999, reported that high bacterial endotoxin levels are present in cigarette smoke. Smoke from one cigarette contained 120 ng (1 200 EU) of bio-

active LPS (40). The committees estimated that smoking of e.g. one pack of 20 cigarettes/day (20 × 120 = 2 400 ng endotoxins) is comparable to an occupational LPS exposure of 240 ng/m^3 for 8 hours a day (assuming a ventilation rate of 10 m^3 per 8 hours).

4.2 Occupational exposure

The exposure data in various occupational environments which were available for the 1998 evaluation were tabulated in the previous report of the Health Council (41). The exposure data, which has become available since, are summarised in Table 2. The exposure data found by Dutkiewicz *et al,* 2001 (27-29), are rather high compared to other studies in similar industry branches, which might be due to the fact that Dutkiewicz *et al* boiled the samples for 15 minutes at 100 °C to dissolve the endotoxins before testing.

5. Kinetics

5.1 Absorption, distribution and elimination

Inhaled endotoxins can deposit at each level of the respiratory tract. If deposited in the trachea and large bronchi, particles are eliminated by mucociliary transport. Smaller particles deposit in the deeper airways where endotoxins can generate inflammatory reactions. Although Hjelle *et al,* 2000, reported that systemic uptake of nanoparticles and nanobacteria is possible, inhaled endotoxins are phagocytised by macrophages and have been assumed not to enter the bloodstream (49, 103, 107). Therefore, systemic effects due to inhaled endotoxins are most likely induced by cytokines that are released from the lung into the blood. There is, however, one recent study in which LPS in plasma were detected after occupational exposure to endotoxins at a mean level of 430 EU/m^3 (114).

For more information on absorption, distribution and elimination of endotoxins, the committees refer to the previous report of the Health Council (41).

5.2 Possibilities for biological monitoring

Markers of the local endotoxin-induced inflammatory response, like cytokines and inflammatory cells, can be investigated in bronchoalveolar lavage (BAL), nasal lavage (NAL), induced sputum and in blood. No attempts to determine endotoxin levels in BAL, NAL and induced sputum have been made (41, 103). In the aforementioned study in which circulating LPS in plasma of exposed workers were detected, LPS levels did not correlate with airborne endotoxin levels. This might be due to the rapid clearance of LPS from blood (114). However, the committees are of the opinion that the usefulness of these methods for monitoring is limited because clinical inflammatory responses are only expected after exposure to *high* endotoxin concentrations.

Table 2. Endotoxin concentrations in various occupational environments.

Source/ industry	Sampling method	No. of samples	Dust (mg/m^3)	Mean/median (range) (EU/m^3) [a]	Mea-sure	Ref.
Vegetable sources						
Cotton						
Mill	Area	5	nd	110 (19–2 230)	MD	(64)
Mill	Personal	4	nd	1 200 (140–9 600)	MD	(64)
Spinning	Personal	31	1.1	4 540 (2 950–6 980)	GM	(116)
Weaving	Personal	36	0.59	50 (30–80)	GM	(116)
Textile factory	Personal	61	1.1	2 566 (5–36 397) [b]	GM	(56)
Hemp/jute						
Jute batching	Personal	3	9.4	23 190 (2 200–44 200)	AM	(15)
Jute spinning	Personal	2	2.2	9 560 (4 400–14 900)	AM	(15)
Jute weaving	Personal	2	1.8	410 (71–750)	AM	(15)
Hemp	Personal	-	29.5 [c]	19 569 [b, c]	AM	(65)
Herbs						
11 herbs [d], 2 sites	Area	10	18	112 000 (2 000–7 568 000)	MD	(28)
Grain						
Storage houses	Area	5	nd	170 000 (17 000–380 000)	MD	(64)
(grain/onions)	Personal	4	nd	56 000 (40 000–80 000)	MD	(64)
Silos/flour mill	Personal	31	4.4	1 150 (550–2 400)	GM	(116)
Silos containing corn	Area	15	3.3	983 (58–77 006) [b]	GM	(10)
	Area	14	1.0 [c]	526 (55–3 733) [b, c]	GM	(10)
Farms cultivating corn	Area	14	3.4	3 175 (499–54 653) [b]	GM	(10)
	Area	16	2.4 [c]	2 534 (284–29 266) [b, c]	GM	(10)
Grain seed and legumes						
Dutch, overall	Personal	188	1.5	580 (2.3–149 060)	GM	(127)
Mushroom						
Cultivation/picking	Personal	30	0.69	70 (50–110)	GM	(116)
Potato						
Processing (sorting,	Personal	7	nd	195 (26–1 123) [b]	MD	(53)
cleaning, trimming)	Area	8	nd	222 (7–5 363) [b]	MD	(53)
Cucumber and tomato						
Nurseries	Personal	70	1.6	320 (5–4 000)	MD	(75)
Wood						
Logging site	Personal	7	0.56	15 (9.9–23)	GM	(1)
Sawmill	Personal	93	1.6	43 (1.9–780)	GM	(1)
Joinery	Personal	66	3.7	24 (1.0–280)	GM	(1)
Sawmill	Personal	37	1.5	190 (130–230)	GM	(116)
Green mill	Personal	55	1.5	66 (1.9–780)	GM	(78)
	Personal	20	0.19 [c]	14 (1–53) [c]	GM	(78)
Dry mill	Personal	28	1.7	16 (5.1–56)	GM	(78)
	Personal	10	0.46 [c]	1.4 (1–3.3) [c]	GM	(78)
Pine sawmill	Area	1	15	2 400	S	(27)
Fir sawmill	Area	1	69	40 000	S	(27)
Fibreboard factory	Area	100	0.4–36	16–1 974 [b]	R	(29)
Chipboard factory	Area	140	1.1–29	< 0.13–217 [b]	R	(29)
3 pulp/paper mills	Area	22	nd	33 (1–510)	MD	(64)
	Personal	11	nd	60 (10–360)	MD	(64)
2 pulp/paper mills	Area	10	0.1–3.9	210 (42–25 000)	MD	(99)

Table 2. Endotoxin concentrations in various occupational environments.

Source/ industry	Sampling method	No. of samples	Dust (mg/m^3)	Mean/median (range) (EU/m^3) [a]	Mea-sure	Ref.
Animal sources						
Animal production						
Dutch, overall	Personal	108	0.7	110 (2.0–8 120)	GM	(127)
Cow						
85 barns (mostly dairy	Personal	194	1.8	647 (25–34 800) [b]	GM	(63)
barns)	Area	216	0.07 [c]	16.8 (0.16–1 380) [b, c]	GM	(63)
Poultry						
Catching/shackling	Personal	33	10.6	84 310 (53 130–133 860)	GM	(116)
Slaughterhouse, 2 sites	Area	10	nd	1 900 (0.2–9 400)	MD	(64)
(reindeer, poultry)	Personal	6	nd	870 (14–5 200)	MD	(64)
Swine						
11 buildings	Personal	27	5.8	6 600 (4 070–10 700)	GM	(116)
8 buildings	Area	8	3.3	390 (215–596) [b]	MD	(20)
Open-style buildings	Area	60	0.24	140 (14–818) [b]	AM	(14)
	Area	95	0.14 [c]	47 (0.02–1 643) [b, c]	AM	(14)
Wool						
Combing/weaving	Personal	28	3.9	830 (360–1 900)	GM	(116)
Other/mixed sources						
Animal feed						
3 plants	Area	13	nd	65 (3–200)	MD	(64)
	Personal	17	nd	190 (2–500)	GM	(116)
	Personal	6	6.0	300 (110–800)	GM	(116)
Fibreglass wool						
	Area	50	nd	10–3 900 (GSD 26–55)	GM [e]	(87)
	Personal	390	nd	58–360 (GSD 26–34)	GM [e]	(87)
Metal working fluid						
	Area	4	nd	67 (16–270)	MD	(64)
	Personal	72	0.18	7.1 (GSD 4.7) [b]	GM	(140)
	Area	9–12	nd	0.5–3 (< 0.1–100)	MD	(71)
Printing						
Printing plant	Area	5	nd	0.5 (0.3–1)	MD	(64)
Sewage/waste water treatment plants						
67 Dutch plants	Personal	460		27 (0.6–2 093)	GM	(123)
Plants	-	-	-	1 000–7 800	R	(91)
8 plants	-	-	-	20–640	R	(91)
9 plants	-	-	-	40–321 700	R	(91)
Waste						
Garbage handling	Area	8	nd	1 200 (9–14 000)	MD	(64)
	Personal	1	nd	2 600	S	(64)
Recycling	Personal	165	0–62	80 (2–1 980)	MD	(37)
Refuse-derived fuel	Personal	78	0.50	29 (5–346) [b]	GM	(77)
Waste collectors	Personal	47	0.58	39 (4–7 182) [b]	GM	(141)
Glass bottle recycling (at point of sale)	Personal	182	0.18	3.6 (< 0.1–180) [b]	GM	(58)

[a] 1 endotoxin unit (EU)/m^3 is approximately 0.1 ng/m^3 endotoxin.

[b] Levels reported in EU/m^3 in the original reference. Remaining levels were calculated using a default conversion factor 10 (1 ng = 10 EU).

[c] Measured in the respiratory fraction.

[d] Nettle, caraway, birch, celandine, marjoram, mint, peppermint, sage, St John's wort, calamus and yarrow.

[e] Range of GMs of 4 areas.

-: no information, AM: arithmetic mean, EU: endotoxin unit, GM: geometric mean, GSD: geometric standard deviation, MD: median, nd: not determined, R: range of means per site, S: single value.

6. Mechanism of action

When endotoxins are inhaled, the lipid A part of the endotoxins is opsonised by a LPS binding protein present in the fluid on the airway surface. This LPS binding protein may act as a transporter to deliver endotoxins to cell membrane protein CD14, present on alveolar macrophages, monocytes and to a lesser extent on neutrophils. CD14 is the primary binding site for LPS, and is also present in a free, soluble form in the extracellular compartment (and thus in normal alveolar fluid) where it facilitates the attachment of endotoxins to endothelial cells, epithelial cells and antigen presenting dendritic cells. Before soluble or membrane-bound CD14-mediated cell-activation takes place, co-activation of a Toll-like-receptor (TLR) seems to be required although the exact mechanism has not yet been revealed. In macrophages and epithelium cells, TLR4, and in dendritic cells, TLR3, play a role in the activation of these cells (107).

Alveolar macrophages and type-II epithelial cells are the predominant airway cells stimulated by inhaled endotoxins. Their stimulation produces many cytokines, chemokines, adhesion molecules and other products that cause inflammation, especially by recruiting and activating polymorphonuclear leukocytes (PMNs), i.e. neutrophils.

When endotoxins are internalised by alveolar macrophages, nuclear factor kappa B (NF-κB) initiates the production of inflammatory cytokines like interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor alpha (TNFα). Production of metabolites of arachidonic acid by macrophages is also up-regulated, as well as the production of inducible nitric oxide synthase, leading to release of nitric oxide. IL-8 is the cytokine that induces the migration of the PMNs into the lung. Elastase produced by activated neutrophils is considered to be the primary factor responsible for the loss of elastic fibres in lung parenchyma and the development of emphysema. Elastase is also a potent stimulus of mucus secretion (103).

Systemic effects are most likely induced by release of the cytokines into the blood. Inhaled endotoxins are assumed not to pass into the vascular department to any great extent, although Hjelle *et al,* 2000, reported that systemic uptake of nanoparticles and nanobacteria is possible (49, 103, 107). The cytokines produced are potential activators of the hepatic acute-phase protein response, as they stimulate hepatocytes. Airway exposure to endotoxins results in elevated blood concentrations of C-reactive protein (CRP) and LPS binding protein within 48 hours (85). This systemic inflammatory response is related to the dose of inhaled endotoxins and to endotoxin-induced fever.

7. Effects

Numerous studies have been published concerning the health effects of occupational exposure to endotoxins. Most of these studies deal with the adverse respiratory health consequences. However, it has also been suggested that exposure

to endotoxins might protect against the development of atopy and atopic (allergic) asthma. Furthermore, repeated exposure to endotoxins might cause tolerance to acute effects.

