Endotoxins

Health-based recommended occupational exposure limit

Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

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Geachte minister,

Graag bied ik u hierbij het advies aan over de gevolgen van beroepsmatige blootstelling aan endotoxinen.

Het maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over endotoxinen is opgesteld door de commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving. Ik onderschrijf de conclusies en aanbevelingen van de Commissie.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport en aan de minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer.

Met vriendelijke groet,

prof. dr. ir. D. Kromhout
waarnemend voorzitter

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Health-based recommended occupational exposure limit

Dutch expert Committee on Occupational Safety
a Committee of the Health Council of the Netherlands
in cooperation with the Nordic Expert Group for
Criteria Documentation of Health Risks from Chemicals

to:

the Minister of Social Affairs and Employment

No. 2010/04OSH, The Hague, July 15, 2010

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in lucht waaraan mensen blootgesteld kunnen worden tijdens de beroepsuitoefening. Die vormen vervolgens de basis voor grenswaarden, vast te stellen door de minister, waarmee de gezondheid van werknemers beschermd kan worden. In dit advies bespreekt de commissie de gevolgen van blootstelling aan endotoxinen en stelt een gezondheidskundige advieswaarde vast.

Dit rapport is opgesteld in samenwerking met de *Nordic Expert Group for criteria documentation of health risks from chemicals*. Het advies is een actualisering van het in 1998 verschenen rapport van de Gezondheidsraad, waarin een eerste evaluatie van de gezondheidskundige implicaties van blootstelling aan endotoxinen werd gemaakt. Het voorliggende rapport bestaat uit een kort overzicht van het vorige rapport, aangevuld met nieuwe literatuur. De conclusies van de commissies zijn gebaseerd op wetenschappelijke publicaties die vóór januari 2010 zijn verschenen.

Fysische en chemische eigenschappen

Endotoxinen maken deel uit van de buitenste membraan van gramnegatieve bacteriën. Ze bestaan uit eiwitten, lipiden en lipopolysachariden. Lipopolysachariden (LPS) van gramnegatieve bacteriën zijn koolwaterstoffen die vrij zijn van eiwit of andere celwandbestanddelen. Ze zijn verantwoordelijk voor het merendeel van de biologische effecten die worden teweeggebracht door bacteriële endotoxinen. LPS zijn in water oplosbaar. Het LPS-molecuul is stabiel en bestaat uit een lipide en een polysacharide-deel. Het lipide-deel, 'lipide A' genoemd, is verantwoordelijk voor de toxiciteit van LPS. Tussen uiteenlopende bacteriesoorten bestaat een opmerkelijke overeenkomst met betrekking tot de samenstelling van lipide A. Daarentegen is er een aanzienlijke variatie in de samenstelling van het hydrofiele polysacharide-deel van LPS.

Het vóórkomen van endotoxinen in de omgevingslucht is gerelateerd aan de aanwezigheid van gramnegatieve bacteriën of celwandfragmenten van deze bacteriën in organische stofdeeltjes in de lucht. Dergelijke bacteriehoudende deeltjes zijn hoofdzakelijk afkomstig van dierlijke fecaliën en van gecontamineerd plantaardig materiaal. Daarom komt beroepsmatige blootstelling aan endotoxinen vooral voor in de agrarische sector en aanverwante bedrijfstakken.

Monitoring

Milieumonitoring vindt plaats door waterige extracten, die uit luchtstof-monsters zijn verkregen, te onderzoeken met de Limulus Amebocyte Lysate (LAL) test. Er bestaan nog geen algemeen geaccepteerde standaarden voor de luchtbemonsterings- en extractieprocedures. Voor het bepalen van de endotoxineconcentratie in de lucht, beveelt de commissie de NEN-EN14031 methodiek met enkele aanpassingen door Spaan e.a. (2007) aan.

Grenswaarden

Noch in Nederland, noch in andere landen is tot dusver een grenswaarde voor beroepsmatige blootstelling aan endotoxinen in lucht vastgesteld.

Kinetiek en toxisch werkingsmechanisme

Endotoxinen die terechtkomen in de bovenste luchtwegen, worden via mucociliair transport verwijderd. Men neemt aan dat dieper doorgedrongen endotoxinen onschadelijk worden gemaakt door macrofagen en polymorfonucleaire leukocy-

ten. Het is zeer waarschijnlijk dat effecten op de longfunctie geïnduceerd worden door ontstekingsreacties in de longen. Systemische effecten worden veroorzaakt door cytokinen die in het bloed terechtkomen; geïnhaleerde endotoxinen komen waarschijnlijk niet zelf in de bloedbaan terecht.

Effecten

Direct na inademing van endotoxinen kunnen zich bij mensen de volgende verschijnselen voordoen: droge hoest, kortademigheid met vermindering van de longfunctie, koorts en algehele malaise. Enkele uren later kunnen optreden: benauwdheid, hoofdpijn en gewrichtsklachten. De acute effecten zijn zowel aangetoond in onderzoek met vrijwilligers als in epidemiologisch onderzoek onder beroepsmatig blootgestelde personen. Bij astmapatiënten en bij mensen met ontstekingen van het neusslijmvlies is aangetoond dat blootstelling aan LPS kan leiden tot obstructie van de bronchiën, gepaard gaand met een toename van de reactiviteit. Uit epidemiologisch onderzoek zijn aanwijzingen verkregen dat langdurige blootstelling aan endotoxinen zou kunnen leiden tot chronische bronchitis en vermindering van de longfunctie. Het is zeer waarschijnlijk dat zowel de acute als de chronische effecten geïnduceerd worden door ontstekingsreacties in de longen, waarbij de macrofagen in de longblaasjes een sleutelrol spelen.

Er zijn geen gegevens die duiden op mutagene, reprotoxische of cardiovasculaire effecten na blootstelling aan endotoxinen. Onderzoek naar het risico op kanker na blootstelling aan endotoxinen in de textielindustrie, suggereert een negatieve relatie tussen longkanker en endotoxine blootstelling. Een verklaring voor deze bevinding is tot nu toe niet gevonden. Recent onderzoek suggereert ook dat blootstelling aan endotoxinen mogelijk beschermt tegen de ontwikkeling van atopie en hooikoorts. Atopie en hooikoorts komen namelijk minder voor bij kinderen die zijn opgegroeid op een boerderij (waar blootsteling aan onder meer endotoxinen kan plaatsvinden). Aan de andere kant is beroepsmatig blootstelling aan endotoxinen wel een risicofactor voor de ontwikkeling van bronchiale gevoeligheid en kortademigheid. Ook zijn astmagerelateerde effecten toegenomen in aan endotoxinen blootgestelde werknemers.

Evaluatie en advies

Een afname in longfunctie wordt beschouwd als het kritische effect van inhalatoire kortdurende en langdurige blootstelling aan endotoxinen. Veranderingen in longfunctie worden het beste gemeten door veranderingen in de FEV_1 (Forced Expiratory Volume in 1 second, dat is de hoeveelheid lucht die binnen 1 seconde

geforceerd uitgeblazen kan worden). Een verandering vóór en na blootstelling op één dag is een maat voor acute effecten, veranderingen in de baseline FEV_1 of jaarlijkse FEV_1 afname is een maat voor chronische effecten.

De basis voor het afleiden van een advieswaarde is een acute studie waarin gezonde vrijwilligers (meermalen) werden blootgesteld aan endotoxinen afkomstig van katoen, een cross-sectional studie naar chronische effecten op de longfunctie van werknemers in de mengvoederindustrie, én een 5 jaar follow-up studie in de mengvoederindustrie.

Op basis van een studie naar de effecten van 6 uur blootstelling aan endotoxinen in vrijwilligers, beschouwt de Commissie GBBS een blootstellingniveau van 90 EU/m^3 als een NOEL (geen waargenomen effect nivo). Aangezien de commissie van mening is dat de groep vrijwilligers een gevoelige groep betreft (ze zijn namelijk geselecteerd op basis van gevoeligheid voor endotoxinen), acht ze een extrapolatiefactor om rekening te houden met individuele gevoeligheid niet nodig. Op basis van deze studie komt de commissie dus tot een gezondheidskundige advieswaarde van 90 EU/m^3 (8 uur tijdgewogen gemiddelde, tgg).

Vervolgens beoordeelt de commissie of deze advieswaarde ook beschermt tegen de effecten van langdurige blootstelling aan endotoxinen. Blootstelling aan 90 EU/m^3 gedurende 40 jaar zou in de cross-sectionele studie in de diervoederindustrie een extra verlaging van 120 ml FEV_1 betekenen. In een studie in katoenmedewerkers is het effect op de longfunctie (FEV_1 daling) minder. De commissie is van mening dat een extra verlaging van de FEV_1 met 120 ml (in 40 jaar) in het algemeen niet geassocieerd wordt met andere gezondheidseffecten (bv cardiovasculaire effecten).

Daarom stelt de Commissie GBBS vast dat een gezondheidskundige advieswaarde voor endotoxinen van 90 EU/m^3 (8 uur tgg) zowel tegen de effecten van acute, kortdurende als langdurige blootstelling beschermt.

De Commissie GBBS heeft verder vastgesteld dat de huidige meetmethodieken van de NEN-EN (met enkele aanpassingen) gevoeliger zijn dan de oudere blootstellingmeetmethoden. De commissie acht het echter niet mogelijk om een standaard conversie factor vast te stellen die in alle situaties van toepassing is. Daarnaast zijn in recentere studies, met recentere meetmethodieken van de blootstelling, respiratoire effecten waargenomen bij blootstellingen hoger dan 100 EU/m^3. De commissie stelt daarom geen standaard factor voor die corrigeert

voor het verschil in gevoeligheid tussen de oudere en meer recente blootstelling-meetmethodieken.

Gezondheidskundige advieswaarde

De Commissie GBBS beveelt een gezondheidskundige advieswaarde voor beroepsmatige blootstelling aan endotoxinen aan van 90 EU/m^3, gemiddeld over een acht urige werkdag (tgg 8 uur). Voor het bepalen van de blootstelling aan endotoxinen adviseert de commissie gebruik te maken van de meest recente NEN-EN 14031 blootstellingmeetmethoden aangevuld met de modificaties die voorgesteld zijn door Spaan e.a. (2007).

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Executive summary

Background

At the request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, recommends health-based occupational exposure limits for airborne substances to which people are exposed in the workplace. These recommendations serve as a basis in setting legally binding occupational exposure limits by the Minister. In this report, the Committee considers the implications of exposure to endotoxins and recommends a health-based occupational exposure limit for these substances.