In this chapter, all these consequences of occupational exposure to endotoxins will be discussed in more detail. Adequate animal studies on effects similar to those observed in humans following endotoxin exposure and allowing a quantitative hazard assessment based on dose-effect/response relationships for endotoxins are not available.

7.1 Introduction

Airborne endotoxin exposure has been shown to generate (local and systemic) biological and clinical effects in man. The main target organ is the lung. Inhaled endotoxins induce an inflammatory response in the lung that is characterised by influx of neutrophils and increased levels of cytokines in the bronchoalveolar compartment. Endotoxins will probably not enter the blood after inhalatory exposure to any great extent. The systemic effects like fever, malaise and headache occur at higher exposure levels. These effects are most likely mediated by locally produced cytokines that are released into the blood and not by the endotoxins themselves.

The inflammatory reaction can lead to acute (respiratory and systemic) effects including fever, shivering, dry cough, chest tightness (byssinosis), dyspnoea, joint aches and influenza-like symptoms, which are all symptoms of the organic dust toxic syndrome (ODTS). Epidemiological and animal studies suggest that chronic exposure to endotoxins may lead to symptoms indicative of chronic bronchitis and asthma and reduced lung function, most likely via chronic inflammation. In case of prolonged exposure, an accelerated decline in lung function and increased bronchial reactivity can lead to chronic obstructive pulmonary disease (COPD). The global initiative for chronic obstructive lung disease (GOLD) has published an extensive review on COPD. COPD has two components, chronic bronchitis and emphysema. In this context, chronic bronchitis is of relevance. Its pulmonary component is characterised by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with abnormal inflammatory response of the lung to noxious particles or gases (101). It can be measured with spirometry. Parameters that are considered as primary indicators of lung function are forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC). FEV_1 is the maximal amount of air that can be expired with force in the first second after full inspiration (measured in litres) and FVC is the total amount of air that can be expired with force after full inspiration (measured in litres). The ratio FEV_1/FVC shows the amount of the FVC that can be expelled in one second. In healthy adults, this should be approximately 80 % or more. In patients with COPD, the FEV_1/FVC ratio is typically below 0.7 and this is accompanied by a $FEV_1 < 80$ % of predicted FEV_1 based on the mean FEV_1 of healthy non-smoking persons at that age, sex and length. COPD is classified as moderate or worse depending on the level of FEV_1.

It was concluded in the previous Health Council's advice that the most critical effects for human risk assessment are local effects in the lung (41). The most sensitive health effect appeared to be acute and chronic bronchial obstruction, which can be measured by a decrease in FEV_1. An acute effect is measured by a change in FEV_1 over exposure on one day, and is denoted by ΔFEV_1. For example, the change in FEV_1 measured before and after a workshift of 4 or 8 hours is denoted as the "across-shift ΔFEV_1". Chronic effects are monitored by the (mean) change in FEV_1 measured over a year (annual FEV_1 change).

7.2 Acute and short-term exposure

Long-term or multiple exposures to endotoxins may lead to some kind of tolerance to acute clinical effects (38). However, after ending the exposure, this tolerance disappears in a few days (e.g. weekend). Tolerance development might obscure the actual dose-effect/dose-response curve. Therefore, effects after acute exposure to endotoxins might differ between workers who have been exposed in the past and healthy volunteers which have not been exposed before. This should be taken into account in the interpretation of the studies described in this section.

Furthermore, in studies where subjects are exposed to single doses of nebulised LPS, comparison with a dose per m^3 is difficult. Different dosing (exposure) and dose (exposure) metrics represent additional challenges for interpreting and comparing various studies of exposure to endotoxin. In some studies, subjects have been exposed to dosing with nebulised agents for short periods (i.e. minutes). While the concentration of the agent in the aqueous medium is typically given, other details (e.g. nebulisation rate and duration) are not provided. Moreover, the agents used in these nebulisation studies differ. Some have used suspensions of organic dust and others have used more purified endotoxin or LPS preparations. Regardless of the details provided for nebulisation studies, their results are difficult to compare with results from studies involving more prolonged (e.g. hours) exposure to airborne endotoxin containing organic dust. The latter studies are much more comparable to endotoxin exposures in occupational settings.

7.2.1 Biological responses

In a number of studies, local endotoxin-induced inflammatory responses have been investigated by studying BAL, NAL and induced sputum after endotoxin exposure. Systemic responses were investigated in blood. Most of these biological responses do not necessarily result in clinical responses.

Single dose studies in healthy volunteers are summarised in Table 3. Biological responses to endotoxin exposure have also been examined in field studies. Effects were measured within a period of one week and were compared to those in healthy non-occupationally exposed controls. Results are shown in Table 4.

Table 3. Biological effects caused by single dose endotoxins in healthy volunteers.

Dose		No. of	Effects	Measured	Ref.
µg	EU	subjects		after	
0.1	1 000	16 [a]	*NAL:* No effect observed	4–24 h	(96)
0.3	3 000		*NAL:* No effect observed (NOAEL)		
1.0	10 000		*NAL:* Eosinophils ↑ (atopics only)		
0.5	5 000	9	*Blood:* PMNs ↓	6 h	(85)
5	50 000		*Blood:* PMNs ↑, CRP ↑		
			Sputum: PMNs ↑, monocytes ↑, MPO ↑		
50	500 000		*Sputum:* Lymphocytes ↑, TNFα ↑, ECP ↑		
5.4	54 000	14	*BAL:* Total cells ↑, TNFα ↑, IL-1β ↑, IL-6 ↑, IL-8 ↑	4 h	(54)
36	360 000		FEV_1 ↓		
40	400 000	21	*Blood:* PMNs ↑, MPO ↑,	24 h	(130)
			Sputum: PMNs ↑, ECP ↑ and MPO ↑		
			FEV_1 2 % ↓		
100	1 000 000	8	*BAL:* PMNs ↑	3 h	(129)

[a] of which 10 atopic subjects.

BAL: bronchoalveolar lavage, CRP: C-reactive protein (acute phase protein), ECP: eosinophilic cationic protein, EU: endotoxin unit, FEV_1: forced expiratory volume in 1 second, IL: interleukin, MPO: myeloperoxidase, NAL: nasal lavage, NOAEL: no observed adverse effect level, PMN: polymorphonuclear leukocyte, i.e. neutrophil, TNFα: tumour necrosis factor alpha.

Table 4. Biological effects caused by acute/short-term endotoxin occupational exposure.

Study design/ population	Work history	Endotoxin level	Parameters measured	Effects	Ref.
4-day follow-up, cotton workers, n = 25 controls, n = 9 (scientists)	> 8 yrs	Range 1–400 EU/m^3 (0.1–40 ng/m^3)	*Blood*: CD14 receptor on monocytes	CD14 ↑ at the end of the 1st day, but back to normal at the end of the week.	(35)
1-week follow-up, domestic waste collectors, n = 47 controls, n = 15 (office workers)	5 yrs	GM (range) 39 (4–7 182) EU/m^3 (3.9 (0.4–718) ng/m^3)	*NAL*: IL-6, IL-8, IL-1β, TNFα, cell counts and differentials. *Serum*: IgE	IL-8 ↑ (1.8×) and total cell ↑ (3.3×) at the end of the week.	(141)
4-day follow-up, waste handlers, n = 31 no controls	1.5 yrs	Median (range) 13 (4–183) EU/m^3 (1.3 (0.4–18.3) ng/m^3)	*NAL*: MPO, ECP, IL-8, cell counts and differentials	ECP ↑ (1.8×) and % PMNs ↑ (1.6×) at the end of the week.	(46)

ECP: eosinophilic cationic protein, EU: endotoxin unit, GM: geometric mean, Ig: immunoglobulin, IL: interleukin, MPO: myeloperoxidase, NAL: nasal lavage, PMN: polymorphonuclear leukocyte, i.e. neutrophil, TNFα: tumour necrosis factor alpha.

7.2.2 Acute effects on lung function

Health Council report, 1998

Studies on acute effects described in the previous Health Council report from 1998 (41) which yielded a no observed adverse effect level (NOAEL) are briefly summarised below and in Table 5.

In a study from 1985 by Rylander *et al*, 15 cotton mill workers (of whom 8 persons had a history of byssinosis) were exposed in an experimental card room to cotton dust for 4 hours on Monday morning. Endotoxin concentrations ranged from 70 to 5 620 ng/m^3 (700–56 200 EU/m^3) (personal sampling). FEV_1 was determined before and after carding. A correlation was found between endotoxin exposure and ΔFEV_1 over the exposure period. The authors calculated an endotoxin concentration of 33 ng/m^3 (330 EU/m^3) at which average FEV_1 changes were zero using individual FEV_1 changes and ambient endotoxin concentrations in a regression analysis (equation: % $\Delta FEV1 = -3.43 \times {}^{e}\log$ (endotoxin concentration in µg/m^3) - 11.68, r = -0.56 (109).

In a study from 1987 by Castellan *et al*, healthy volunteers (smoking and non-smoking) were selected from the general population. They were not occupationally exposed to substances known to affect airway response and had an FEV_1 above 80 % of the predicted value. In addition, the volunteers were pre-tested by exposure to 100 ng/m^3 of LPS in order to select sensitive subjects. Only volunteers that responded with an FEV_1 decrease of at least 5 % (and not more than 30 %) were accepted for the main study. The main study started with 61 (34 smokers) subjects, but during the 20-month study period the number of participating subjects decreased to 33 (16 smokers) for a variety of reasons, none of which were related to the responsiveness to cotton dust. In 108 different exposure sessions, volunteers (24–35 subjects) were exposed to cotton dust during 6 hours, with airborne endotoxin concentrations ranging from 6 to 779 ng/m^3 (60–7 790 EU/m^3). Each session was followed by at least two full days without exposure. The authors found an exposure-effect relation between ΔFEV_1 and endotoxin concentration (ng/m^3) of: % $\Delta FEV_1 = 3.84 - 4.02$ ($^{10}\log$ endotoxin), $r = 0.85$ ($r^2 = 0.72$), $p < 0.0001$. Another 66 sessions of exposure of the same subjects to clean air resulted in a mean ΔFEV_1 of ± 0 %. The linear regression model yielded a zero percentage change in FEV_1 at 9 ng/m^3 (90 EU/m^3). In contrast, dust exposure (instead of endotoxin exposure) was not correlated with ΔFEV_1 (13).

The difference between the calculated zero-change level in Rylander's study (33 ng/m^3) (109) and the one in Castellan's study (9 ng/m^3) (13) might be due to different exposure times (4 vs. 6 hours). In addition, in Castellan's study the responsiveness was enhanced for the assessment of acute airway responses by selecting responsive subjects during pre-screening. Furthermore, the population of Rylander's study consisted of cotton mill workers who had been occupationally exposed to the same agent for years. As long-term exposure might cause short-term tolerance to effects of endotoxins, this might obscure the actual dose-effect relationship, as might also the "healthy worker effect" in Rylander's study. Finally, it cannot be ruled out that other constituents of cotton dust may also be of im-

portance in the development of acute pulmonary effects. This was suggested by the results of a study performed by Buck *et al*, 1986, in which changes in lung function were demonstrated when healthy volunteers were exposed to an endotoxin-free eluate of cotton dust (11).

In a study performed in an experimental cardroom, Haglind and Rylander, 1984, demonstrated that a dose-related decrease in FEV_1 was more pronounced in smoking cotton mill workers (n = 4) resulting in a threshold of 80 ng/m^3 versus 170 ng/m^3 in non-smoking (n = 13) volunteers (39). This suggests an increased risk for smokers.

Endotoxin-related acute lung function changes as reported in the above summarised experimental studies have been confirmed in the following two observational studies (21, 86, 88).

Donham *et al,* 1989, found a relationship between endotoxin exposure and an across-shift decrement of FEV_1 and the maximal expiratory flow rate at 25 % of FVC (MEF_{25}, same as FEF_{75}) in 41 non-smoking swine confinement workers. The mean 2–8 hour endotoxin exposure, characterised by area sampling of total dust, was 180 ng/m^3 (1 800 EU/m^3). By regression analysis, it was estimated that decrements in FEV_1 over a workshift began at 180 ng/m^3 (21).