This report has been compiled in collaboration with the *Nordic Expert Group for criteria documentation of health risks from chemicals.* It updates an earlier Health Council report, published in 1998, which set out the Council's initial evaluation of the health implications of exposure to endotoxins. The present report consists of a brief summary of the earlier report, plus information gleaned from literature published since 1998. The committees' conclusions reflect the content of scientific publications that have appeared prior to January 2010.

Physical and chemical properties

Endotoxins are substances found in the outer membranes of gram-negative bacteria. They consist of proteins, lipids and lipopolysaccharides. Lipopolysaccha-

rides (LPSs) from gram-negative bacteria are hydrocarbons that are free of protein and other cell wall materials. They are responsible for most of the biological effects of bacterial endotoxins. LPSs are soluble in water. The LPS molecule is a stable combination of a lipid component and a polysaccharide component. It is the lipid component, known as 'lipid A', that is responsible for the toxicity of LPSs. The composition of lipid A is remarkably similar in a wide variety of species of bacteria. By contrast, the composition of the hydrophilic polysaccharide LPS component varies considerably.

The presence of endotoxins in ambient air is related to the presence of gram-negative bacteria or cell wall fragments from such bacteria in airborne organic dust particles. Such bacteria-containing particles originate mainly from animal faeces and contaminated plant material. Occupational exposure to endotoxins consequently occurs principally in the agricultural industry and related sectors.

Monitoring

There are no generally accepted standards for the air sampling and extraction procedures. For the determination of endotoxin concentrations in the air, the Committee recommends using the NEN-EN14031 method, with adjustments by Spaan *et al*. (2007).

Exposure limits

To date, no occupational exposure limits have been defined for airborne endotoxins, either in the Netherlands or elsewhere.

Kinetics and toxic effect mechanism

Endotoxins that enter the upper respiratory tract are expelled by means of mucociliary transportation. It is believed that endotoxins that penetrate further into the respiratory tract are rendered harmless by macrophages and polymorphonuclear leukocytes. The effects that these substances can have on lung function are in all probability induced by inflammatory responses in the lungs. The systemic effects that occur are attributable to cytokines that find their way into the blood; it is not thought that inhaled endotoxins themselves enter the bloodstream.

Effects

In humans, the inhalation of endotoxins may cause the following acute symptoms: dry cough, dyspnoea accompanied by diminished lung function, fever and general malaise. After several hours, the following symptoms may develop: bronchoconstriction, headache and aching joints. The acute effects have been observed in the context of research with volunteers and reported in the context epidemiological research amongst occupationally exposed people. It has been demonstrated that, in asthma sufferers and people with inflammations of the nasal mucosa, exposure to LPSs can lead to bronchial obstruction, accompanied by increased reactivity. Epidemiological research has produced evidence to suggest that prolonged exposure to endotoxins may lead to chronic bronchitis and diminished lung function. It is highly likely that both the acute and the chronic effects are induced by inflammatory reactions in the lungs, in the context of which the macrophages in the alveoli play a key role.

No evidence of mutagenic, reproduction toxic or cardiovascular effects has been reported following exposure to endotoxins. The findings of research into the risk of cancer following exposure to endotoxins in the textiles industry suggest a negative relationship between lung cancer and endotoxin exposure. No convincing explanation for this relationship has been provided. Recent research results also suggest that exposure to endotoxins protects against the development of atopy and hay fever, which are less prevalent in children who grow up on farms (where exposure to endotoxins and other substances can occur). On the other hand, occupational exposure to endotoxins is a risk factor for the development of bronchial sensitivity and dyspnoea. Furthermore, asthma-related conditions are more common in endotoxin-exposed workers.

Evaluation and recommendations

Diminished lung function is regarded as the critical effect of both short and long-term inhalatory exposure to endotoxins. Changes in lung function are best measured by measuring the FEV_1 (forced expiratory volume in one second, i.e. the amount of air that can be forcibly exhaled in the space of a second). Divergence between the pre-exposure and post-exposure FEV_1 over a single day is indicative of acute effects, while change in the baseline FEV_1 or decline in the annual FEV_1 is indicative of chronic effects.

The exposure limit recommended by DECOS is based upon an acute study, in which healthy volunteers were exposed to endotoxins from cotton, a cross-sec-

tional study of the chronic effects on the lung function of animal feed mill workers and a five-year follow-up study of such workers.

On the basis of a study of the effects of six-hour exposure to endotoxins in volunteers, DECOS regards an exposure level of 90 EU/m^3 as a NOEL (no observed effect level). DECOS regards the volunteers used in the study as a sensitive group, because they were selected on the basis of their sensitivity to endotoxins. Hence, DECOS does not believe that an extrapolation factor is necessary to take account of variations in individual sensitivity. On the basis of this study, DECOS' health-based recommended exposure limit is 90 EU/m^3 (eight-hour time-weighted average).

DECOS has additionally considered whether the health-based recommended exposure limit referred to above affords adequate protection against the effects of prolonged exposure to endotoxins. According to the findings of the cross-sectional study of animal feed mill workers, exposure to 90 EU/m^3 for forty years would result in an additional reduction of 120 ml in the average worker's FEV_1. A study of cotton workers indicated a less pronounced effect on lung function (smaller FEV_1 reduction). DECOS does not consider an additional FEV_1 reduction of 120 ml to constitute an adverse effect; a reduction of this size is not generally associated with other health effects (e.g. cardiovascular effects).

Hence, DECOS takes the view that a health-based recommended exposure limit of 90 EU/m^3 (eight-hour time-weighted average) affords adequate protection against the effects of both acute and chronic exposure to endotoxins.

DECOS has also established that, subject to certain modifications, the test methods currently described in NEN-EN are more sensitive than the exposure measurement methods used in the past. Nevertheless, DECOS does not believe it is possible to specify a standard conversion factor that is applicable in all circumstances. Furthermore, in more recent studies, which used more recent methods to measure exposure, respiratory effects were observed in subjects who were exposed to concentrations higher than 100 EU/m^3. DECOS does therefore not propose the use of a standard factor to correct for differences in the sensitivity of older and newer methods for measuring exposure.

Health-based recommended exposure limit

DECOS proposes a health-based recommended exposure limit (HBROEL) of 90 EU/m^3 (eight-hour time-weighted average) for endotoxins in the workplace. Fur-

thermore, DECOS recommends using the method currently described in NEN-EN 14031, modified as suggested by Spaan *et al*. (2007), to measure exposure to endotoxins.

Chapter 1

Scope

1.1 Background

At the request of the Minister of Social Affairs and Employment (Annex A), the Dutch Expert Committee on Occupational Safety (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations of the toxicity of substances that are used at the workplace. The purpose of these evaluations is to recommend health-based occupational exposure limits for concentrations in the air, provided the database allows the derivation of such values.

1.2 Committee and procedure

The advice is a co-production of the DECOS and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). It is a result of an agreement between both groups to prepare jointly scientific criteria documents, which can be used by the national regulatory authorities in the Netherlands and the Nordic countries for establishing exposure limits. This document contains an assessment of the health hazard of occupational exposure to endotoxin by DECOS and NEG, hereafter called the committees. The recommendation of the health based occupational exposure limit (see section 9.2) is, however, only the responsibiilty of DECOS. The members of both committees are listed in Annex B.

In 2009, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex C. These comments are taken into account in deciding on the final version of the report. It is to be noted that this report is an update of the previous report of the Health Council of the Netherlands on endotoxins published in 1998.[1]

1.3 Data

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. Relevant references (ie. references with quantification of exposure) were included in the advice. Finally, a list of abbreviations and symbols can be found at the end of this report in Annex D.

Chapter 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.[1]

2.1 Chemical identity

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 Health Council's report 1998.[1]

Physical and chemical properties

LPS are stable water-soluble molecules composed of lipids and polysaccharides. In water, LPS usually converts 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 KDO (2-keto-3-deoxy-D-manno-octulosonic acid). 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 Health Council's report 1998.[1]

Endotoxins are relatively heat stable; the temperature reported to inactivate LPS is 177°C,[2] and LPS is stable at 121°C for at least 1 h.[3] It is estimated that a single cell of *Salmonella* contains 10 femtograms (10×10^{-15} g) of LPS,[3] 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 SDS (sodium dodecyl sulphate), individual monomers may form with molecular weights of approximately 2,500 to 25,000 Dalton.[4]

2.2 EU Classification and labeling

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 either be measured 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 unit (EU)/m^3. In the LAL-assay, the reaction is measured between endotoxins in the sample and a pro-enzyme purified from horseshoe crab (*Limulus*) amebocytes (blood cells). Subsequent coagulation can be evaluated by an increase in optical density measured spectrophotometrically. Test values are read off 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.[4,5] 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 level in air.

Table 1 A comparison between different analytical methods used determining occupational endotoxin levels (adapted from industox).

part of the method	aspect	method described by Spaan *et al.* 2007[6]	method used by Castellan *et al.* (1987)[7]	method used by Smid *et al.* (1992)[8] and Post *et al.* (1998)[9]
sample	dust-fraction	inhalable dust	inhalable dust	inhalable dust
	filter type	glass fiber	teflon	glass fiber
extraction and storage	storage temp filter	-18°C	+4°C	+4°C
	extraction solution	pyrogenic water with 0.05% TWEEN	pyrogenic and sterile water	pyrogenic and sterile water
	storage temp	-18°C	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	kabivitrium test

As endotoxin levels in air measured in a functional assay correlate better with toxic effects than measured in weight/m^3,[10] the chromogenic LAL-test is the most accepted assay for endotoxin exposure measurements. The detection limit of the present airborne environmental endotoxin measurement is approximately 0.005 EU/m^3.

In 2003, a NEN-EN 14031 protocol was published concerning a standardized method for the extraction and analyses of endotoxin concentrations in the environment.[11]

Despite the specificity of the LAL-method, significant differences in calculated levels of exposure have been reported by different laboratories analysing the same samples, demonstrated by several round robin studies. A round robin study allows an evaluation of a test method by examining two parameters critical to any test method: inter-laboratory and intra-laboratory variation. One study compared 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). This comparison revealed tenfold 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 labs and all dust types.[12] In another round robin study, thirteen laboratories measured endotoxin concentrations that

also varied up to a factor 10, despite the use of a common extraction procedure for extraction of endotoxins from cotton dust.[13] The variation in endotoxin concentration in twenty samples measured by three laboratories, all using the NEN-EN 14031 protocol for extraction and analyses, were smaller, maximally a factor 4.2 between the different laboratories.[11] Therefore, several studies conclude that a reliable assessment of exposure to endotoxins activity is only possible when standard operation procedures (SOPs) for sampling and determination are established.[14,15] On the other hand, Spaan *et al*. (2008) also showed using a endotoxin exposure database (with a fairly similar protocol for exposure measurement) that the analytical error for endotoxin 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.[15]

According to Rylander,[16] the results of the LAL-test also depend on the physical state of the endotoxins in the sample. If it is present in a water solution, the values represent all of the endotoxins present in the sample. If the analysis is made on a dust sample, where endotoxins are still part of fragments of an intact bacterial cell walls, 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.[16]

In conclusion, the present NEN-EN-procedure still leaves some aspects of the protocol open for interpretation by individual laboratories. Nevertheless, the committee emphasizes that airborne endotoxin exposure should be assessed using standardized 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 (eg. 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.[6,14,17]

The committees recommend to adapt these adjustments of Spaan *et al*. (2007)[6] in the NEN-EN protocol.