Milton *et al,* 1995 and 1996, showed a dose-effect relationship with across-shift changes over 4 hours in self-recorded peak expiratory flow (PEF) of 37 fibreglass workers exposed to 0.4–759 ng/m^3 (4–7 590 EU/m^3) of endotoxins (personal sampling). An effect on across-shift changes in FEV_1 was also suggested but was not as strong as that demonstrated for PEF. In the medium exposure group (geometric mean 8.4 ng/m^3, range 4–15 (84 EU/m^3, range 40–150)), a ≥ 5 % decline in PEF was observed. Therefore, the authors defined 8.4 ng/m^3 as the lowest adverse observed effect level (LOAEL) and 1.7 ng/m^3 as the NOAEL (86, 88).

Later data

Later studies are described below and those showing dose-effect relationships are also summarised in Table 5 (except the challenge study).

In a study from 1999 by Kline *et al*, 72 healthy volunteers (non-atopic, non-asthmatic, non-smoking) were exposed (within several hours) in sequence to increasing single doses of nebulised LPS: 0.5, 1.0, 2.0, 3.0, 5.0, 10 and 20 µg LPS/person by inhalation challenge. Lung function was examined 1, 10, 20 and 30 minutes after inhalation of each dose. The inhalation challenge was continued with the next dose of LPS if the FEV_1 decrease of the subject was less than 20 % 30 minutes after exposure. Marked differences in the response to inhaled LPS were observed. Eight "sensitive" subjects had at least a 20 % decline in FEV_1 after inhaling 6.5 µg LPS or less per person (cumulative dose). Eleven "hyposensitive" persons maintained an $FEV_1 > 90$ % after inhaling 41.5 µg LPS/person. The three most sensitive responders reached an FEV_1 decrease of 20 % at the second dose (1.5 µg LPS/person, cumulative dose) (61).

Donham *et al*, 2000, studied poultry workers (n = 257) and found a statistically significant dose-effect relation between lung function decrement (FEV_1 and

forced expiratory flow at 25–75 % of lung volume ($FEF_{25–75}$)) over a workshift (2–4 hours) and each quartile of exposure to endotoxins and dust levels (both total and respirable fraction). The exposure-effect correlations were weak with correlation coefficients (r) of 0.16 ($r^2 = 0.026$) and 0.19 ($r^2 = 0.036$) for respirable and total endotoxins, respectively. These low coefficients indicate that only 3–4 % of the variation in lung function is explained by endotoxin exposure. This is explained by the relatively small changes over the workshift relative to the measurement error of 1–3 % for an individual lung function measurement. Correlation and multiple regression were used to calculate the levels at which a 3 % across-shift change in FEV_1 was statistically significant. This was the case at concentrations of 2.4 mg/m^3 total dust, 0.16 mg/m^3 respirable dust, 614 EU/m^3 (61.4 ng/m^3) endotoxins and 0.35 EU/m^3 (0.035 ng/m^3) respirable endotoxins. The combination of 614 EU/m^3 endotoxins and 0.35 EU/m^3 respirable endotoxins is remarkable, as 3.7 % of total endotoxins was respirable. This might, however, be due to division of individual exposure in four groups, each containing a quartile of the exposure level. For each quartile, the odds ratio (OR) and its 95 % confidence interval (CI) for 3 % ΔFEV_1 was calculated. If the OR was significantly different from 1, the lower limit of the group was proposed as no effect level. The relatively arbitrary NOAELs in combination with weak correlations limit the usefulness of this study (22).

In 25 organic waste collectors, FEV_1 was significantly reduced on Thursday pre-shift as compared to Monday pre-shift. Personal full-shift measurements revealed endotoxin levels in the range 7–180 EU/m^3 with an arithmetic mean of 31 and a median of 13 EU/m^3. There was also co-exposure to bacteria (1.2×10^6/m^3) and fungal spores (4×10^5/m^3) (47).

Bønløkke *et al,* 2009, investigated the health effects in swine farm workers during summer and winter. Twenty-four workers underwent lung function testing and blood sampling before and after work. The mean endotoxin exposure of the workers was highest during winter (25 690 vs. 6 553 EU/m^{3}during summer, $p = 0.004$). Although exposure to endotoxins varied between the seasons, no differences in lung function were found between the seasons (12). Earlier results also showed seasonal differences in endotoxin levels in pig houses (100) and in intensive livestock production (111). On the other hand, Seedorf *et al* did not observe a significant seasonal variation in airborne endotoxin concentrations in livestock buildings (cattle, pigs and poultry) (112).

Dosman *et al,* 2006, investigated if inflammatory response can be predicted by FEV_1 response following swine barn exposure. Twenty naïve males were exposed at baseline, low (452 EU/m^3) and high (3 984 EU/m^3) swine dust levels for 5 hours. There appeared to be a dose-effect relationship between the endotoxin levels and lung function decrements. However, no formal statistical testing was presented. The subjects were classified as more or less responsive based on a reduction in FEV_1 over a workshift following the high swine dust exposure. High responders also had significantly greater across-shift FEV_1 reductions at the low exposure level (23).

Table 5. Effects on lung function after acute and short-term endotoxin exposure.

Study design and population	Exposure source	Endotoxin exposure mean (range)	Effects reported	Reference
Experimental exposure (6 h) of healthy volunteers, pre-selected for being reactive to endotoxin, n = 33–61	Cotton	6–779 ng/m^3 (60–7 790 EU/m^3) (108 sessions)	Linear regression: 0 % across-shift change in FEV_1 at 9 ng/m^3 (90 EU/m^3).	(13)
Experimental exposure (4 h) of cotton mill workers, n = 15, of which 8 had a history of byssinosis	Cotton	70–5 620 ng/m^3 (700–56 200 EU/m^3)	Linear regression: 0 % across-shift change in FEV_1 at 33 ng/m^3 (330 EU/m^3).	(109)
Experimental exposure (4 h) of: - non-smoking volunteers, n = 13 and - smoking cotton mill workers, n = 4	Washed cotton	80–1 206 ng/m^3 (80–12 060 EU/m^3)	Linear regression: 0 % across-shift change in FEV_1 at 170 ng/m^3 (1 700 EU/m^3) in the volunteers and 80 ng/m^3 (800 EU/m^3) in cotton mill workers.	(39)
Experimental exposure (5 h), non-smoking male volunteers, n = 20	Swine dust [a]	baseline, low (452 ± 66 EU/m^3) and high exposure (3 984 ± 498 EU/m^3)	There appeared to be a dose-related decline in across-shift FEV_1, however, no formal statistical test data were presented. Two groups of responders appeared (low and high) after both exposures.	(23)
Observational study in non-smoking swine farm workers exposed 2–8 h, n = 41	Swine dust [a]	180 (4–330) ng/m^3 (1 800 (40–3 300 EU/m^3))	Across-shift decline in FEV_1 appeared at 180 ng/m^3 (1 800 EU/m^3).	(21)
Observational study in waste handlers, n = 25	Org. waste [b]	31 (7–180) EU/m^3	FEV_1 decline of 120 ml from Monday to Thursday morning.	(47)
Observational study in poultry workers exposed 2–4 h, n = 257	Poultry faeces and feed [a]	0.24–39 167 EU/m^3 (2.4–3 917 ng/m^3) mean ± SD: 1 589 ± 3 394 EU/m^3 (159 ± 339 ng/m^3)	> 3 % across-shift decline in FEV_1 at > 61.4 ng/m^3 (614 EU/m^3).	(22)
Observational study in glass wool manufacturers exposed 4 h, n = 37	Recycled wash water	0.4–759 ng/m^3 (4–7 590 EU/m^3)	≥ 5 % across-shift decline in self-recorded PEF at 8.4 ng/m^3 (84 EU/m^3). No significant changes in across-shift FEV_1. No effects observed at 1.7 ng/m^3 (17 EU/m^3).	(86, 88)

[a] Co-exposure to e.g. ammonia, [b] Co-exposure to bacteria and fungal spores.
EU: endotoxin unit, FEV_1: forced expiratory volume in 1 second, PEF: peak expiratory flow, SD: standard deviation.

7.3 Long-term exposure

7.3.1 Effects on lung function and respiratory symptoms
Health Council report, 1998
Studies on long-term effects described in the previous Health Council report (41) that yielded dose-effect relationships are described below and in Table 6.

Kennedy *et al,* 1987, performed a cross-sectional study investigating the relationship between endotoxin and dust exposure and lung disease in 443 cotton workers from Shanghai, China and 439 control subjects from a silk mill nearby (57). Pre- and post-shift FVC and FEV_1 were determined for each worker. In 130 area samples (aerodynamic diameter < 15 μm), the endotoxin concentrations varied from 1 to 920 ng/m^3 (10–9 200 EU/m^3) and dust concentrations varied from 0.15 to 2.5 mg/m^3. The cotton worker population was stratified by current endotoxin exposure into four groups with median endotoxin exposures of 2, 100, 230 and 520 ng/m^3 (20, 1 000, 2 300 and 5 200 EU/m^3). Groups were then compared for FEV_1, FVC, FEV_1/FVC %, across-shift ΔFEV_1 and prevalences of chronic bronchitis and byssinosis. All analyses were adjusted for confounders such as age, height and smoking habits. Dose-effect/dose-response trends were seen for the current endotoxin level and FEV_1, ΔFEV_1, and prevalence of chronic bronchitis and byssinosis, except for the highest exposed group in which a reversal of the trend was seen, most likely to be caused by a "healthy workers effect". The dose-effect relation for current exposure was statistically significant for measured pre-shift FEV_1 and was calculated to be -0.242 ml per ng/m^3 (or per 10 EU/m^3) ($p < 0.10$), or, when the highest endotoxin exposure category was excluded, the coefficient increased to -0.778 ml per ng/m^3 (or per 10 EU/m^3) ($p < 0.01$) for workers with a mean work history of 15 years. No correlation coefficient was given. Mean pre-shift FEV_1 in group 1 (median 2 ng/m^3) and group 2 (median 100 ng/m^3) were higher than that in the control group of silk workers (FEV_1 set on 100 %). FEV_1 in group 3 (median 230 ng/m^3) was 96.7 % and in group 4 (median 520 ng/m^3) 98.5 % for non-smokers (both not statistically significantly different from the control group) (57). The authors attempted to assess the presence of a threshold level of endotoxin exposure by comparing the silk workers (controls) to the cotton workers who had always worked in an area with "low endotoxin" levels (less than 20 ng/m^3 or 200 EU/m^3). They found no difference in baseline (= pre-shift) spirometry, but based on the increased prevalence of byssinosis and chronic bronchitis and the augmented across-shift change in FEV_1, the authors suggested that even exposure to the lowest level of endotoxins at 1–20 ng/m^3 (10–200 EU/m^3) constitutes an "adverse respiratory health effect" (57).

Smid *et al,* 1992, performed a similar cross-sectional study in 315 subjects working in 14 animal feed mills in the Netherlands (120). The average 8-hour personal inhalable dust (< 30 μm) exposure was 9 mg/m^3 (range 0.2–150) grain dust and 25 ng/m^3 (range 0.2–470) (250 EU/m^3) endotoxins based on 530 personal dust samples. An external control group was selected without exposure to agents that may affect the respiratory system. This group was, however, not used in the epidemiological analyses as they differed with respect to variables other than

exposure. Further analyses were then performed only with exposed workers and internal control subjects who consisted of non-production animal feed workers. Analyses were adjusted for confounders such as age, height and smoking habits. All studied lung function variables (FVC, FEV_1, PEF, maximal expiratory flow rate at 75 % and 50 % of FVC (MEF_{75} and MEF_{50})) showed significantly reduced values with increasing current exposure to both dust and endotoxins. The differences between the endotoxin categories appeared to be somewhat greater than those between the dust categories. The stronger relationship for endotoxins was also indicated by similar or lower p-values than those for dust exposure. Mean current exposure levels per job title ranged from 6 to 68 ng/m^3 (60–680 EU/m^3) for endotoxins and from 1.7 to 29.7 mg/m^3 for dust. The dose-effect relation for current endotoxin exposure and FEV_1 was calculated to be -4.91 ml per ng/m^3 (or per 10 EU/m^3) for workers with a mean work history of 13 years (annual FEV_1 change -0.38 ml per ng/m^3 based on present exposure and -0.34 ml per ng/m^3 based on cumulative exposure). No clear differences in symptom prevalences existed between different exposure groups. The estimated cumulative exposures of both dust and endotoxins were significantly related to lung function impairment (120).