Finally, in the earlier versions of the LAL assays, the relation between 1 EU and the amount of endotoxins in weight per cubic metre, is that 1 EU is usually considered equivalent to 0.1 ng. This is dependent on the potency of the specific species of endotoxins used to create the standard curve.[5]

Moreover, the committees are aware that the more recent versions of the LAL-assay (NEN-EN protocol and Spaan *et al*. (2007)[6] are more sensitive in measuring the endotoxin exposure. Compared to these recent protocols, the older assays most likely underestimated the exposure levels of endotoxins in the past.[6,12,17,18]

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 micro-organisms, 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 committee recommends to monitor endotoxin air levels more frequently than normally applied for workplace control measurements.[17]

2.5 Recommendations

In the former report of the Health Council[1], recommendations on procedures for collection, storage, extraction and analysis of airborne dust samples for endotoxins were made. In 2004, a NEN-EN 14031 protocol was published. Spaan *et al*. (2007)[6] investigated the gaps in this NEN-EN protocol and presented several adjustments to the guideline protocol for further standardisation. The committees recommend to adapt these adjustments in the NEN-EN protocol and recommends further standardization.

Chapter 3

Sources

The protection of workers against toxic effects of endotoxin 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 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.[1]

Chapter 4

Exposure

4.1 Environmental levels

Water

In the report of Health Council (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 0.2 to 1.0 µg endotoxin/ml. These concentrations were exceptionally high and situations like that were considered not likely to occur in the Netherlands.[1]

Since 1998 new data has become available. In studies reviewed by Anderson *et al.* (2002),[3] it was indicated that endotoxin concentration in raw (untreated) water ranged from <1 to 1050 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), where 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, but chlorination reduced endotoxin concentrations and ozonation maximally by only 10%.

Food

In the report of the Health Council (1998), it was stated that actual endotoxin levels in human diet were not known. In a pig study described in Health Council (1998), high dietary doses of endotoxins did not cause clinical symptoms. 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 Health Council (1998), it was concluded that relevant airborne exposure was mainly limited to occupational environments and may occasionally occur in a outside situation in the vicinity of agricultural and related industry. Since 1998, no further data has become available.

4.2 Human exposure

General population

The general population is exposed to endotoxins to a small extent, as endotoxins are a component of house dust. The population may further be exposed to endotoxins when living in the vicinity of industries that emit organic dust in 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 (US), once every 6 weeks for 1 year. The geometric mean of endotoxin levels associated to PM_{10} (particulate matter < 10 µm in aerodynamic diameter) was 0.44 EU/m^3 (GSD 3.1, range 0.03-5.5 EU/m^3). Endotoxin concentrations differed significantly across regions as shown by the fact that geometric mean concentrations by sampling site ranged from 0.19 to 1.85 EU/m^3.[19] More data on endotoxin outdoor levels are limited. A small measurement series has been reported by Schulze *et al*. (2006).[20]

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).[21] Endotoxin in indoor air is suspected of playing a role in ‘sick building syndrome’.[22] Concentrations were highest in the spring and lowest in the winter and were not well cor-

related with endotoxin concentrations in settled dust. Similar air levels have been observed in Dutch homes[23] Woskie *et al.* (1996) reported an exposure of 1.9±6.4 EU/m^3 (0.19 ng/m^3 geometric mean, SD 0.64 ng/m^3,) in office-workers (n=34),[24] Wan *et al.* (1999) reported a mean endotoxin concentration of 0.065 ng/m^3 (0.65 EU/m^3) in houses.[25]

Hasday *et al.* (1999) reported that high levels of bacterial endotoxins are present in cigarette smoke. Smoke from one cigarette contained 120 ng (1200 EU) of bioactive LPS.[2] The committee estimated that smoking of, for instance, one pack of 20 cigarettes a day (20 x 120 = 2400 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 8h).

Working population

The exposure data in various occupational environments which were available for the 1998 evaluation were tabulated in the report of the Health Council of the Netherlands.[1] The exposure data which has become available since, are summarised in Table 2.

Table 2 Endotoxin concentrations measured in various occupational environments.

source / industry	sampling[a]	n	dust (mg/m^3)	mean endotoxin contamination (in EU/m^3 (range))[b]	outcome[c]	ref.
vegetable sources						
cotton						
mill	A	5	n.d.	110 (19-2,230)	MD	10
mill	P	4	n.d.	1,200 (140-9,600)	MD	10
spinning	P	31	1.1	4,540 (2,950-6,980)	GM	26
weaving	P	36	0.59	50 (30-80)	GM	26
textile factory	P	61	1.1	2,566* (5-36,397)	GM	27
hemp/jute						
jute batching	P	3	9.4	23,190 (2,200-44,200)	AM	28
jute spinning	P	2	2.2	9560 (4,400-14,900)	AM	28
jute weaving	P	2	1.8	410 (71-750)	AM	28
hemp	P	n.i.	29.5 (r)	19,569* (r)	AM	5
herbs						
11 herbs[d]; 2 sites	A	10	18	112,000 (2,00-7,568,000)	MD	29
grain						
storage houses	A	5	n.d.	170,000 (17,000-380,000)	MD	10
(grain/onions)	P	4	n.d.	56,000 (40,000-80,000)	MD	10
silos/flour mill	P	31	4.4	1,150 (550-2,400)	GM	26
silos containing	A	15	3.3	983* (58-77,006)	GM	30
corn	A	14	1.0(r)	526(r)* (55-3,733)	GM	30
farms cultivating corn	A	14	3.4	3,175* (499-54,653)	GM	30
	A	16	2.4(r)	2,534(r)* (284-29,266)	GM	30

grain seed and legumes						
overall	P	188	1.5	580 (2.3-149,060)	GM	[17]
mushroom						
cultivation/picking	P	30	0.69	70 (50-110)	GM	[26]
potato						
processing (sorting,	P	7	n.d.	195* (26-1,123)	MD	[31]
cleaning, trimming)	A	8	n.d.	222* (7-5,363)	MD	[31]
cucumber and tomato	P	70	1.6	320 (5-4,000)	MD	[32]
nurseries	A					
wood						
logging site	P	7	0.56	15 (9.9-23)	GM	[33]
sawmill	P	93	1.6	43 (1.9-780)	GM	[33]
joinery	P	66	3.7	24 (1.0-280)	GM	[33]
sawmill	P	37	1.5	190 (130-230)	GM	[26]
green mill	P	55	1.5	66 (1.9-780)	GM	[34]
	P	20	0.19 (r)	14(r) (1-53)	GM	[34]
dry mill	P	28	1.7	16 (5.1-56)	GM	[34]
	P	10	0.46 (r)	1.4(r) (1-3.3)	GM	[34]
pine sawmill	A	1	15	2,400	S	[35]
fir sawmill	A	1	69	40,000	S	[35]
fibreboard factory	A	100	0.4-36	16-1,974*	R	[29]
chipboard factory	A	140	1.1-29	< 0.13-217*	R	[29]
3 pulp/paper mills	A	22	n.d.	33 (1-510)	MD	[10]
	P	11	n.d.	60 (10-360)	MD	[10]
2 pulp/paper mills	A	10	0.1-3.9	210 (42-25,000)	MD	[36]
animal sources						
animal production overall	P	108	0.7	110 (2.0-8,120)	GM	[17]
cow						
85 barns (mostly	P	194	1.8	647* (25-34,800)	GM	[37]
dairy barns)	A	216	0.07 (r)	16.8 (r)* (0.16-1,380)	GM	[37]
poultry						
catching/shackling	P	33	10.6	84,310 (53,130-133,860)	GM	[26]
slaughterhouse						
2 sites	A	10	n.d.	1,900 (0.2-9,400)	MD	[10]
(reindeer, poultry)	P	6	n.d.	870 (14-5,200)	MD	[10]
swine						
11 buildings	P	27	5.8	6,600 (4,070-10,700)	GM	[26]
8 buildings	A	8	3.3	390* (215-596)	MD	[38]
open-style	A	60	0.24	140* (14-818)	AM	[39]
buildings	A	95	0.14 (r)	47(r)* (0.02-1,643)	AM	[39]
wool						
combing /weaving	P	28	3.9	830 (360-1,900)	GM	[26]
other / mixed sources						
animal feed						
3 plants	A	13	n.d.	65 (3-200)	MD	[10]
	P	17	n.d.	190 (2-500)	GM	[26]
	P	6	6.0	300 (110-800)	GM	[26]

fibreglass wool						
	A	50	n.d.	10-3,900 (GSD 26-55)	GM	40
ranges (means of 4 areas)	P	390	n.d.	58-360 (GSD 26-34)	GM	40
metal working fluid						
	A	4	n.d.	67 (16-270)	MD	10
	P	72	0.18	7.1* (GSD=4.7)	GM	24
	A	9-12	n.d.	0.5-3 (<0.1-100)	MD	41
printing						
printing plant	A	5	n.d.	0.5 (0.3-1)	MD	10
sewage						
STP's	n.i.	n.i.	n.i.	1,000-7,800	R	42
9 WTP's	n.i.	n.i.	n.i.	20-640	R	42
8 STP's	n.i.	n.i.	n.i.	40-321,700	R	42
waste						
garbage handling	A	8	n.d.	1,200 (9-14,000)	MD	10
	P	1	n.d.	2,600	S	10
recycling	P	165	0 – 62	80 (2-1,980)	MD	43
refuse-derived fuel	P	78	0.50	29* (5-346)	GM	44
waste collectors	P	47	0.58	39* (4-7,182)	GM	45
glass bottle recycling (at point of sale)	P	182	0.18	3.6* (<0.1-180)	GM	46
waste water						
67 dutch sewage treatment plants	P	460		27 (0.6-2,093)	GM	47

[a] Sampling method: P= personal sample; A= area sample.
[b] 1 endotoxin unit (EU) is approximately 0.1 ng/m^3 endotoxin.
[c] Mean, median, or range: AM= arithmetic mean; GM= geometric mean; MD= median; R= range of means per site; S= single value.
[d] Herbs: nettle, caraway, birth, celandine, marjoram, mint, peppermint, sage, St-Johns wort, calamus, yarrow.

n.d. = not determined; n.i. no information $^{(r)}$ = measured in the respirable fraction
STP = sewage treatment plant; WTP = waste water treatment plant
* Asterisks are marking the values expressed in the unit as reported in literature, indicating that the other value is calculated, using a default factor 10 (ng to EU) or 0.1 (EU to ng).
In gray: Dutch occupational environments.

Exposure data found by Dutkiewicz *et al.* (2001)[29,35,48] are rather high compared to other studies in similar industry branches; this might be due to the fact that Dutkiewicz *et al.* boiled the samples for 15 min at 100°C to dissolve the endotoxins before testing.

The American Society for Testing and Materials (ASTM) has recently approved an endotoxin standard, ie. '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 sam-

ples, it does not address the issue of airborne endotoxin aerolized 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’.[49]

Chapter 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 are assumed not to enter the bloodstream.[16,50,51] Therefore, systemic effects due to inhaled endotoxins are most likely induced by cytokines that are released from the lung into the blood.

For more information on absorption, distribution and elimination of endotoxins the committee refers to the previous report of the Health Council.[1]

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; in blood no endotoxin was measured after inhalatory exposure.[1,51] However, the committee is of the opinion that the usefulness of these methods for monitoring is limited

because the inflammatory reponses are only expected after exposure to *high* endotoxin concentrations.

Chapter 6

Mechanism of action

When endotoxins are inhaled, the lipid A part of the endotoxins is opsonised by a lipopolysaccharide binding protein (LBP) present in the fluid on the airway surface. This LBP may act as an 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 (sCD14) 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 been revealed yet. In macrophages and epithelium cells TLR-4, and in dendritic cells TLR-3 play a role in the activation of these cells.[16]

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 neutrophils (PMNs).

When endotoxins are internalised by alveolar macrophages, nuclear factor κB (NF-κB) initiates the production of inflammatory cytokines like IL-1β, TNFα, IL-6 and IL-8. Production of metabolites of arachidonic acid by macrophages is

also up regulated, as well as the production of inducible nitric oxide synthase (iNOS), 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.[51]

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, although Hjelle *et al*. (2000) reported that systemic uptake of nanoparticles and nanobacteria is possible.[16,50,51] 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 LBP within 48h.[52] This systemic inflammatory response is related to the dose of inhaled endotoxins and to endotoxin-induced fever.

Chapter 7

Effects

Numerous studies have been published concerning the health effects of occupational exposure to endotoxins. Most of these studies regard the adverse respiratory health consequences. However, it has also been suggested that exposure to endotoxins might protect against the development of atopy and 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 studying the effects of endotoxins similarly to human and allowing a quantitative hazard assessment based on dose-response relationship 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. 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 characterized 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 gasses.[53] It can be measured with spirometry. Parameters that are considered as primary indicators of lung function are FEV_1 and FVC. FEV_1 (forced expiratory volume in 1 second) is the maximal amount of air that can be expired with force in the first second after full inspiration (measured in litres), FVC (forced vital capacity) 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. 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 work shift 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 exposure to endotoxins may lead to some kind of tolerance for acute clinical effects.[54] However, after ending the exposure, this tole-

rance disappears in a few days (e.g. weekend). Tolerance development might obscure the actual 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 the paragraphs '*Health Council report (1998)*' and '*New data*'.

Furthermore, in studies where subjects are exposed to single doses of nebulized 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 nebulized agents for short periods (i.e. minutes). While the concentration of the agent in the aqueous medium is typically given, other details (e.g., nebulization rate and duration) are not provided in reports of these studies. Moreover, the agents used in these nebulization 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 nebulization 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 *Health Council report (1998)*

Studies on acute effects described in the previous advice from the Health Council (1998), which yielded a NOEL (no-observed-effect level), are briefly summarized below.

In a study of Rylander *et al.* (1985)[55], 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 70-5,620 ng/m^3 (700-56,200 EU/m^3) (personal sampling). Before and after carding, FEV_1 was determined. 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*.

* The authors used the following equation:
$(\%\Delta FEV_1) = -3.43\ ^e\log(\text{endotoxin concentration } (\mu g/m^3)) - 11.68$ ($r = -0.56$).

In a study of Castellan (1987)[7], 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 a FEV_1 above 80 percent of the predicted value. In addition, the volunteers were pre-tested by exposure to 100 ng/m^3 LPS, in order to select sensitive subjects; only volunteers that responded with a FEV_1 decrease of at least 5% (and not more than 30%) (n=33, of which 16 smokers) 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, non 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-response relation between ΔFEV_1 and endotoxin concentration of: % ΔFEV_1 = 3.84 - 4.02 (^{10}log endotoxin (ng/m^3)); 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%. Using linear regression modelling, the authors calculated the zero percentage change in FEV_1 during exposure to endotoxins to be 9 ng/m^3 (90 EU/m^3). In contrast, dust exposure (instead of endotoxin exposure) was not correlated with ΔFEV_1.

The difference between the calculated zero-change level in Rylander's study (33 ng/m^3) and the one in Castellan's study (9 ng/m^3) might be due to different exposure times (4 v. 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 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 for effects of endotoxins, this might obscure the actual dose-response 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 importance in the development of acute pulmonary effects. This was suggested by the results of a study performed by Buck *et al*. in which changes in lung function were demonstrated when subjects were exposed to an endotoxin-free eluate of cotton dust.[56]

Haglind and Rylander (1984)[57] demonstrated that a dose-related decrease in FEV_1 was more pronounced in smoking cotton mill workers resulting in a thres-

hold of 80 ng/m^3 versus 170 ng/m^3 in non-smoking (n=13) workers. 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 field studies.[58-60]

Donham *et al.* (1989)[58] found a relationship between endotoxin exposure and an across-shift decrement of FEV_1 and the maximum expiratory flow rate at 25% of vital capacity (MEF_{25}) in non-smoking swine confinement workers (n=41). The mean 2 to 8 hours endotoxin exposure, characterised by area sampling of total dust, was 180 ng/m^3 (1800 EU/m^3). A no-effect level of 180 ng/m^3 (1800 EU/m^3) was estimated.

Milton *et al.* (1995 and 1996)[59,60] showed a dose-response relationship with cross-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) 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. In the medium exposure group (geometric mean = 8.4 ng/m^3 (84 EU/m^3), range 4-15 ng/m^3 (40-150 EU/m^3)) acute effects on PEF were measured. Therefore, the authors defined 8.4 ng/m^3 as the LOEL (lowest-observed-effect-level) and 1.7 ng/m^3 as the no observed adverse effect level (NOAEL) in this study.

7.2.2 New data

Biological responses

In a number of studies, local endotoxin-induced inflammatory responses have been investigated by studying bronchoalveolar lavage (BAL), nasal lavage (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. Therefore, these effects (at relatively high exposure levels) are less suitable for the deriving an occupational exposure limit for endotoxins.

Single-dose studies in healthy volunteers are summarised in Table 3.

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

exposure		effects	measured after	n	NOEL	ref.
mg/m³	EU/m³					
100	1,000,000	PMN ↑	3 h	8	No	61
40	400,000	sputum: PMN ↑, ECP ↑, MP0 ↑, blood: PMN ↑, MP0 ↑, FEV_1 2% ↓	24 h	21	No	62
0.5	5,000	PMN ↓	6 h	9	No	52
5	50,000	blood: PMN ↑, CRP ↑ sputum: PMN ↑, MPO ↑, monocytes ↑				
50	500,000	sputum: lymphocytes ↑, TNFα ↑, ECP ↑				
0.1	1,000	-	4-24 h	16[a]	No	63
0.3	3,000	-				
1.0	10,000	NAL: eosinophils ↑ (only in atopics)				
5.4	54,000	BAL: total cells, TNF-α, IL-1β, IL-6 and IL-8 ↑;	4 h	14	No	64
36	360,000	FEV_1 ↓				

CRP = C-reactive protein (acute phase protein); ECP = eosinophilic cationic protein; IL = interleukin; MPO = myeloperoxidase; NAL = nasal lavage; PMN = neutrophils (polymorphonuclear leukocytes); TNFα= tumor necrosis factor alpha.

a of which 10 atopic subjects

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

Table 4 One-week epidemiological studies of biological effects.

ref	study design	control	work history	endotoxin exposure; mean (range)	parameters measured	effects measured
Fishwick *et al.* (2002)[65]	four days follow-up of cotton workers, n=25	scientists n=9	>8 years	1-400 EU/m³ (0.1- 40 ng/m³)	CD14 on monocytes in blood	CD14 ↑ at the end of first day of the week, but back to normal at the end of the week.
Wouters *et al.* (2002)[45]	one-week follow-up of domestic waste collectors n=47	office workers n=15	5 years	GM = 39 (4-7,182) EU/m³; (3.9 ng/m³, range 0.4-718 ng/m³)	cells, IL-6, IL-8, IL-1β, TNFα in NAL; IgE in serum	IL-8 ↑ (1.8x) and cells ↑ (3.3) in NAL at the end of the week
Heldal *et al.* (2003)[66]	four days follow-up of waste handlers n=31	No	1.5 years	MD = 13 (4-183) EU/m³ (1.3 ng/m³, range 0.4-18.3 ng/m³)	MPO, ECP, IL-8 and cell diff. in NAL	ECP ↑ (1.8x) and %PMN ↑ (1.6x) in NAL at the end of the week.

CD14 = CD14 receptor; ECP = eosinophilic cationic protein; IL = interleukin; MPO = myeloperoxidase; NAL = nasal lavage fluid; %PMN = % neutrophils (polymorphonuclear leukocytes) of total cells; TNFα= tumor necrosis factor alpha.