In 1993, Smid calculated a safe threshold level based on the studies on animal feed workers. Both acute and chronic lung function effects were demonstrated in the intermediate exposure group (40 ng/m^3 or 400 EU/m^3) as compared to the low exposure group (< 15 ng/m^3 or < 150 EU/m^3). The upper limit of the lower exposure group was chosen as the LOAEL. It was estimated from regression models that 40 years of exposure to 15 ng/m^3 (150 EU/m^3) may lead to a decrease in FEV_1 of approximately 200 ml (which is equivalent to approximately 5 %). For MEF_{75}, the effect would be 1 200 ml/second (approximately 16 %). Thus, the author suggested that the NOAEL would be below 15 ng/m^3 (150 EU/m^3). Taking into account selection and attenuation leading to downward bias, the author applied a safety factor to the LOAEL and proposed a "safe" level between 3 and 7.5 ng/m^3 (30–75 EU/m^3) (119), as cited in (41).

Later data

Later studies concerning effects on lung function after long-term exposure are presented in Table 6. For studies in which dose-relationships were examined, a more detailed description follows below.

Post *et al,* 1998, followed up 140 workers in the grain processing and animal feed industry for 5 years (98). This study was a follow-up of the study population of the previously described cross-sectional study by Smid *et al*, 1992 (120). During the first survey, 520 personal exposure samples were gathered (120), and another 179 samples were gathered during the second survey. Mean exposures per job title ranged from 3.6 to 99 ng/m^3 (36–990 EU/m^3) for endotoxins. FEV_1 and maximal midexpiratory flow (MMEF, the average expiratory flow over the middle half of the FVC) were measured on Mondays at the beginning of the study and approximately 5 years later. The annual decline in FEV_1 and MMEF (both corrected

for age, height and smoking) were statistically significantly related to occupational exposure to dust and to endotoxins. An FEV_1 decrease of 0.33 ml (standard error, SE = 0.14) (0.0077 % ± 0.0033) per ng/m^3 endotoxins (or 10 EU/m^3) per year of exposure ($r^2 = 0.12$) was calculated. Fourteen percent of the workers had a rapid (> 90 ml/ year) annual decrease in FEV_1 during the 5 years of the study. Workers with an endotoxin concentration > 20 ng/m^3 (200 EU/m^3) had a statistically significantly higher risk (OR 3.3, 95 % CI 1.02–10.3) of rapid decline in FEV_1. Increasing working years was related to decreasing annual decline in FEV_1 (-18 ml) for over 20 working years and to fewer people with rapid decline in FEV_1.

Christiani *et al*, 1999, performed an 11-year follow-up study in cotton (n = 349) and silk workers (n = 319, both active and retired) recruited in Shanghai, China (17) (same cohort as in the study by Kennedy *et al* (57)). Mean exposure per work area (area sampling) of the cotton workers ranged from 0.2 to 1.6 mg/m^3 for dust and was 42–12 038 EU/m^3 (4.2–1 204 ng/m^3, mean approximately 1 500 ± 1 900 EU/m^3 or 150 ± 190 ng/m^3) for endotoxins, compared to a mean of 0.2 mg/m^3 dust and no (< 1 EU/m^3) endotoxins for silk workers (17). At the beginning of the study, respiratory symptoms occurred more often in cotton workers than in silk workers; byssinosis and chest tightness at work (both 8 % in cotton workers vs. 0–0.2 % in silk workers); chronic bronchitis (22 % vs. 8 %); chronic cough (20 % vs. 7 %) and dyspnoea (grade 2 +) (15 % vs. 4 %) (16). A total of 730 air samples were collected over the 11-year survey period. Mean years of employment at the end of the study were 25 years. The average annual FEV_1 loss was the same for cotton and silk workers. Though initially the FEV_1 loss in cotton workers was (statistically non-significantly) higher with 40 ml/year compared to 30 ml/year in silk workers, after 5 years of follow up, the FEV_1 loss in the last 6 years of study was only 18 ml/year in cotton workers compared to 27 ml/year in silk workers. The total FEV_1 decrease in 11 years was 0.31 litres in both cotton and silk workers and FEV_1 values were 100 % (of predicted) in cotton and silk workers both at baseline in 1981 (99.6 % cotton and 100.6 % silk), as well as 11 years later in 1992 (100.0 % and 100.3 %). After adjustment for confounders, the 11-year loss in FEV_1 was associated with cumulative dust but not with endotoxin exposure (17).

In 2001, Christiani *et al* published the results of another 4-year follow-up, in total 15 years (same cohort). A total of 802 air samples were collected over the 15-year survey period, the medium cumulative endotoxin exposure was 48 000 EU/m^3 × years (4 800 ng/m^3 × years). A small but statistically significantly higher annual FEV_1 loss was found in cotton workers (-32.3 ± 1.0 ml, or 1.1 %) compared to silk workers (-29.4 ± 1.0 ml or 1.0 %) (16). However, the annual decrease in FEV_1 in this study was rather similar to that found in the previous study, and the FEV_1 remained 100 % of the predicted value. It can therefore be assumed that despite the significantly higher decrease in FEV_1 in cotton workers, the FEV_1 will still not be significantly lower than 100 % of predicted. The difference was found only in smokers, as non-smokers had similar annual FEV_1 losses in both groups. A statistically significant relation was found between the annual change in FEV_1 and the across-shift change in FEV_1 (ΔFEV_1). An acute FEV_1 change of -1 % was

associated with an average annual decline in FEV_1 of 0.061 % ($p < 0.001$, r^2 not given). A statistically significant relation of accelerated chronic loss in FEV_1 to byssinosis or chest tightness at work was observed.

In 2005, Wang *et al* published a 20-year follow-up of the same cohort and also presented results from the previous surveys. The cohort now consisted of 346 cotton and 342 silk textile workers. Cotton workers had a mean cumulative endotoxin exposure of 49 123 (± 45 284) EU/m^3 × year. The cotton workers had a greater annual decline in FEV_1 (32.4 ± 1.0 ml/year) compared to the silk workers (27.3 ± 0.9 ml/year) (135).

Kirychuk *et al*, 1998, studied 42 swine-confinement workers in a longitudinal study for 5 years. ΔFEV_1, annual rate change in FEV_1 and FVC, and only the respirable fraction of personal endotoxin exposure were measured at baseline and after 5 years. The mean exposure to respirable endotoxins was about 65 EU/m^3 (6.5 ng/m^3). The mean annual rate change between baseline and follow-up for FEV_1 was -54 ± 62 ml/year (-1.2 ± 1.4 %) and for FVC -49 ± 72 ml/year (-0.9 ± 1.3 %) (60). No statistically significant relation was found between annual FEV_1 or FVC decrease and airborne respirable endotoxins, probably due to the low number of workers studied (resulting in a low power). Therefore, the committees consider the use of this study limited. Furthermore, the committees noted that the authors incorrectly adjusted the calculation for the initial FEV_1 level.

Laitinen *et al,* 2001, assessed associations between self-reported symptoms and exposure to endotoxins of workers in several industries. Among 77 workers, the number of workers with respiratory complaints or fever/shivering was statistically significantly higher when the concentration of biologically active endotoxins in the air was over 25 ng/m^3 (250 EU/m^3). Reporting of eye symptoms and chest tightness was higher when the airborne concentration of biologically active endotoxins was over 150 ng/m^3 (1 500 EU/m^3). Excluding workers with atopy or symptoms of chronic bronchitis from the analysis did not change the results (64). Division of exposed workers into 2 groups (below or above 25 and 150 ng/m^3 (250 and 1 500 EU/m^3), respectively) seemed arbitrary as no statements were made about the origin of these limits.

In 2004, Latza *et al* performed a cross-sectional study in 114 male employees of a cotton mill in western Germany. Airborne endotoxin exposures were classified as low (< 100 EU/m^3), medium (100–450 EU/m^3) or high (> 450 EU/m^3). The dose-response relationships between endotoxin exposure and prevalences of wheezing (medium exposure group: OR 2.15, 95 % CI 0.48–9.62; high exposure group: OR 5.49, 95 % CI 1.17–25.81) and cough (medium exposure group: OR 2.11, 95 % CI 0.59–7.56; high exposure group: OR 3.93, 95 % CI 1.02–15.12) during the last 12 months were significant for the highest exposure group (> 450 EU/m^3) (69).

In 2007, Oldenburg *et al* performed a cross-sectional study in which 150 employees (114 male and 36 female) of the same German cotton spinning mill underwent lung function testing. Airborne endotoxin exposures were classified as low (< 100 EU/m^3), medium (100–450 EU/m^3) and high (> 450 EU/m^3). The dose-

response relationship between current endotoxin exposure and prevalence of an obstructive ventilation pattern was significant (OR 11.2, 95 % CI 1.03–121.2 for the highest exposure group). No significant deviation was observed in mean lung function parameters in the different exposure groups (94).

In a large study of Simpson *et al,* 1998, prevalence of symptoms and endotoxin exposure levels were measured for 1 032 workers in several occupations and industries. Lower respiratory tract symptoms recorded were cough, phlegm, shortness of breath, wheeze and chest tightness. ODTS was identified in people reporting recurrent episodes of at least two of the following symptoms: fever, shivering, malaise, weakness and joint or muscle pain. Byssinosis, work-related chronic bronchitis and eye and nasal irritation were also registered. A relation between prevalence of symptoms and endotoxin exposure levels was shown (115). The authors showed a figure with percentage of workers with lower respiratory tract symptoms plotted against the mean endotoxin level for that group of workers, and a log-linear regression line was drawn. However, the plotted data indicated that an exponential curve would give a better fit and that symptoms were noticeably increased at endotoxin levels above approximately 50 ng/m^3 (500 EU/m^3). However, as raw data were not available, no calculations could be performed and no quantitative conclusions can be drawn. The study showed that workers with symptoms had consistently higher exposures to dust and endotoxins compared to their counterparts working in the same occupations, though the difference was not statistically significant. Highest prevalences of lower respiratory tract symptoms and nasal and eye symptoms were found in poultry workers. Despite high levels of exposure to endotoxins (up to 50 μg/m^3 (500 000 EU/m^3)) only 1.3 % of all workers suffered from ODTS (115).

Vogelzang *et al,* 1998, performed a 3-year follow-up study in 171 pig farmers. Those were selected out of a larger group of pig farmers in a way that half the farmers included in the study would be symptomatic. The mean endotoxin concentration was 105 ng/m^3 (1 050 EU/m^3). A decrease in baseline FEV_1 of 73 ml/year (compared to a normal age-related decrease of 29 ml/year) and a decrease in FVC of 55 ml/year were found (138). In an additional paper, bronchial responsiveness was measured. Provocative histamine concentrations (PCs) were measured for 10 % and 20 % FEV_1 falls. PC_{10} and PC_{20} decreased in both symptomatic and asymptomatic groups within 3 years of additional exposure to ammonia and dust (137).

Smit *et al,* 2008, conducted a cross-sectional study in 877 Dutch farmers and agricultural industry workers in 2006. They investigated exposure-response relationships between current endotoxin exposure and allergic and respiratory symptoms in adults, taking into account farming exposures during childhood. Based on 249 full-shift personal airborne endotoxin samples, a job-exposure matrix was constructed to assign endotoxin exposure levels to all participants. Associations between endotoxin exposure and questionnaire data on symptoms were studied by multiple logistic regressions. Adjusted ORs for an interquartile range increase in endotoxin levels were elevated for respiratory symptoms such

Table 6. Effects on lung function after long-term occupational endotoxin exposure.

Study design and population	Control population	Work history[a]	Endotoxin exposure mean (range)	Parameters measured	Effects reported	Reference
Cross-sectional, animal feed workers, n = 265	Non-exposed colleagues n = 50	13 yrs	< 15, 30–40 and 67 ng/m^3 (range 0.2–470 ng/m^3) (< 150, 300–400 and 670 EU/m^3)	Stratified exposure-response analyses for FEV_1 and respiratory symptoms	Regression[b]: annual FEV_1 change = -0.34 ml per ng/m^3 or per 10 EU/m^3.	(120)
5-year follow-up, grain and animal feed industry workers, n = 140 (same cohort as above)	No	12.5 ± 8 yrs	3.6–99 ng/m^3 (36–990 EU/m^3)	Annual decline in FEV_1, FVC, MMEF, PEF, MEF_{25}, MEF_{50}, MEF_{75} (over 5 yrs)	Regression[b]: annual FEV_1 change = -0.33 ml (SE = 0.14, r^2 = 0.12) per ng/m^3 or per 10 EU/m^3.	(98)
Cross-sectional, cotton mill workers, n = 443	Silk workers n = 439	16–17 yrs	2, 100, 230 and 520 ng/m^3 (20, 1 000, 2 300 and 5 200 EU/m^3)	Stratified exposure-response analyses for FEV_1 and respiratory symptoms	Regression[b]: annual FEV_1 change = -0.016 to -0.052 ml per ng/m^3 or per 10 EU/m^3.[c]	(57)
11-year follow-up, employed and retired cotton workers, n = 349 (same cohort as above)	Silk workers n = 319	16–17 yrs	≈ 40 000[d] EU/m^3 × yr (cumulative) 1 500 (27–12 038) EU/m^3 (150 ng/m^3)	Annual FEV_1 loss	No cumulative endotoxin effects on FEV_1.	(17)
15-year follow-up, employed and retired cotton workers, n = 346 (same cohort as above)	Silk workers n = 338	16–17 yrs	48 000 EU/m^3 × yr (median cumulative) 1 500 EU/m^3 (150 ng/m^3)	Annual FEV_1 loss	Greater annual FEV_1 decline as compared to controls (1.1 % per yr vs. 1.0 %); correlation between ΔFEV_1 and annual FEV_1 decline. Findings independent from endotoxin exposure.	(16)
20-year follow-up, employed and retired cotton workers, n = 346 (same cohort as above)	Silk workers n = 342	16–17 yrs	49 000 EU/m^3 × yr	Annual FEV_1 loss	Greater annual FEV_1 decline (32.4 ± 1.0 ml/yr) as compared to controls (27.3 ± 0.9 ml/yr).	(135)

Table 6. Effects on lung function after long-term occupational endotoxin exposure.

Study design and population	Control population	Work history[a]	Endotoxin exposure mean (range)	Parameters measured	Effects reported	Reference
9-year follow-up, RDF workers with rotating jobs, n = 87	No	9–10 yrs	Geometric mean: 28 ± 3.8 EU/m^3 (2.8 ± 0.38 ng/m^3)	FVC and FEV_1 change over 9 yrs	No effect observed.	(76)
Cross-sectional, pig farmers of which 50 % symptomatic, n = 40 poultry farmers of which 58 % symptomatic, n = 36	General population	20 yrs	Median (range): 58 (1–1 101) ng/m^3 (580 EU/m^3) 258 (19–1 635) ng/m^3 (2 580 EU/m^3)	FEV_1, MMEF and FVC	% predicted FEV_1: > 100 % in both groups. % predicted MMEF: 101 % in pig farmers. 89 % in poultry farmers.	(102)
Longitudinal, cotton workers in 3 mills, newly hired young (average 18 yrs) non-smoking females, n = 101	No	0.0 yrs	220, 1 360 and 1 070 ng/m^3 (2 200, 13 600 and 10 700 EU/m^3) at 3 months	Change in FEV_1, FVC and ΔFEV_1 after 3, 12, 18 months as compared to 1st day of work	FEV_1 and FVC: 2 % ↑ after 3 months. FVC: 5 % ↓ after 12 and 18 months. FEV_1: 2.8 % ↓ after 12 months and 1.3 % ↓ after 18 months.	(134)
3-year follow-up, pig farmers selected so that 50 % had symptoms, n = 171	No	16.7 yrs	105 ng/m^3 (1 050 EU/m^3)	FEV_1 and FVC	Decrease in baseline FEV_1 of 73 ml/yr and a decrease in FVC of 55 ml/yr.	(137, 138)
11-year follow-up, paper workers (males employed 1989–2000), n = 97	n = 55	-	69 (6–370) EU/m^3	ΔFEV_1 and ΔFVC yearly for 11 yrs	No increase in loss of lung function in workers exposed up to 200 EU/m^3.	(113)
Cross-sectional, paper workers (bark cleaning and paper recycling in paper factory), n = 77	Office workers n = 40	12 yrs	Low-exposure group: 2–20 ng/m^3 (20–200 EU/m^3) High-exposure group: 21–98 ng/m^3 (210–980 EU/m^3)	Serum MPO and ECP, airway responsiveness, symptoms and baseline FEV_1	Decreased baseline FEV_1, increased airway responsiveness, elevated serum MPO and ECP levels and nose irritation in both groups. Flu-like symptoms in the high-exposure group.	(110)

Table 6. Effects on lung function after long-term occupational endotoxin exposure.

Study design and population	Control population	Work history[a]	Endotoxin exposure mean (range)	Parameters measured	Effects reported	Reference
Cross-sectional, veterinarians, non-smoking and without asthma, n = 66	General population	nd	2.8–3.6 hours/day: 18–28 ng/m^3 (180–280 EU/m^3) 8-hour TWA: 9 ng/m^3 (90 EU/m^3)	% of subjects with diurnal PEF variation > 20 %	No effects observed.	(31)

[a] At the start date of the study.
[b] Linear regression: annual $\Delta FEV_1 = \beta \times$ endotoxin exposure (ng/m^3 or EU/m^3).
[c] For comparability with other studies, -0.242 to -0.778 ml FEV_1 change per ng/m^3 over 15 years has been converted to annual FEV_1 change.
[d] Calculated by NEG from a cumulative mean exposure of 23 826 EU/m^3 × yr *at baseline* and an estimated annual exposure of ca. 1 500 EU/m^3 for 11 years.
ECP: eosinophilic cationic protein, EU: endotoxin unit, FEV_1: forced expiratory volume in 1 second, FVC: forced vital capacity, MEF_x: maximal expiratory flow rate at x % of FVC, MMEF: maximal midexpiratory flow (average flow over middle half of FVC), MPO: myeloperoxidase, nd: not determined, PEF: peak expiratory flow, RDF: refuse-derived fuel, SE: standard error, TWA: time-weighted average.

as wheezing (OR 1.41, 95 % CI 1.16–1.72), wheezing with shortness of breath (OR 1.50, 95 % CI 1.18–1.90) and daily cough (OR 1.29, 95 % CI 1.03–1.62). In contrast, endotoxin exposure was strongly associated with a decreased prevalence of hay fever (OR 0.62, 95 % CI 0.49–0.78). Workers who had grown up on a farm had a lower prevalence of hay fever, but no evidence was found of effects modification by farm childhood. Smit *et al* concluded that occupational endotoxin exposure in adulthood was associated with an increased risk of asthma-like symptoms but a reduced prevalence of hay fever (121).

7.4 Carcinogenicity

Cancer risks have been investigated in relation to occupational exposure to endotoxins. In the 1970s, findings in several occupational cohort studies suggested reduced risks in mortality studies for lung cancer among textile workers (48, 50, 66, 79). More recent findings suggest an inverse dose-response for lung cancer.

Astrakianakis *et al,* 2007, observed a dose-dependent reduction in lung cancer risk in a cohort of female textile workers in Shanghai (4). The authors stated that the study had several limitations. A potential source of bias is the "healthy worker effect". However, the authors concluded that a healthy worker effect was probably not an important bias in their study. Only limited epidemiological evidence for this relation is available in other industries with endotoxin exposure.

In a recent review concerning the relation between exposure to endotoxins and cancer, Lundin *et al* concluded that epidemiological studies of cotton textile and other endotoxin exposed occupational groups have consistently demonstrated reduced lung cancer risks. However, absence of data on potentially confounding factors has been a limitation of most studies (74).

7.5 Reproductive effects

There were no data available.

7.6 Immunological effects

CD14 is a regulator of T-cell activity, which may have great relevance to the pathogenesis of allergic asthma. The ligation of endotoxin to CD14 depends on the presence of a transporter protein, the LPS binding protein. The LPS binding protein is an acute-phase protein that circulates in plasma and binds to endotoxins forming high affinity complexes that enhance the capacity of low-concentrations of endotoxins to bind to and activate macrophages and neutrophils. Under normal conditions, little LPS binding protein is present in the lung. After inhalation of antigen by atopic subjects, extravasations of LPS binding protein and soluble CD14 to this compartment occur, due to rapid increase in bronchial microvascular permeability. This allows the endotoxin that was inhaled with the antigen to amplify the inflammatory response to the antigen (80), while the other way round, the

simultaneous presence of antigens leads to an exaggerated response to endotoxins in asthmatic subjects (61).

In a study performed by Michel *et al,* 1989, bronchial obstructive responses (associated with an increase in non-specific bronchial reactivity) were demonstrated in asthmatic and rhinitis patients at an inhalatory dose of 20 µg endotoxins (LPS) (200 000 EU) per person, while no bronchial constriction at this level was observed in healthy subjects. Healthy subjects responded at a dose level of 200 µg (2 000 000 EU) endotoxin/person. No significant response was observed in the asthmatic and rhinitis patients at dose levels up to 2 µg (20 000 EU) endotoxin/ person. Endotoxin-induced bronchial obstruction was reflected in decreased forced expiratory values (e.g. FEV_1) (83).

Low levels of endotoxin exposure significantly augment the inflammatory response to allergen exposure in sensitised subjects with asthma (72, 103), in subjects with allergic rhinitis (33), and in skin test wheal-and-flare response to allergen (80). In metropolitan households, higher house dust endotoxin levels have been associated with increased asthma symptoms (84, 139). Higher house dust endotoxin levels are also associated with more wheezing symptoms in the first year of life (36). Possible explanations for this association of endotoxin exposure with increased asthma symptoms at any age include an adjuvant-like effect of endotoxin exposure on airway inflammation, increased susceptibility to viral respiratory tract infections caused by endotoxin exposure, and respiratory manifestations after endotoxin inhalation in normal and asthmatic subjects (32, 33, 61, 62, 80, 139). Significant blood leukocytosis and neutrophilia were observed 4–8 hours after inhalation of endotoxins in normal and asthmatic subjects. In *in vitro* studies, it is observed that small amounts of endotoxins (< 1 ng/ml) activate human airway macrophages, releasing several pro-inflammatory cytokines (TNFα, IL-1, IL-6) and metabolites of arachidonic acid (80). The presence of LPS binding protein and the soluble fraction of CD14 receptor in the airways increases the macrophage activation by endotoxins (26). After inhalation of endotoxin-containing dust (6 hours), high concentrations of IL-1, IL-1 RA, IL-6, IL-8 and TNFα, and their mRNA were measured in BAL (19) (as cited in (80)). Also after exposure to endotoxin containing swine dust for 3 hours, IL-8 was induced in BAL and NAL fluid of non-smoking subjects (68). Increased neutrophil recruitment was also observed in BAL (51) (as cited in (26)). A significant increase in neutrophils in the induced sputum occurred in asthmatic subjects after inhalation of 5–60 µg (50 000–600 000 EU) endotoxin/person, which was also seen to a lesser extent in normal subjects exposed to endotoxins (84, 92, 130). The sputum concentrations of myeloperoxidase (MPO, from neutrophils), eosinophilic cationic protein (ECP) and TNFα rose significantly 6 hours after endotoxin inhalation.

Some published data suggests that environmental endotoxins could be a synergistic factor on the amplitude of an immunoglobulin E (IgE) mediated response. Allergic asthmatics exposed to air with low levels of endotoxins (250 ng/m^3 or 2 500 EU/m^3) for 4 hours before bronchial challenge with allergen show an increased bronchial reactivity and antigen-induced airway eosinophilia. While de-

toxified allergen extract results in bronchial eosinophil recruitment, endotoxin contamination (1 ng/ml) causes recruitment of neutrophils. In sensitised subjects, inhalation of allergen leads to airway plasma exudation including extravasations of soluble CD14 and LPS binding protein (81). Asthmatic subjects exposed to endotoxins show a significant decrease in lung function, reflected in a decreased FEV_1 and FEV_1/FVC ratio, and PEF (24, 33, 84, 92). The decrease in FEV_1 and systemic response were smaller in atopic than in non-atopic subjects, suggesting a link between atopic status and endotoxin responsiveness (7, 81, 82).