Acute effects on lung function

Seventy-two 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 min after inhalation of each dose. The inhalation challenge was continued with the next dose of LPS when 30 min (or more) after exposure, the FEV_1 of the subject was less than 20% decreased. 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 a $FEV_1 > 90\%$ after inhaling 41.5 µg LPS/person. The three most sensitive responders reached a FEV_1 decrease of 20% at the second dose (1.5 µg/person cumulative).[67]

In poultry workers (n=257), statistically significant dose-response relations were observed between lung function decrement (FEV_1 and FEF_{25-75}) over a work-shift (2 to 4 hours), and each quartile of exposure to endotoxins and dust levels (both total and respirable fraction). The exposure-response correlations were weak; the correlation coefficients (r) were 0.16 (r^2=0.026) and 0.19 (r^2=0.036) for respirable and total endotoxin respectively. These low coefficients indicate that only 3-4% of the variation in lung function is explained by exposure to endotoxins. This is explained by the relative small changes over the work shift relative to the measurement error between 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% 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 and 0.35 EU/m^3 respirable endotoxins is remarkable, as 3.7% of total endotoxin was respirable. This might however be due to division of individual exposure in four groups, each containing a quartile of the exposure level, and for each quartile the odds ratio for 3% ΔFEV_1 and its 95% confidence interval was calculated; if the odds ratio was statistically significantly different from 1, the lower limit of the group was proposed as no effect level. The relatively arbitrary NOEL's in combination with weak correlations limit the usefulness of this study.[68]

Bonlokke *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 65,53 EU/m^3; p = 0.004). Although exposure to endotoxins varied between the seasons, no differences in lung function were found between the seasons.[69] Earlier results also found seasonal differences in endotoxin levels in pig houses[70] and in intensive livestock production.[20] On the other hand, Seedorf *et al.* did not observe a significant seasonal variation in airborne endotoxin concentrations for cattle, pigs and poultry.[71]

7.3 Long-term exposure

7.3.1 Health Council data 1998

Studies on long-term effects from the Health Council's advices (1998) that yielded dose-response relationships are briefly repeated below.

Kennedy *et al.* (1987)[72] performed a cross-sectional study investigating the relationship between endotoxin and dust exposure and lung disease in 443 cotton workers and 439 control subjects from a silk mill. Pre- and post shift FVC and FEV_1 were determined for each worker. In 130 area samples (<15 µgm), the endotoxin concentrations varied from 1-920 ng/m^3 (10-9200 EU/m^3) and dust concentrations varied from 0.15-2.5 mg/m^3. The cotton worker population was stratified by current endotoxin exposure into 4 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) endotoxin. 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. A dose-response trend was seen with the current endotoxin level and FEV_1, change in FEV_1 over the shift and prevalence of chronic bronchitis and byssinosis, except for the highest exposure level group in which a reversal of the trend was seen most likely to be caused by a 'healthy workers effect'. The dose-response 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 (-2.4 ml per 100 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 (-7.8 ml per 100 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 or 20 EU/m^3) and group 2 (median 100 ng/m^3 or 1,000 EU/m^3) were higher than FEV_1 in the control group of silk workers (FEV_1 set on 100%); FEV_1 in group 3 (median 230 ng/m^3 or 2,300 EU/m^3) was 96.7% and in group 4 (median 520 ng/m^3 or 5,200 EU/m^3)

98.5% for non-smokers (both not statistically significantly different from control group). The authors attempted to assess the presence of a threshold level of endotoxin exposure by comparing the (control) silk workers with 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 cross-shift change in FEV_1, the authors suggested that even exposure to lowest level of endotoxins at 1 to 20 ng/m^3 (10-200 EU/m^3) constitutes an 'adverse respiratory health effect'.

Smid *et al.* (1992)[8] performed a similar cross-sectional study in 315 workers working in 14 animal feed mills in the Netherlands. The average 8-h personal inhalable dust (<30 µm) exposure was 9 mg/m^3 grain dust (range 0.2-150 mg/m^3) and 25 ng/m^3 (250 EU/m^3) endotoxins (range 0.2-470 ng/m^3) 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 epidemiologic analyses because the external control subjects differed with respect to variables other than exposure. Further analyses were then performed with only exposed workers and internal control subjects who existed 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, MEF_{75}, MEF_{50}) showed significantly reduced values with increasing current exposure to both dust and endotoxins. Dose-response relations between different endotoxin exposure categories appeared to be greater than for 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 to 680 EU/m^3) for endotoxin and from 1.7 to 29.7 mg/m^3 for dust. The dose-response relation for current endotoxin exposure and FEV_1 was calculated to be -4.91 ml per ng/m^3 (-49.1 ml per 100 EU/m^3) for workers with a mean work history of 13 years. No clear differences in symptom prevalences existed between different exposure groups. In the study, the estimated cumulative exposure of both dust and endotoxins was significantly related to lung function impairment.

In 1996, Smid calculated a safe threshold level* between 3 and 7.5 ng/m^3 (30-75 EU/m^3) based on the animal feed studies.[73] Both acute and chronic lung func-

* The authors calculated that exposure to these levels of endotoxin for 40 years will lead to an estimated effect on FEV_1 of approximately 200 ml. This effect is considered a no effect level.

tion 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 LOEL (lowest-observed-effect-level). 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% FEV_1). For MEF_{75}, the effect would be 1200 ml/s (approximately 16%). The author suggested that the NOEL 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 on the LOEL en proposed a 'safe' level between 3 and 7.5 ng/m^3.

7.3.2 New data

Lung function

Studies in which no dose-response relationships were examined, are summarized in Table 5.

For studies in which dose-relationships were examined, a more detailed description is followed below.

Post *et al.* (1998)[9] followed up 140 workers in the grain processing and animal feed industry for 5 years. This study was a follow-up of the study population of the previously described cross-sectional study by Smid *et al*. (1992)[8]. During the first survey 520 personal exposure samples were gathered[8], 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 to 990 EU/m^3) for endotoxins. The annual decline in FEV_1 and in maximal mid-expiration flow (MMEF, the average expiratory flow over the middle half of the FVC) was 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. A FEV_1 decrease was calculated of 0.326 ml (SE=0.139) per ng/m^3 endotoxin (or 10 EU/m^3) per year of exposure (r^2=0.12). Fourteen percent of workers had a rapid (>90 ml/y) 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 (odds ratio = 3.3; 95% C.I. =1.02 to 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 years of working years and fewer people with rapid decline in FEV_1.

Table 5 Epidemiological studies without dose-response investigations.

ref	study design	control	work history[a]	endotoxin exposure; mean (range)	parameters measured	critical effect and (no) effect level
Mahar (2002) [74]	9–years follow-up of RFD workers with rotating jobs n=87	no	± 9.5 years	GM: 28 EU/m^3 ±3.8 EU/m^3 (2.8 ng/m^3 ± 0.38)	FVC and FEV_1 change over 9 years	no effects observed
Elbers *et al.* (1996)[75]	cross sectional, in veterinarians, non-smoking without asthma n=66	general population	n.d.[b]	18-28 ng/m^3 (180-280 EU/m^3) 2.8-3.6 h/day TWA_8=9 ng/m^3 (90 EU/m^3)	% of subjects with diurnal PEF variation >20%	no effects observed
Radon *et al.* (2001)[76]	cross sectional, in pig farmers, n=40; and in poultry farmers, n =36	general population	20 years	pig: 58 ng/m^3 (1-1,101) (580 EU/m^3) poultry:258 ng/m^3 (19-1,635) (2,580 EU/m^3)	FEV1, MMEF and FVC	%predicted FEV_1 > 100% in both groups; %predicted MMEF: 101% in pig farmers and 89% in poultry
Rylander *et al.* (1999)[77]	bark cleaning and paper recycling in paper factory n=77	office workers n=40	12 years	low: 2-20 ng/m^3 (20-200 EU/m^3) high: 21-98 ng/m^3 (210-980 EU/m^3)	serum MPO and ECP, airway responsiveness, symptoms and baseline FEV_1	serum MPO and ECP in both groups, flu-like symptoms in high group, nose irr. in both groups
Wang *et al.* (2003)[78]	newly hired young female non-smoking cotton workers (18 yr) n=101	no	0.0 years	220, 1,360 and 1,070 ng/m^3 (2,200, 13,600 and 10,700 EU/m^3) for 3, 12, 18 months	change in FEV_1, FVC and ΔFEV_1 after 3, 12, 18 months as compared to first day of work	in all 3 groups: FEV_1 and FVC were 2% after 3 months; FVC 5% after 12 and 18 months; FEV_1 2,8% after 12 m. and 1.3% after 18 m.
Sigsgaard *et al.* (2004)[79]	97 male paper workers from 1989 to 2000	n=55		69 (6-370) EU/m^3	ΔFEV_1 and ΔFVC_1 yearly for 11 years.	no increase in loss of lung function among workers exposed up to 200 EU/m^3.
Dosman *et al.* (2006)[80]	experimental study with 20 non-smoking male subjects exposed to endotoxins from swine barn room	n=20	naive unexposed	each subject was exposed to successively baseline, low exposure (452 +/- 66 EU/m^3), dust and high exposure (3,984 +/- 498 EU/m^3)	FVC, FEV_1, FEV_1/FVC ratio, IL6 and IL-8 in nasal lavage	two groups of responders appeared (low and high responders) after both exposures

[a] work history at the start date of the study.
[b] not determined

Christiani *et al.* (1999)[81] performed an 11-year follow-up study in cotton (n=349) and silk workers (n=319, both active and retired). Mean exposure per work area (area sampling) of the cotton workers ranged from 0.2 to 1.6 mg/m^3 for dust and 42-12038 EU/m^3 (4.2-1204 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. 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 versus 0-0.2% in silk workers); chronic bronchitis (22% v. 8%); chronic cough (20% v. 7%) and dyspnoea (2+) (15% in cotton workers versus 4% in silk workers).[82] 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 insignificantly) higher with 40 ml/y compared to 30 ml/y in silk workers after 5 years of follow up, the FEV_1 loss in the last 6 years of study was only 18 ml/y in cotton workers, compared to 27 ml in silk workers. Total FEV_1 decrease in 11 years was 0.31 L in both cotton and silk workers and FEV_1 values measured were 100% (of predicted) in cotton and silk workers both at baseline in 1981 (99.6% cotton; 100.6% silk), as well as 11 years later in 1992 (100.0% cotton; 100.3% silk). After adjustment for confounders the 11-year loss in FEV_1 was associated with cumulative dust but not with endotoxin exposure.

In 2001, Christiani *et al.* (2001)[82] published the results of another 4 years 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 (4800 ng/ m^3 · yr). 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%). However, the annual decrease in FEV_1 in this study is rather similar to that found in the previous study, while 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 ($p<0.001$) was found between change in FEV_1 and the across-shift change in FEV_1 (ΔFEV_1). A statistically significant relation of accelerated chronic loss in FEV_1 to byssinosis or chest tightness at work was observed.

Kirychuk *et al.* (1998)[83] 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). Mean annual rate change between baseline and follow-up for FEV_1 was -54 ml ± 62 ml/year (-1.2±1.4%) and for FVC -49±72 ml/year (-0.9±1.3%). 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 committee considers the use of this study limited. Furthermore, the committee noted that the authors incorrectly adjusted the calculation for the initial FEV_1 level.