Besides exacerbation of the adverse effects of asthma on the lungs in adults, it is hypothesised that exposure to endotoxins early in life (no particular dose levels given) has a protective effect on the early allergic response and thus the early development of atopic (allergic) asthma (72). This is thought to be mediated by binding of endotoxin to innate immune cells, which are thereby stimulated to produce cytokines supportive of T-helper cell type 1 (Th1) development, i.e. IL-12 and interferon-gamma (IFNγ) (72, 73). The induction of a Th1 response in early life, separate from serious infection, down-regulates Th2-type immune development, which is relevant in preventing atopy and possibly atopic asthma (25, 73). Although the studies on the protective effect of endotoxins in early development of allergy in children gave more insight into the mode of action, it is not directly relevant to the occupational exposure of endotoxins and the possible adverse health effects in adults including asthmatic persons.

Endotoxins also induce up-regulation of CD14 expression by macrophages (67, 128). The membrane bound CD14 is a multifunctional receptor constitutively expressed primarily on the surface of monocytes, macrophages and neutrophils and serves as a receptor for the LPS - LPS binding protein complex (5, 9). CD14 participates in regulation of IL-8 and IL-6 release by bronchial epithelial cells (128). The soluble form of CD14 is abundant in serum and is apparently derived both from secretion of soluble CD14 and from enzymatically cleaved glycosyl-phosphatidylinositol-anchored membrane-bound CD14 (2), suggesting that polymorphism in the CD14 gene promoter region could influence the differentiation of T-cells and the levels of serum IgE. In a large cohort of allergic and non-allergic children, two alleles were found. Children exhibiting the TT allele presented higher levels of circulating soluble CD14, and lower IgE and IL-4 levels (2). Those children appeared to benefit from the protective effect of higher levels of soluble CD14, which allows stronger stimulation of Th1 during bacterial infections of endotoxin exposure in early life, hereby reducing the chances of becoming atopic.

As described before, there is evidence for increased allergic response after endotoxin exposure. Furthermore, an increased response to endotoxin exposure has been observed in patients with symptomatic atopy or allergic asthma. However, no support is found for the hypothesis that chronic inhalatory endotoxin exposure may non-specifically encourage sensitisation to antigens in man (adjuvant effect). On the other hand, there is growing compelling evidence that even endotoxin exposure at adult age protects against the development of atopic responses. This evidence comes from occupationally exposed work force based studies in farmers

(45) and from general population samples. Despite the increased response to endotoxins in symptomatic atopics, the attribution of atopy to the increased prevalence of respiratory symptoms in exposed workers is rather small. The positive association between endotoxin exposure and respiratory effects in non-atopics therefore predominates. Finally, the role of confounding effects due to atopy might even underestimate the association between endotoxin exposure and respiratory effects as was probably the case in the study of Smit *et al*, 2008 (121).

In 2009, Smit *et al* conducted a case-control analysis with unrelated subjects to investigate whether single nucleotide polymorphisms (SNPs) in CD14, TLR2, TLR4 and TLR9 genes were associated with asthma in adults. The role of atopy was evaluated by conducting separate analyses for atopic and non-atopic subjects. The authors concluded that TLR2 and CD14 single nucleotide polymorphisms were associated with asthma and atopic asthma, respectively. In addition, CD14, TLR2, TLR4 and TLR9 single nucleotide polymorphisms modified the association between country living and asthma (122).

Atopic asthmatics are more sensitive to endotoxin exposure than healthy subjects. A possible explanation for the enhanced sensitivity of atopic asthmatics might be the fact that soluble CD14 and LPS binding protein levels in the lungs are severely increased (26). LPS binding protein and soluble CD14 are normally present in human plasma in concentrations of 5–10 μg/ml (132) (as cited in (26)) and ~ 6 μg/ml (6) (as cited in (26)), respectively. Extravagation of LPS binding protein and soluble CD14 into the bronchoalveolar compartment after antigen inhalation, due to increase in bronchial microvascular permeability, might enhance the capacity of inhaled endotoxins to activate an inflammatory response. On the other hand, atopic, but asymptomatic, subjects may have a genetically lower response to LPS, e.g. by lower levels of expression of soluble CD14 (2), lower expression of TNFα (89) or lower expression of LPS binding protein. Because of the lower response of macrophages to endotoxins, lower levels of IL-12 did stimulate the Th2 cell expression when the immune system was developing (72), which stimulates B-cell production of IgE by IL-4. So, hyporesponsiveness to endotoxins might have caused the atopy.

7.7 Other effects

Sjögren *et al,* 2003, compared the occurrence of ischaemic heart disease (IHD) among male and female livestock and agricultural workers in Sweden. The IHD mortality among the livestock and agricultural workers was compared to that of gainfully employed men and women. The standardised mortality ratio (SMR) for livestock male workers was 1.06 (95 % CI 0.95–1.18) and for female workers 1.10 (95 % CI 0.98–1.23). Agricultural workers had lower SMRs. Adjustments for smoking habits increased the SMR by about 9 % in male workers and about 5 % in female workers (118).

No data were available regarding neurological and endocrine effects.

7.8 Summary and evaluation

Acute and short-term exposure

Acute health effects in humans after inhalation of endotoxins are dry cough and shortness of breath accompanied by a decrease in lung function, fever reactions and malaise, and sometimes dyspnoea, headache and joint aches occurring a few hours after the exposure. Acute effects have been demonstrated in laboratory studies with human volunteers and in epidemiological studies in exposed workers.

In a number of studies performed in volunteers and in workers, biological responses (not necessarily reflecting clinically manifest effects) were measured. In one study, the first responders in a group of healthy volunteers showed a ΔFEV_1 decline of at least 20 % after exposure to 1.5 µg (15 000 EU) (3 × 0.5 µg) endotoxin, whereas exposure to 0.5 µg (5 000 EU) did not lead to significant decline in ΔFEV_1 in any of them (61).

In another study, a clear exposure-effect relationship ($\% \Delta FEV_1 = 3.84 - 4.02$ (^{10}log endotoxin in ng/m^3) with an $r^2 = 0.72$ and $p < 0.0001$) was found in a group of healthy volunteers (smoking and non-smoking) who were exposed to endotoxin concentrations up to 779 ng/m^3 (7 790 EU/m^3) for 6 hours. The linear regression model yielded a zero percentage across-shift change in FEV_1 during exposure to endotoxins at 9 ng/m^3 (90 EU/m^3) (13). In a study of fibre-glass workers, an across-shift decline over 4 hours in self-recorded PEF ≥ 5 % was shown for the group with a GM exposure of 8.4 ng/m^3 (84 EU/m^3) (LOAEL). The authors defined 1.7 ng/m^3 (17 EU/m^3, GM of low-exposure group) as the NOAEL (86, 88).

Long-term exposure

Epidemiological studies suggest that chronic endotoxin exposure may lead to chronic bronchitis and reduced lung function. Only in three studies was a quantitative dose-effect relationship between endotoxin exposure and lung function parameters reported.

Post *et al*, 1998, found a dose-effect relationship with an annual FEV_1 decline of 0.33 ± 0.14 ml (mean ± SE) per ng/m^3 (or per 10 EU/m^3) endotoxin exposure in a 5-year follow-up study in animal feed workers (98). Post *et al*, 1998, used the same cohort as Smid *et al,* 1992 (120). In the cross-sectional study of Smid *et al,* an annual FEV_1 decline of 0.34 ml per ng/m^3 was calculated.

A third dose-effect relationship was found in the cross-sectional study by Kennedy *et al*, 1987. They found an annual FEV_1 change in cotton workers of - 0.052 ml per ng/m^3 (or per 10 EU/m^3) (as converted from -0.778 ml FEV_1 per ng/m^3 after a mean of 15 working years) (57). This value is lower than those found in the first two studies.

A possible explanation for the different outcomes might be the presence of other constituents in the air that also influenced lung function. Furthermore, in the studies of Post *et al* and Smid *et al*, but not in that of Kennedy *et al*, a dose-effect relationship was also found for FEV_1 changes and exposure to dust. A quick scan of the amount of endotoxins per µg dust in the air revealed remarkable differences. In the Kennedy *et al* study (origin of endotoxin is cotton), the ratio of

ng endotoxin per µg dust is much higher than in the study of Smid *et al* (origin of endotoxin is grain). Endotoxin levels co-varied with dust levels in both studies. Therefore, it is assumed that (some specific constituents of) (grain-) dust contributed to the steeper decline in FEV_1 in subject exposed in the studies of Post *et al* and Smid *et al* and that the most accurate dose-effect relationship between endotoxin exposure and FEV_1 changes is revealed by Kennedy *et al*.

Several epidemiological studies have been performed in which workers were stratified by exposure levels. Workers with average work histories of 10–20 years were exposed to average endotoxin concentrations varying from 2.8 to 520 ng/m^3 (28–5 200 EU/m^3). Effects of exposure were found on baseline FEV_1 for workers exposed to 230 ng/m^3 (2 300 EU/m^3) or higher (57), but not in workers exposed to average endotoxin levels at or below 150 ng/m^3 (1 500 EU/m^3) (16, 17). In newly hired cotton workers, lung function parameters were affected after one year of exposure to average endotoxin levels of 220 ng/m^3 (2 200 EU/m^3) or more (134).

In most studies, workers had respiratory complaints. However, respiratory symptoms are quite common as the incidence of respiratory symptoms in the normal population can be higher than 30 %. Associations between symptoms and endotoxin exposure, or symptoms and lung function changes were highly inconsistent and, therefore, conclusions will not be based on uncertain differences in the occurrence of symptoms.

Immunological effects

Several studies indicated that some people are more sensitive to endotoxins than others. This concerns especially atopic asthmatics and other symptomatic atopics, as even generally low endotoxin levels in house dust can aggravate asthma or other respiratory tract effects. The literature also indicates that asymptomatic atopics are equally or even less sensitive to endotoxins than healthy persons.

No evidence is found for the hypothesis that chronic inhalatory endotoxin exposure may non-specifically encourage sensitisation to antigens in man (adjuvant effect). In contrast, endotoxin exposure even at mature age seems to protect against the development of atopy.

In conclusion, the committees note a number of different interactions between exposure to endotoxins and atopy or atopic asthma. These different interactions comprise 1) an increased susceptibility to endotoxin of symptomatic atopics; 2) the protection against development of atopy after exposure to endotoxins. In addition, gene-environment interactions; 3) have been observed for respiratory symptoms (wheezing). The committees believe that there are several possible mechanisms behind the various interactions between exposure to endotoxins and atopy or atopic asthma.

Carcinogenic, reproductive, neurological or endocrine effects

In the literature, no evidence is found for possible reproductive, neurological or endocrine effects. Endotoxins probably do not enter the bloodstream to any great extent, which makes an effect on reproductive, neurological or endocrine end-

points unlikely. Reduced lung cancer rates as a beneficial effect of occupational exposure to LPS have been suggested in textile workers.

8. Existing guidelines, standards and evaluations

8.1 General population

There is no recommended exposure limit for airborne endotoxins for the general population.

8.2 Working population

In 1994, an evaluation on occupational endotoxin exposure was conducted by the International Committee on Occupational Health (ICOH), through its Committee on Organic Dusts. It was stated that ODTS is elicited at exposure levels of 1 000–2 000 ng/m^3 (10 000–20 000 EU/m^3), while acute bronchoconstriction occurs at levels of 100–200 ng/m^3 (1 000–2 000 EU/m^3), and mucous membrane irritation at levels of 20–50 ng/m^3 (200–500 EU/m^3). It was also stated that these levels may be lower for sensitive subjects (52).

In the Netherlands, an advice report was written (1998) in order to set a maximal accepted concentration (MAC) for endotoxins, coming up with a health based recommended occupational exposure level of 5 ng/m^3 (50 EU/m^3) (41). However, no legally binding limit has yet been established.

9. Hazard assessment

9.1 Assessment of the health hazard

Airborne endotoxin exposure, occurring in certain occupational settings, has convincingly been shown to generate biological and clinical effects in man. Exposure to endotoxins can cause acute and chronic health effects. The lung appears to be the main target organ in which these adverse effects occur.

Endotoxin exposure has been associated with decreased lung function in several experimental and epidemiological studies. The committees consider an across-shift FEV_1 change as a sensitive and important parameter to indicate lung function changes due to inhalation endotoxin exposure in a dose-dependent manner. FEV_1 is known to be the parameter most consistently affected by endotoxin exposure, and small decrements in FEV_1 are sensitive indicators of respiratory impairment and mortality.

Consequences of decreased lung function in general
For a good interpretation of data on FEV_1, the following considerations are important:
- FEV_1 decreases with age.

- FEV_1 decrease is not linear, but the annual decrease in FEV_1 increases with age and is dependent on sex and standing height (34) (i.e. ± 0.5 % per year for 20-year old women (34) and ± 0.8 % per year as the mean in men and woman from 30 to 60 years (8)).
- The average FEV_1 decline during 40 years is approximately 1 litre in the non-smoking population, which corresponds to approximately 25–30 ml/year (59).
- FEV_1 exhibits variability over 24-hour periods (diurnal variation) (8).
- The annual FEV_1 decline due to endotoxin exposure decreases with increasing working years in an endotoxin-rich environment (17, 98). The annual FEV_1 decrease is higher in chronically symptomatic workers than in asymptomatic workers (16, 98).

A WHO working group has recommended criteria for classifying individual workers with respect to ventilatory effects caused by exposure to organic dust. The working group defined chronic ventilatory effects for an individual as "mild to moderate" when FEV_1 (measured after an absence from exposure of at least two days) is between 60 and 79 % of the reference level, and as "severe" when it is less than 60 % of the reference level. Chronic ventilatory impairment is defined "absent" in an individual as long as the FEV_1 level is over 80 % of the reference level (as mentioned in Chattopadhyay *et al,* 2003 (15)). Using individual FEV_1 values expressed as percentage of predicted for that person (considering its sex, standing height and age) can prevent difficulties in interpretation of the data and automatically corrects differences due to confounding by one of those parameters.

Several studies indicate that an average decline in FEV_1 (on *group* level) may be a predictor of respiratory morbidity and mortality (3, 30, 44, 106).

In a 20-year follow-up study in a population of 668 men, an average FEV_1 loss of 620 ml (compared to the predicted FEV_1) was associated with a higher relative risk (RR) of developing chronic non-specific lung disease (RR 1.8, 95 % CI 1.27–2.67), a higher mortality from chronic non-specific lung disease (RR 3.35, 95% CI 1.23–9.11) and a higher total mortality (RR 1.32, 95 % CI 1.03–1.71). Workers with an FEV_1 reduction greater than 1 240 ml below the reference level had a considerably higher risk for developing chronic lung disease (RR 12.8, 95 % CI 5.96–27.5), a higher mortality due to chronic lung disease (RR 25.5, 95 % CI 8.69–75.0) and a higher total mortality (RR 2.86, 95 % CI 1.82–4.49) (44).

Ryan *et al*, 1999, found that the average FEV_1 was significantly associated with all-cause mortality and cardiovascular disease mortality in both sexes. An extra decline in FEV_1 of 50 ml/year increased the risk of death for all causes in women by 1.23 (95 % CI 1.06–1.44). In men, the effect of decline in FEV_1 on death rate was less (106).

In a study of Sin *et al,* 2005, it has been shown in a population (n = 1 861, 40–60 years) that a mean decline of FEV_1 to 88 % of the predicted FEV_1, is statistically significantly associated with cardiovascular events. This association was not found for a mean decline to 96 % of the predicted FEV_1 (117). Assuming a mean predicted FEV_1 of 3 litres, the committees estimate that no association with cardio-

vascular effects has to be expected when FEV_1 is declined with 120 ml (4 % of FEV_1).

However, for the hazard assessment of occupational exposure to endotoxins, DECOS and NEG emphasise that effects on lung function on a population level should be weighed differently than effects on an individual level. In other words, although, for example, a 5 % decrease in FEV_1 for an individual person is not considered an adverse effect by the WHO, as cited in (15), DECOS and NEG are of the opinion that such a decrease in FEV_1 *on the population level* should be considered adverse because the population will include individuals with considerably higher (and lower) decreases.

Acute and short-term exposure

Studies concerning effects on lung function of acute and short-term exposure to endotoxins are summarised in Table 5 (Section 7.2). Critical studies are compiled in Table 7.

In a study from 1987 by Castellan *et al,* healthy volunteers, pre-selected for being sensitive to LPS, were exposed to cotton dust during 6 hours, with airborne endotoxin concentrations ranging from 6 to 779 ng/m^3 (60–7 790 EU/m^3). A zero percentage across-shift change in FEV_1 at an exposure level of 9 ng/m^3 (90 EU/m^3) was calculated (13).

Milton *et al,* 1995 and 1996, showed a dose-effect relationship with across-shift decreases (> 5 %) over 4 hours in self-recorded PEF of 37 fibreglass workers exposed to 0.4–759 ng/m^3 (4–7 590 EU/m^3) endotoxin (personal sampling). An effect on across-shift changes in FEV_1 was also suggested but was not as strong as that demonstrated for PEF. A LOAEL of 8.4 ng/m^3 and a NOAEL of 1.7 ng/m^3 was defined in this study (86, 88).

The committees consider an across-shift change in FEV_1 a more reliable indicator of lung function change than a small decline in self-reported PEF.

Long-term exposure

Studies on long-term exposure to endotoxins and adverse human effects on lung function are summarised in Table 6 (Section 7.3). Critical studies are compiled in Table 7.

Chronic exposure to endotoxins is a causal factor for chronic airway responses. Three studies reported quantitative dose-effect relationships. Smid *et al*, 1992 (120) and Post *et al,* 1998 (98), studying the same cohort of animal feed workers (exposed to grain), observed a correlation between annual FEV_1 change and endotoxin exposure, i.e. -0.34 ml (0.0077 %) per ng/m^3 (or per 10 EU/m^3) endotoxins. Kennedy *et al*, 1987, however, found a less steep dose-effect relation in cotton workers, i.e. -0.052 ml FEV_1 annually per ng/m^3 (or per 10 EU/ m^3) endotoxins (57). A clear explanation for this difference was not given but a remarkable difference in dust per endotoxin ratio in air is noted. The dust per endotoxin ratio was approximately 100 times higher in the studies of Smid *et al* (120) and Post *et al* (98) than in that of Kennedy *et al* (57). This might suggest that other con-

stituents in the air are responsible for the steeper decrease in FEV_1 in the study of Smid *et al* and Post *et al*. In addition, also some methodological differences between the studies may have played a role.

Christiani *et al*, 2001, found a statistically significant relationship between acute (across-shift) changes in FEV_1 and annual changes in FEV_1 (16). The findings were, however, independent from exposure to endotoxins. It is thought that the correlation between chronic and acute FEV_1 decrease is more likely caused by variability in sensitivity of the respiratory tract between subjects than by the level of endotoxin exposure. This leads to changes in acute ΔFEV_1 and in the long run to chronic FEV_1 decreases.

Latza *et al,* 2004, found a dose-effect relation between exposure to endotoxins and respiratory symptoms (wheezing and coughing) (69). The result suggests a clear dose-dependent increase in respiratory symptoms after exposure to endotoxin levels between 100 and 450 EU/m^3, with significant effects after exposure to levels that exceeded 450 EU/m^3. The low-exposure group (< 100 EU/ m^3) was used as controls. Therefore, NOAELs or LOAELs cannot be derived.

Other adverse or beneficial effects after long-term exposure

No information is available concerning adverse effects of inhalatory exposure to endotoxins on reproduction, neurological, endocrine or other systemic parameters.

Cancer risks have been investigated in relation to occupational exposure to endotoxins. Reduced risks for lung cancer have been reported in several epidemiological studies since the 1970s. The most recent finding of Astrakianakis *et al* from 2007 suggests an inverse relation between cancers of the lung and endotoxin exposure in the textile industry as well (4). No biological explanation for this finding has been given yet.

Recent studies suggest that environmental endotoxin exposure might protect against the development of atopy and asthma. A lower prevalence for atopy and hay fever has been observed in farmer's children and in adolescents with farmers' background compared to those without a farmer's background. Negative exposure response relations have also been observed (97). However, on the other hand, occupational exposure to endotoxins is a risk factor for wheeze and bronchial hyperresponsiveness and these symptoms have most often a non-atopic background, independent on the presence of allergy. Asthma-like disorders are induced by occupational exposure to endotoxins in the absence of atopic sensitisation and a recent analysis indicated that the attribution of symptoms to atopy is small in highly endotoxin exposed populations (97, 121).

In conclusion, there is considerable reason for caution for interpreting associations between atopy and endotoxin exposure. Furthermore, DECOS and NEG do not take the possible health benefits into account for the quantitative health assessment after occupational exposure.

Conclusion
The critical study regarding lung function effects from acute exposure is the study by Castellan *et al* (13), in which linear regression analysis indicated that 90 EU/m^3 (9 ng/m^3) is the highest level at which no across-shift change in FEV_1 occurs (NOAEL). By combining this result with those from long-term studies, it is possible to judge whether this level can also be regarded as a NOAEL for lung function effects after chronic occupational exposure. Applying the results by Castellan *et al* to the data of Smid *et al* and Post *et al* (98, 120) implies that 40 years of exposure to 90 EU/m^3 (9 ng/m^3) endotoxins cause an extra decline in FEV_1 of 120 ml. If one instead applies the data of Kennedy *et al* (57), in the same way, a more than 10-fold lower decline is implied. The committees consider an extra decline of 120 ml over 40 years to be a non-adverse effect, in view of the average age-related decrease of about 1 litre in non-smokers over the same period.

9.2 Groups at extra risk

Groups suffering from COPD and groups with atopic asthma and atopic respiratory disease have an increased risk of aggravation of respiratory symptoms and other acute pulmonary effects at endotoxin levels that would not affect "normal" healthy workers. Furthermore, smokers may be more sensitive for endotoxin insults than non-smokers.

9.3 Scientific basis for an occupational exposure limit

The critical effect of endotoxin inhalation exposure is considered to be impaired lung function. Data indicates that no adverse FEV_1 decline is expected from long-term exposure up to approximately 90 EU/m^3 (9 ng/m^3).

It is recommended to measure endotoxins by using the most recent version of the LAL-assay (NEN-EN 14031 procedure with adjustments by Spaan *et al*, 2007) as older methods tend to underestimate the exposure.

10. Recommendations for research

There are no recommendations for further research.

Table 7. Critical studies with a dose-effect relation between endotoxin exposure and adverse effects on lung function.

Study design and population	Work history[a]	Endotoxin exposure, range	Effect/NOAEL based on	Reference
Experimental exposure (6 h) of healthy volunteers, pre-selected for being reactive to endotoxins, n = 33–61	-	6–779 ng/m^3 (60–7 790 EU/m^3)	0 % across-shift change in FEV_1 at 9 ng/m^3 (90 EU/m^3).	(13)
Cross-sectional, cotton mill workers, n = 443 silk workers, n = 439 (controls)	15 yrs	2, 100, 230, 520 ng/m^3 (20, 1 000, 2 300, 5 200 EU/m^3)	Annual FEV_1 change: -0.016 to -0.052 ml per ng/m^3 or per 10 EU/m^3. [b]	(57)
Cross-sectional, animal feed workers, n = 265 non-exposed colleagues, n = 50	13 yrs	0.2–470 ng/m^3 < 15, 30–40, 67 ng/m^3 (< 150, 300–400, 670 EU/m^3)	Annual FEV_1 change: -0.34 ml per ng/m^3 or per 10 EU/m^3.	(120)
5-year follow-up animal feed industry workers, n = 140 no controls (same cohort as above)	12.5 ± 8 yrs	3.6–99 ng/m^3 (36–990 EU/m^3)	Annual FEV_1 change: -0.33 ml per ng/m^3 or per 10 EU/m^3.	(98)

[a] At the start date of the study.
[b] For comparability with other studies, -0.242 to -0.778 ml FEV_1 per ng/m^3 over 15 years has been converted to annual FEV_1 change.
EU: endotoxin unit, FEV_1: forced expiratory volume in 1 second, NOAEL: no observed adverse exposure level.