Laitinen *et al.* (2001)[10] 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. Division of exposed workers among 2 groups (> or < 25 and 150 ng/m^3 (250 and 1,500 EU/m^3)) seemed arbitrary as no statements were made about the origin of these limits.

One hundred fourteen male employees of a cotton mill in western Germany participated in a cross-sectional study.[84] 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 the endotoxin exposure and prevalence 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 was significant for the highest exposure group (> 450 EU/m^3).

In 2007, Oldenburg *et al*. performed a cross-sectional study in which one hundred fifty (114 male and 36 female) employees of the same German cotton spinning mill underwent lung function testing.[85] 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.

Wang *et al*. (2005) evaluated the chronic effects of longterm exposure to cotton dust on respiratory health, and lung function (annual decline FEV_1 and FVC).[86] The results from five surveys over a 20-year period were reported. This follow-up study consisted of 447 cotton and 472 silk textile workers, recruited in Shanghai, China. Cotton workers had a mean endotoxin exposure of 49122,60 (+/- 45284) EU/m^3. The cotton workers had a greater annual decline in FEV_1 (32.4 +/- 1.0 ml/yr) in comparison with the silk workers (27.3 +/- 0.9 ml/yr).

In a large study of Simpson *et al.* (1998)[87], prevalence of symptoms and the exposure level to endotoxins 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. Organic dust toxic syndrome (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 the exposure level to endotoxins was shown. 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 indicate that an exponential curve would give a better fit and that symptoms are noticeably increased when endotoxin levels are 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 found that, compared to their counterparts working in the same occupations, workers with symptoms had consistently higher exposures to dust and endotoxins , thought 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.[87]

Vogelzang *et al.* (1998)[88] performed a 3-yr 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/y) and a decrease in FVC of 55 ml/y were found.[88] In an additional paper[89], bronchial responsiveness was measured. Provocative histamine concentrations (PC's) were measured for 10% and 20% fall in FEV_1. PC_{10} and PC_{20} decreased in both symptomatic and asymptomatic groups within 3 years of additional exposure to ammonia and dust.

Smit *et al.* (2008)[90] explored exposure-response relationships in Dutch farmers and agricultural industry workers. They investigated exposure-response relationships between current endotoxin exposure and allergic and respiratory symptoms in adults, taking into account farming exposures during childhood. A cross-sectional study was conducted among 877 Dutch farmers and agricultural industry workers in 2006. 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 odds ratios (OR) for an interquartile range increase in endotoxin levels were elevated for respiratory symptoms such as wheezing (OR 1.41 (95% 1.16-1.72)), wheezing with shortness of breath (OR 1.50 (95% 1.18-1.90)) and daily cough (OR 1.29 (95% 1.03-1.62)). In contrast, endotoxin exposure was strongly associated with a decreased prevalence of hay fever (OR 0.62 (95% 0.49-0.78)). Workers who had grown up on a farm had a lower prevalence of hay fever, but no evidence was founds 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.

Carcinogenicity

Cancer risks have been investigated in relation to occupational exposure to endotoxins. In the 1970's findings in several occupational cohort studies suggested reduced risks in mortality studies for lung cancer among textile workers.[91-94] More recent findings suggest an inverse dose-response for lung cancer. Astrakianakis *et al.* (2007)[95] observed in a cohort of female textile workers in Shanghai a dose-dependent reduction in lung cancer risk. The authors stated that the study also has several limitations. A potential source of bias is the healthy worker effect. However, the authors concluded that a healthy workers 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.[96]

Reproductive effects

There were no data available.

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 (LBP). LBP 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 endotoxin to bind to and activate macrophages and neutrophils. Under normal conditions little LBP is present in the lung. After inhalation of antigen by atopic subjects, extravasations of LBP and sCD14 to this compartment occurs, 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,[97] while the other way around the simultaneous presence of antigens lead to an exaggerated response to endotoxins in asthmatic subjects.[67]

In a study performed by Michel *et al.* (1989)[98], bronchial obstructive responses (associated with an increase in non-specific bronchial reactivity) were demonstrated in asthmatic and rhinitis patients at a inhalatory dose of 20 µg endotoxins (LPS) (200,000 EU) per person, while at this level no bronchial-constriction 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 a decreased forced expiration values (e.g. FEV_1). Such effect was seen at a lower dose in asthmatic patients in the previous endotoxin report of the Health Council of the Netherlands.[1]

Low levels of endotoxin exposure significantly augment the inflammatory response to allergen exposure in sensitised subjects with asthma,[51,99] in subjects with allergic rhinitis,[100] and in skin test wheal-and-floare response to allergen.[97] In metropolitan households, higher house dust endotoxin levels have been asso-

ciated with increased asthma symptoms.[101,102] Higher house dust endotoxin levels are also associated with more wheezing symptoms in the first year of life.[103] Possible explanations for this association of endotoxin exposure with increased asthma symptoms at any age, include an adjuvant-like effect of endotoxins exposure on airways inflammation, increased susceptibility to viral respiratory tract infections caused by endotoxin exposure, and respiratory manifestations after endotoxin inhalation in normal and asthmatic subjects.[67,97,100,102,104,105] 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 (tumour necrosis factor-α [TNF α], interleukin [IL-1, IL-6] and metabolites of arachidonic acid).[97] The presence of LPS-binding protein and the soluble fraction of CD14 receptor in the airways increases the macrophage activation by endotoxins.[106] After inhalation of endotoxin-containing dust (6-hr) high concentrations of IL-1, IL-1 RA, Il-6, IL-8 and TNF-α, and their mRNA were measured in bronchial lavage (BAL).[107 as cited by 97] Also after exposure to endotoxins containing swine dust for three hours, IL-8 was induced in BAL fluid and nasal lavage fluid of non-smoking subjects.[108] Increased neutrophil recruitment was also observed in BAL.[109, as cited by 106] A significant increase in neutrophils in the induced sputum occurred in asthmatic subjects after 5-60 µg (50,000-600,000 EU) endotoxin/person, which was also seen to a lesser extent in normal subjects exposed to endotoxins.[62,101,110] The sputum concentration in myeloperoxidase (MPO, from neutrophils), eosinophilic cationic protein and TNF-α concentration rose significantly 6 hours after endotoxin inhalation. Some published data suggest that environmental endotoxins could be a synergistic factor on the amplitude of an IgE mediated response.[111] 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 IgE. While detoxified allergen extract results in bronchial eosinophil recruitment, endotoxin contamination (1 ng/ml) causes recruitment of neutrophils.[111]Inhalation of allergen in sensitised subjects leads to airway plasma exudation including extravasations of sCD14 and lipopolysaccharide binding protein.[111] 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.[100,101,110,112] Decrease in FEV_1 and systemic response were inversely associated with the atopic status, suggesting a link between atopy and endotoxin responsiveness.[111,113,114]

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 asthma.[99] 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 1 (Th_1) development., i.e. interleukin 12 (IL-12) and Interferon-γ (IFNγ).[99,115] The induction of a T-helper type 1 (Th_1) response in early life, separate from serious infection, down-regulates Th_2-type immune development, which is relevant in preventing atopy and possibly asthma.[115,116] Although the studies on the protective effect of endotoxins in early development of allergy in children gave more insight in 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.[117,118] CD14 is a multifunctional receptor constitutively expressed primarily on the surface of monocytes, macrophages, and neutrophils (mCD14) and serves as a receptor for the LPS-LBP complex.[119,120] CD14 participates in regulation of IL-8 and IL-6 release by bronchial epithelial cells.[118] A soluble form of CD14, sCD14 is abundant in serum and is apparently derived both from secretion of sCD14 and from enzymatically cleaved glycosyl-phosphatidylinositol-anchored mCD14,[3] suggesting that polymorphism in the CD14 gene promoter region could influence the differentiation of T-cells and the levels of serum immunoglobulin E (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 (sCD14), and lower IgE levels and IL-4 levels.[3] Those children appear to benefit from the protective effect of higher levels of sCD14, which allows stronger stimulation of Th_1 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 atopy or allergic asthma. However, no support is found for the hypothesis that chronic inhalatory endotoxin exposure may encourage non-specifically 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 general population samples and from occupationally exposed work force based studies in farmers (Smit *et al*. 2008).[114] Despite the increased

response to endotoxin in subjects with atopy, 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).[90]

In 2009, Smit *et al.*[121] conducted a case-control analysis with unrelated subjects to investigate whether SNP's (single nucleotide polymorphisms) in CD14, TLR2, TLR4 and TLR9 genes are 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 TRL2 and CD14 SNP's were associated with asthma and atopic asthma respectively. In addition, CD14, TRL2 TRL4 and TRL9 SNP's modified he association between country living and asthma.

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 sCD14 and LBP levels in the lungs are severely increased.[106] LBP and sCD14 are normally present in human plasma in 5-10 µg/ml[122, as cited by 106] and ~6 µg/ml,[123, as cited by 106] respectively. Extravagation of LBP and sCD14 into the broncho alveolar compartment after antigen inhalation, due to increase in bronchial micro vascular permeability, might enhance the capacity of inhaled endotoxins to activate an inflammatory response. On the other hand, atopic, but a-symptomatic, subjects may have a genetically lower response to LPS, e.g. by lower levels of expression of sCD14,[3] lower expression of TNFα,[124] or lower expression of LBP. Because of the lower response of macrophages to endotoxins, lower levels of IL-12 did stimulate the T_H2-cell expression when the immune system was developing[99], which stimulates B-cell production of IgE by IL-4. So, hypo responsiveness to endotoxins might have caused the atopy.

Cardiovascular effects

Sjogren *et al.* (2003) compared the occurrence of ischaemic heart disease (IHD) among male and female livestock and agricultural workers in Sweden.[125] The IHD mortality among the livestock and agricultural workers was compared with that of gainfully employed men and women. The standardized mortality ratio 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 SMR's (standardized

mortality ratio). Adjustments for smoking for smoking habits increased the SMR by about 9% in male workers and about 5% in female workers.

Neurological effects

No data were available.

Endocrine effects

No data were available.

7.4 Summary and evaluation

Acute and short-term exposure

Acute health effects in humans after inhalation of endotoxin 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 epidemiological studies in exposed workers.

In a number of studies performed in volunteers and in workers, biological responses were measured, but as biological responses do not necessarily result in clinical responses, most of these studies are not suited to establish a NOAEL (no-observed-adverse-effect level). In a study, the first responders in a group of healthy volunteers showed a ΔFEV_1 decline of at least 20% after a single exposure to 1.5 µg (15,000 EU) endotoxin, where exposure to 0.5 µg (5,000 EU) did not lead to significant declines in ΔFEV_1 in any of them. A clear exposure-response relationship ($\%FEV_1 = 3.84 – 4.02$ (log endotoxin), 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.