11. Summary

The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Safety. 144. Endotoxins. Arbete och Hälsa 2011;45(4):1-53.

Endotoxins are components of the outer membrane of Gram-negative bacteria and are a biologically active component in most organic dusts. Occupational exposure occurs primarily in the agriculture industry and related sectors.

Endotoxin levels in air can be measured either in absolute terms (weight/m^3) or as functional (bioactive) levels. The latter can be measured by use of the *Limulus* amebocyte lysate assay and levels are expressed as endotoxin units (EU)/m^3.

Endotoxins that enter the upper respiratory tract are expelled by means of muco-ciliary transport. Smaller particles deposit in the deeper airways and are locally phagocytised by macrophages, a process that may result in inflammatory reactions. Therefore, systemic effects due to inhaled endotoxins are most likely induced by cytokines that are released from the lung into the blood.

Airborne endotoxin exposure has been shown to generate biological and clinical effects in man. The lung appears to be the main target organ and endotoxin exposure has been associated with decreased lung function in several experimental and epidemiological studies. Acute effects in humans after endotoxin inhalation are dry cough and shortness of breath accompanied by decreased lung function, fever reactions and malaise, and sometimes dyspnoea, headache and joint aches occurring a few hours after exposure. Chronic endotoxin exposure may lead to chronic bronchitis and reduced lung function.

Forced expiratory volume in 1 second (FEV_1) is known to be the parameter most consistently affected by endotoxin exposure, and small decrements in FEV_1 are early and sensitive indicators of respiratory impairment.

A clear exposure-effect relationship for the percentage change in FEV_1 was found in healthy volunteers (pre-selected for being sensitive to endotoxin) exposed to endotoxins for 6 hours. The study indicated that 90 EU/m^3 (9 ng/m^3) is the highest level at which no acute across-shift change in FEV_1 occurs. This result was combined with those from long-term studies. The extra decline in FEV_1 after 40 years of exposure to 90 EU/m^3 endotoxin was calculated to be up to 120 ml, which was considered as a non-adverse effect.

In conclusion, no adverse health effects are expected after chronic occupational exposure at 90 EU/m^3.

Keywords: endotoxin, lipopolysaccharide, lung function, occupational exposure limit, respiratory effects, review, risk assessment, toxicity

12. Summary in Swedish

The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and the Dutch Expert Committee on Occupational Safety. 144. Endotoxins. Arbete och Hälsa 2011;45(4):1-53.

Endotoxiner är beståndsdelar i cellväggen hos Gram-negativa bakterier och är en biologiskt aktiv komponent i de flesta typer av organiskt damm. Yrkesmässig exponering för endotoxiner förekommer framför allt inom jordbrukssektorn och därtill knutna industrier.

Nivåerna av endotoxiner i luft kan mätas i absoluta termer (vikt/m^3) eller som funktionella, bioaktiva nivåer. De senare kan mätas med hjälp av *Limulus* amöbocytlysat-metoden och anges i endotoxinenheter (EU)/m^3.

Vid inandning av endotoxiner deponeras dessa i andningsvägarna. Vid deponering i luftstrupen och bronkerna transporteras partiklarna bort av flimmerhåren. Mindre partiklar fastnar i de nedre luftvägarna där de fagocyteras av makrofager vilket kan utlösa inflammatoriska reaktioner. Systemiska effekter orsakas därför troligen av cytokiner som frisätts från lungan ut i blodet.

Luftburna endotoxiner ger upphov till biologiska och kliniska effekter på människa. Lungorna tycks vara det viktigaste målorganet och exponering för endotoxiner har satts i samband med försämrad lungfunktion i flera experimentella och epidemiologiska studier. Akuta hälsoeffekter efter inandning är torrhosta och andfåddhet åtföljt av försämrad lungfunktion, feber och sjukdomskänsla, samt ibland andnöd, huvudvärk och ledvärk som uppträder några timmar efter exponeringen. Epidemiologiska studier antyder att kronisk exponering för endotoxiner kan leda till kronisk bronkit och försämrad lungfunktion.

Forcerad exspiratorisk volym under första sekunden (FEV_1) anses vara en tillförlitlig parameter för att påvisa effekter av endotoxiner och små försämringar av FEV_1 är tidiga och känsliga indikatorer på luftvägsbesvär. Tydliga dos-effekt-samband för den procentuella förändringen i FEV_1 observerades hos friska frivilliga (utvalda för att vara känsliga för endotoxiner) som exponerades för endotoxiner i 6 timmar. Studien indikerade att 90 EU/m^3 (9 ng/m^3) är den högsta nivå som inte ger upphov till akuta förändringar i FEV_1 över ett arbetspass. I kombination med data från långtidsstudier kan det beräknas att exponering i 40 år för 90 EU/m^3 ger en extra sänkning av FEV_1 på upp till 120 ml, vilket inte anses vara skadligt.

Sammanfattningsvis bedöms yrkesmässig långtidsexponering för 90 EU/m^3 inte ge upphov till skadliga hälsoeffekter.

Nyckelord: endotoxin, hygieniskt gränsvärde, lipopolysackarid, lungfunktion, respiratoriska effekter, riskbedömning, toxicitet, översikt

13. References

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14. Data bases used in the search for literature

This report has been based on scientific data, which are publicly available. Data were mainly obtained from the online database MEDLINE, using endotoxins and LPS as main key words and many additional search terms for refinement. The search was performed for the period January 1996 till May 2004. An additional search was performed in January 2010 using the following keywords: endotoxin, health effects and occupational.

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Appendix 1. The committees

Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG)

Gunnar Johanson, *chairman*	Institute of Environmental Medicine, Karolinska Institutet, Sweden
Kristina Kjærheim	Cancer Registry of Norway
Anne Thoustrup Saber	National Research Centre for the Working Environment, Denmark
Tiina Santonen	Finnish Institute of Occupational Health
Vidar Skaug	National Institute of Occupational Health, Norway
Mattias Öberg	Institute of Environmental Medicine, Karolinska Institutet, Sweden
Jill Järnberg and Anna-Karin Alexandrie, *scientific secretaries*	Swedish Work Environment Authority

Dutch Expert Committee on Occupational Safety (DECOS)

GJ Mulder, *chairman*	Leiden University
RB Beems	formerly employed at the National Institute for Public Health and the Environment
PJ Boogaard	Shell International BV
JJAM Brokamp, *advisor*	Social and Economic Council
DJJ Heederik	Institute for Risk Assessment Sciences, Utrecht University
R Houba	Netherlands Expertise Centre for Occupational Respiratory Disorders (NECORD)
H van Loveren	Maastricht University and National Institute for Public Health and the Environment
TM Pal	Netherlands Center for Occupational Diseases
AH Piersma	National Institute for Public Health and the Environment
HPJ te Riele	VU University Amsterdam
I.M.C.M. Rietjens	Wageningen University and Research Centre
H Roelfzema, *advisor*	Ministry of Health, Welfare and Sport
GMH Swaen	Dow Benelux N.V.
RCH Vermeulen	Institute for Risk Assessment Sciences
RA Woutersen	TNO Quality of Life and Wageningen University and Research Centre
PB Wulp	Labour Inspectorate
ASAM van der Burght, *scientific secretary*	Health Council of the Netherlands

Appendix 2. Previous NEG criteria documents

NEG criteria documents published in the scientific serial Arbete och Hälsa (Work and Health):

Substance/Agent	Arbete och Hälsa issue
Acetonitrile	1989:22, 1989:37*
Acid aerosols, inorganic	1992:33, 1993:1*
Acrylonitrile	1985:4
Allyl alcohol	1986:8
Aluminium	1992:45, 1993:1*
Ammonia	1986:31, 2005:13*
Antimony	1998:11*
Arsenic, inorganic	1981:22, 1991:9, 1991:50*
Arsine	1986:41
Asbestos	1982:29
Benomyl	1984:28
Benzene	1981:11
1,2,3-Benzotriazole	2000:24*D
Boric acid, Borax	1980:13
1,3-Butadiene	1994:36*, 1994:42
1-Butanol	1980:20
γ-Butyrolactone	2004:7*D
Cadmium	1981:29, 1992:26, 1993:1*
7/8 Carbon chain aliphatic monoketones	1990:2*D
Carbon monoxide	1980:8
Ceramic Fibres, Refractory	1996:30*, 1998:20
Chlorine, Chlorine dioxide	1980:6
Chloromequat chloride	1984:36
4-Chloro-2-methylphenoxy acetic acid	1981:14
Chlorophenols	1984:46
Chlorotrimethylsilane	2002:2
Chromium	1979:33
Cobalt	1982:16, 1994:39*, 1994:42
Copper	1980:21
Creosote	1988:13, 1988:33*
Cyanoacrylates	1995:25*, 1995:27
Cyclic acid anhydrides	2004:15*D
Cyclohexanone, Cyclopentanone	1985:42
n-Decane	1987:25, 1987:40*
Deodorized kerosene	1985:24
Diacetone alcohol	1989:4, 1989:37*
Dichlorobenzenes	1998:4*, 1998:20
Diesel exhaust	1993:34, 1993:35*
Diethylamine	1994:23*, 1994:42
2-Diethylaminoethanol	1994:25*N
Diethylenetriamine	1994:23*, 1994:42
Diisocyanates	1979:34, 1985:19
Dimethylamine	1994:23*, 1994:42
Dimethyldithiocarbamates	1990:26, 1991:2*
Dimethylethylamine	1991:26, 1991:50*
Dimethylformamide	1983:28
Dimethylsulfoxide	1991:37, 1991:50*
Dioxane	1982:6
Enzymes, industrial	1994:28*, 1994:42
Epichlorohydrin	1981:10

Substance/Agent	Arbete och Hälsa issue
Ethyl acetate	1990:35*
Ethylbenzene	1986:19
Ethylenediamine	1994:23*, 1994:42
Ethylenebisdithiocarbamates and Ethylenethiourea	1993:24, 1993:35*
Ethylene glycol	1980:14
Ethylene glycol monoalkyl ethers	1985:34
Ethylene oxide	1982:7
Ethyl ether	1992:30* N
2-Ethylhexanoic acid	1994:31*, 1994:42
Flour dust	1996:27*, 1998:20
Formaldehyde	1978:21, 1982:27, 2003:11*D
Fungal spores	2006:21*
Furfuryl alcohol	1984:24
Gasoline	1984:7
Glutaraldehyde	1997:20*D, 1998:20
Glyoxal	1995:2*, 1995:27
Halothane	1984:17
n-Hexane	1980:19, 1986:20
Hydrazine, Hydrazine salts	1985:6
Hydrogen fluoride	1983:7
Hydrogen sulphide	1982:31, 2001:14*D
Hydroquinone	1989:15, 1989:37*
Industrial enzymes	1994:28*
Isoflurane, sevoflurane and desflurane	2009;43(9)*
Isophorone	1991:14, 1991:50*
Isopropanol	1980:18
Lead, inorganic	1979:24, 1992:43, 1993:1*
Limonene	1993:14, 1993:35*
Lithium and lithium compounds	2002:16*
Manganese	1982:10
Mercury, inorganic	1985:20
Methacrylates	1983:21
Methanol	1984:41
Methyl bromide	1987:18, 1987:40*
Methyl chloride	1992:27*D
Methyl chloroform	1981:12
Methylcyclopentadienyl manganese tricarbonyl	1982:10
Methylene chloride	1979:15, 1987:29, 1987:40*
Methyl ethyl ketone	1983:25
Methyl formate	1989:29, 1989:37*
Methyl isobutyl ketone	1988:20, 1988:33*
Methyl methacrylate	1991:36*D
N-Methyl-2-pyrrolidone	1994:40*, 1994:42
Methyl-tert-butyl ether	1994:22*D
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* in English, remaining documents are in a Scandinavian language.
D = collaboration with the Dutch Expert Committee on Occupational Safety (DECOS).
N = collaboration with the US National Institute for Occupational Safety and Health (NIOSH).

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