Long-term exposure

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

Post *et al.* (1998)[9] found a dose-response relationship with an annual FEV_1 decline of 0.33±0.14 ml (SE) (0.0077%±0.0033) per ng/m^3 (or 10 EU/m^3) endotoxin exposure in a 5-year follow-up study in animal-feed workers. Post *et al.* used the same cohort as Smid *et al*. (1992). In the cross-sectional study of Smid *et al.* (1992) a value of 0.34 ml FEV_1 decline per year·ng/m^3 was calculated.

A third dose-response relationship was found in the cross-sectional study by Kennedy *et al*. (1987). They found an FEV_1 change in cotton workers of -0.052 ml per year·ng/m^3 (or yr·10 EU/m^3) (as converted from -0.778 ml FEV_1 per ng/m^3 endotoxin after a mean of 15 working years). 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-response 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 that 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-response relationship between endotoxin exposure and FEV_1 changes is revealed by Kennedy *et al*.

Besides the dose-response studies, several epidemiological studies have been performed with only one or two exposure groups, in which workers with average work histories of 10 to 20 years were exposed to average endotoxin concentrations varying from 2.8 to 520 ng/m^3 (28 to 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, but not in workers exposed to average endotoxin levels at or below 150 ng/m^3 (1,500 EU/m^3). 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.

In most studies, workers had respiratory complaints. However, respiratory symptoms are quite common as the incidence of respiratory symptoms in the normal population can 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 encourage non-specifically 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 committee notes 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 subjects with atopy/ atopic; (2) the protection against development of atopy after exposure to endotoxin. In addition, gene-environment interactions 3) have been observed for respiratory symptoms (wheezing). The committee believes 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, which makes an effect on reproductive, neurological or endocrine endpoints unlikely. Reduced lung cancer rates as beneficial effect of occupational exposure to LPS have been suggested in textile workers.

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

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

8.2 Working population

In 1994, an evaluation on occupational endotoxin exposure was conducted by an international organisation. The International Committee on Occupational Health (ICOH), through its Committee on Organic Dusts, reported that endotoxins may provoke different reactions when exposure occurs at different levels. As an example, the report states that organic dust toxic syndrome (ODTS) is elicited at level of 1,000 to 2,000 ng/m^3 (10,000 to 20,000 EU/m^3), while acute broncho-constriction occurs at levels of 100 to 200 ng/m^3 (1,000 to 2,000 EU/m^3), and mucous membrane irritation at levels of 20-50 ng/m^3 (200 to 500 EU/m^3). The report states that these levels may be lower for sensitive subjects.

In the Netherlands, an advice report was written in order to set a MAC-value for endotoxin, coming up with a health based recommended occupational exposure level of 5 ng/m^3 (50 EU/m^3).[1] However, no legally binding limit has yet been established.

Chapter 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 committee considers 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. Decrease in 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[126] (± 0.5%/year for 20 year

old women[126]; ± 0.8%/year as the mean in men and woman from 30 to 60 years[127])
- The average FEV_1 decline during 40 years is approximately 1 litre in the non-smoking population, this corresponds to approximately 25-30 mL per year[128]
- An average FEV_1 decrease up to 200-300 mL during 40 year is, in general, considered to be a normal aging event in non-smokers[129]
- FEV_1 exhibits variability over 24-hour periods (diurnal variation)[127]
- Annual FEV_1 decline due to endotoxin exposure decrease with increasing working years in an endotoxin-rich environment.[9,81] Annual FEV_1 decrease is higher in chronically symptomatic workers than in asymptomatic workers.[9,82]

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 reference level. Chronic ventilatory impairment is defined 'absent' in an individual as long as the FEV_1 level is over 80% of the reference FEV_1 level (as mentioned in Chattopadhyay *et al.* (2003)[28]). 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.[130-133]

In a 20-year follow-up study in a population of 668 men, an average loss of FEV_1 of 620 mL (compared to the predicted FEV_1) was associated with a higher risk 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 1240 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).

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 per 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.[133]

In a study of Sin *et al.* (2005)[129], it has been shown in a population (n=1861, 40-60 years), that a mean decline of FEV_1 to 88% of the predicted FEV_1, is statistically significantly associated with cardiovascular events.[129] This association was not found for a mean decline to 96% of the predicted FEV_1. Assuming a mean predicted FEV_1 of 3 litres, the committee estimates that no association with cardiovascular effects has to be expected when FEV_1 is additionally declined with 120 ml (4% of FEV_1). As Sin *et al.* delineated in the systematic review part of the paper, also other studies show that an additional FEV_1 loss of 200-300 mL is not related with cardiovascular or other health effects.

However, for the hazard assessment of occupational exposure to endotoxins, DECOS and NEG emphasize 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[28], 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

In Table 6, studies concerning the effects on lung function of acute and short-term exposure to endotoxins are summarized.

Table 6 Suggested no-effect levels after acute and short-term exposure.

study design and population	exposure source	n	suggested NOEL	effect / NOEL based on	ref.
observational study in glass wool manufacturers exposed for 4 h	recycled wash water	130	<8.4 ng/m^3 (<84 EU/m^3) (LOAEL)	across-shift self-recorded PEF change $\geq$ 5%. No sign. changes in spirometry	Milton *et al.* (1995-1996)[59,60]
experimental exposure (6 h) of healthy volunteers, pre-selected on being reactive to cotton dust (endotoxin)	cotton	33-61	9 ng/m^3 (90 EU/m^3)	calculated 0% across-shift change in FEV_1	Castellan *et al.* (1987)[7]
experimental exposure (4 h) of cotton mill workers, of which 8 had a history of byssinosis	cotton	15	33 ng/m^3 (330 EU/m^3)	calculated[a] 0% across-shift change in FEV_1	Rylander *et al.* (1985)[55]
epidemiological study in poultry workers exposed 2-4 h	poultry faeces and feed	257	61.4 ng/m^3 (614 EU/m^3)	decline in across-shift FEV_1 >3%	Donham *et al.* (2000)[68]
- non-smoking volunteers (experimental exposure, 4 h)	washed cotton	13	170 ng/m^3 (1,700 EU/m^3)	calculated 0% across-shift change in FEV_1	Haglind *et al.* (1984)[57]
- smoking cotton mill workers (experimental exposure, 4 h)		4	80 ng/m^3 (800 EU/m^3)		

[a] calculated 0% across-shift change in FEV_1: The zero percentage change in across-shift FEV_1 could be calculated using linear regression on exposure-response curves

Long-term exposure

Studies showing a dose-response relation between long-term exposure to endotoxins and adverse human health effects on lung function are summarised in Table 7.

Three studies reported quantitative dose-response relationships. Smid *et al.* (1992)[8] and Post *et al.* (1998)[9], studying the same cohort of animal feed workers (exposed to grain) observed a correlation between annual FEV_1 change with endotoxin exposure, i.e., -0.34 ml (0.0077%±0.0033) per ng/m^3 (or per 10 EU/m^3) endotoxin.

Table 7 Effects on lung function after long-term occupational endotoxin exposure.

study design and population	control	work history[a]	endotoxin exposure; mean (range)	parameters measured	effects reported[b]	calculated level[c] (ng/m^3)	ref.
cross-sectional study in cotton mill workers n=443	silk-workers n=439	15 years	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: annual FEV_1 β (ng/m^3 or 10 EU/m^3) endotoxin exposure) = -0.016 to -0.052 ml FEV_1 [d]	75-150 ng/m^3	Kennedy *et al.* (1987)[72]
cross-sectional study in animal feed workers n=315	non-exposed colleagues n=50	13 years	<15, 30-34 and 67 ng/m^3 (range 0.2-470) (<150, 300-400 and 670 EU/m^3)	stratified exposure-response analyses for FEV_1 and respiratory symptoms	regression: annual FEV_1 β (ng/m^3 or 10 EU/m^3) endotoxin exposure) = -0.34 ml FEV_1 [e]	7.5 ng/m^3	Smid *et al.* (1992)[8]
9–years follow-up of RFD workers with rotating jobs n=87	no	± 9-10 years	GM: 28 EU/m^3 (2.8 ng/m^3) ±3.8 EU/m^3	FVC and FEV_1 change over 9 years	no effect	n.d.[f]	Mahar (2002)[74]
5-years follow-up of grain and animal feed industry workers n= 140 (310-170)	no	12.5 ±8 years	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 years)	regression: annual FEV_1 β (ng/m^3 endotoxin exposure) = -0.326 SE 0.139, R^2=0.12	7.5 ng/m^3	Post *et al.* (1998)[9]
follow-up (11 years) of employed and retired cotton workers n=349	silk workers n=319	16-17 years	32,000 EU/m^3 .y = 1,500 EU/m^3 (42-12,038 EU/m^3) (150 ng/m^3)	annual FEV_1 loss	no cumulative endotoxin effects could be detected on FEV_1	n.d.	Christiani *et al.* (1999)[81]
follow-up (15 years) of employed and retired cotton workers n=346	silk workers n=338	16-17 years	median cumulative: 48,000 EU/m^3 .y ≈ 1,500 EU/m^3 (150 ng/m^3)	annual FEV_1 loss	9% excess annual FEV_1 decrease (i.e. 1.1% instead of 1.0% in control group); correlation between ΔFEV_1 and annual FEV_1 decline. Findings independent from endotoxin exposure	n.d.	Christiani *et al.* (2001)[82]
pig farmers of which 50% symptomatic n=40; poultry farmers, of which 58% symptomatic, n=36	general population	20 years	1-1,101 ng/m^3, MD: 58 ng/m^3 (580 EU/m^3) 19-1,635 ng/m^3 MD: 258 ng/m^3 (2,580 EU/m^3)	FEV_1 and FVC	% predicted FEV_1 > 100% in both groups; % predicted MMEF: 101% in pig farmers and 89% in poultry	n.d.	Radon *et al.* (2001)[76]

newly hired female non-smoking cotton workers (18y) n=101	no	0.0 years	220, 1,360 and 1,070 ng/m^3 (2,200, 13600 and 10,700 EU/m^3)	change in FEV_1, FVC and ΔFEV_1 after 3, 12, 18 months	in all 3 groups: FEV_1 and FVC were 2% ↑ after 3 months; FVC 5% ↓ after 12 and 18 months; FEV_1 2,8% ↓ after 12 m. and 1.3% after 18 m	n.d.	Wang *et al.* (2003)[78]
3-year follow-up in pig farmers selected so that 50% had symptoms n=171	no	16.7 years	105 ng/m^3 (1,050 EU/m^3)	FEV_1 and FVC	decrease in baseline FEV_1 of 73 ml/y and a decrease in FVC of 55 ml/yr	not possible to calculate level because extrapolation is outside the curve	Vogelzang *et al.* (1998-2000)[88,89]
cross-sectional study with 114 male employees in the cotton spinning mill in Western Germany	general population	-	'low'(L): <100 EU/m^3 'medium' (M): 101-450 EU/m^3 and 'high' (H): >450 EU/m^3	cough, weezing, chest tightness, shortness of breath	*cough* OR=1.0 (L) OR=2.11 (0.59-7.56) (M) OR= 3.93 (1.02-15.12) (H) *wheezing* OR=1.0 (L) OR=2.15 (0.48-9.62) (M) OR=5.49 (1.17-25.81) (H)	n.d.	Latza *et al.* (2004)[84]

[a] Work history at the start date of the study
[b] Linear regression: annual $\Delta FEV_1 = \beta *$ endotoxin exposure (ng/m^3 or EU/m^3)
[c] From the regression models, the committee calculated the concentration in air (ng/m^3) corresponding to an excess population mean decline in FEV_1 of 100 mL after occupational endotoxin exposure for 40 years.
[d] For comparability with other studies, the regression of -0.242--0.778 mL FEV_1 per ng/m^3 over 15 years has been converted to annual FEV_1 changes.
[e] The annual FEV_1 change was stated to be -0.34 mL per ng/m^3 in the Health Council's report (1998) (-4.91 ml FEV_1 per ng/m^3 over 13 years of work is -0.38 ml)
[f] n.d.= not determined

Kennedy *et al.* (1987)[72], however, found a less steep dose-response relation in cotton workers, i.e. -0.052 ml FEV_1 per year·ng/m^3 (or yr·10·EU/m^3) endotoxin. 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 study of Smid *et al.* and Post *et al.* than in that of Kennedy *et al*. This might suggest that other constituents 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)[82] found a statistically significant relationship between acute (across-shift) changes in FEV_1 and annual changes in FEV_1. Christiani *et al.* found that a ΔFEV_1 change of -1% was associated with an average annual decline in FEV_1 of 0.061% ($p<0.001$, r^2 not given). 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-response relation between exposure to endotoxins and respiratory symptoms (wheezing and coughing).[84] The result suggest a dose-dependent increase in respiratory symptoms after exposure to endotoxin levels between 100 en 450 EU/m^3, with significant effects after exposure levels that exceeded 450 EU/m^3.

Other adverse or beneficial effects after long-term exposure

No information is available concerning the 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 1970-ies. The most recent finding of Astrakianakis *et al.* (2007)[95] suggest an inverse relation between cancers of the lung and endotoxin exposure in the textile industry as well. 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 (Portengen *et al.*).[134] However, on the other hand, occupational exposure to endotoxins is a risk factor for wheeze and bronchial hyper responsiveness and these symptoms have been 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 analyses indicated that the attribution of symptoms to atopy is small in high endotoxin exposed populations.[90,134]

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.

9.2 Quantitative hazard assessment*

DECOS concludes that a health-based occupational exposure limit (HBROEL) for endotoxins should be based on the avoidance of effects after both acute, short-term and chronic airway exposure. The committee is of the opinion that effects on mean (across-shift) changes in FEV_1, measured on a population level, should be avoided. DECOS is aware that this is a conservative starting point. However, taking into account the uncertainties in the exposure assessment of endotoxins, DECOS deems this starting point reasonable.

The committee uses both the experimental study of Castellan *et al*. (see table 6) and the studies of Smid *et al*. (1992) and Post *et al*. (1998) as a starting point for deriving an HBROEL (8-hours TWA).

Starting point for deriving HBROEL: Respiratory effects after acute or short-term exposure

Using the dose response curve in the study of Castellan *et al*. (1987), DECOS calculates that exposure to 90 EU/m^3 for 6 hours is the highest exposure concentration which causes no shift change in FEV_1. The committee therefore considers this exposure level of 90 EU/m^3 (for 6 hours) as a No-Observed-Adverse-Effect Level (NOAEL). As in this experimental study the volunteers were preselected of being reactive to cotton dust (containing endotoxin), DECOS considers these volunteers as a sensitive group. Therefore, the committee is of the opinion that it is unnecessary to apply an extrapolation factor to compensate for interindividual differences.

Subsequently, based on the study of Castellan, DECOS recommends an HBROEL of 90 EU/m^3 (8-hour time weight average) and is of the opinion that this level will protect workers against the respiratory effects after acute as well as short-term occupational exposure to endotoxins.

* For the recommendation of a health-based occupational exposure limit only DECOS (and not NEG) takes responsibility.

Does the HBROEL also protect against effects after long-term exposure?

Chronic exposure to endotoxins is a causal factor for chronic airway responses. Several studies show a relation between chronic occupational exposure to endotoxins (EU/m^3) and effects on lung function (e.g., excess decrease in FEV_1 (ml)). Therefore, DECOS has to judge whether the proposed OEL of 90 EU/m^3 (based on the prevention of acute effects) also protects against the effects on lung function after chronic occupational exposure. However, the available epidemiological data do not allow the committee to derive, in analogy to the effects after short-term exposure, a level at which the excess decrease in FEV_1 after 40 years is nil. Therefore, DECOS chooses another approach.

Starting with the proposed HBROEL of 90 EU/m^3, DECOS estimates what the additional decrease in FEV_1 would be after 40 years of exposure to this exposure level. In the studies of Smid *et al.* and Post *et al*., 40 years of exposure to 90 EU/m^3 endotoxin will result in an extra decline in FEV_1 of 120 mL. In the study of Kennedy *et al*., the extra decline is more than a factor 10 less, ie below 12 mL.

Subsequently, DECOS has judged whether an additional FEV_1 loss of 120 mL after 40 years of exposure to endotoxins should be considered an adverse health effect or not. The study of Sin *et al.* (2005)[129] suggests that a mean FEV_1 decline of 120 mL is not statistically significantly associated with cardiovascular events. Also other studies show that an additional FEV_1 loss of 200-300 mL is not related with cardiovascular or other health effects.[129] Therefore, DECOS assumes that an additional FEV_1 loss of 120 mL after 40 years of exposure in the non-smoking population should not be regarded as an adverse effect.

Therefore, DECOS is of the opinion that an OEL of 90 EU/m^3 will also protect workers against long-term exposure. DECOS has chosen a worst case approach by taking the study of Smid *et al.* and Post *et al.* for the estimation of the effect after chronic exposure. Co-exposure to other constituents may have played an important role in developing respiratory effects as well. Moreover, DECOS noticed that the study of Latza *et al*. (2004) confirm the HBROEL of 90 EU/m^3 as well by showing no respiratory symptoms after exposure to concentrations of endotoxins lower than 100 EU/m^3.

In conclusion, DECOS recommends an HBROEL of 90 EU/m^3 for both chronic and short-term exposure to inhalable endotoxins. In addition, to measure the endotoxin exposure, the committee recommends the NEN-EN 14031 method with the adjustments described by Spaan *et al*. (2007).

DECOS is aware of the fact that the HBROEL is based on studies predominantly using the older LAL assays for exposure measurements. The more recent kinetic assays, described by NEN-EN 14031 and Spaan *et al.*, are more sensitive versions of the earlier endpoint versions of the LAL-assay. However, DECOS is not able to determine a standard factor which compensates for this difference in sensitivity in all occupational situations for all endotoxin origins. Therefore, the older epidemiological studies have probably underestimated exposure levels to some extent, resulting in a lower, more conservative, HBROEL. However, DECOS noticed that in the more recent study of Latza *et al.* (2004) respiratory effects appeared after exposure to endotoxin levels exceeding 100 EU/m^3. In this study, a more recent version of the LAL-assay was used.

9.3 Groups at extra risk

Groups suffering from COPD and groups with asthma and atopic respiratory disease (hay fever) 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.

9.4 Health based recommended occupational exposure limit

DECOS recommends a health-based occupational exposure limit for inhalable endotoxins of 90 EU/m^3 based on personal inhalable dust exposure, measured as an eight-hour time-weighted average and using the most recent version of the LAL assay (see NEN-EN 14031 procedure and the adjustments by Spaan *et al.* (2007).

Chapter 10

Recommendations for research

There are no recommendations for further research.

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a ‘exposure versus tumour incidence range’ and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the committee is given in Annex B.

Annex **B**

The Committees

Dutch Expert Committee on Occupational Safety (DECOS)

- G.J. Mulder, *chairman*
  Emeritus Professor of toxicology, Leiden University, Leiden
- R.B. Beems
  Toxicologic pathologist, formerly employed at the National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, Shell International BV, The Hague
- J.J.A.M. Brokamp, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
  Professor of health risk analysis, Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- R. Houba
  Occupational hygienist, Netherlands Expertise Centre for Occupational Respiratory Disorders (NECORD), Utrecht
- H. van Loveren
  Professor of immunotoxicology, Maastricht University, Maastricht; National Institute for Public Health and the Environment, Bilthoven

- T.M. Pal
  Occupational physician, Netherlands Center for Occupational Diseases, Amsterdam
- A.H. Piersma
  Professor of reproductive toxicology, National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  Professor of molecular biology, VU University Amsterdam, Amsterdam
- I.M.C.M. Rietjens
  Professor of toxicology, Wageningen University and Research Centre, Wageningen
- H. Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- G.M.H. Swaen
  Epidemiologist, Dow Benelux N.V., Terneuzen
- R.C.H. Vermeulen
  Epidemiologist, Institute for Risk Assessment Sciences, Utrecht
- R.A. Woutersen
  Toxicologic pathologist, TNO Quality of Life, Zeist; Professor of translational toxicology, Wageningen University and Research Centre, Wageningen
- P.B. Wulp
  Occupational physician, Labour Inspectorate, Groningen
- A.S.A.M van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague

Nordic Expert Group for Criteria Documentations on Health Risks from Chemicals (NEG)

- G. Johanson, *chairman*
  Professor of occupational toxicology, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden
- K. Kjærheim
  MD, PhD, Cancer Registry of Norway, Oslo, Norway
- A.T. Saber
  Toxicologist, Ph D, National Research Centre for the Working Environment, Copenhagen, Denmark

- T. Santonen
  MD, PhD, MSc in applied toxicology, Finnish Institute of Occupational Health, Helsinki, Finland
- V. Skaug
  Toxicologist, occupational physician, National Institute of Occupational Health, Oslo, Norway
- M. Öberg
  Toxicologist, Ph D, Institute of Environmental Medicine, Karolinska Institute Stockholm Sweden
- J. Järnberg
  Swedish Work Environment Authority, Stockholm, Sweden, scientific secretary
- A. Alexandrie
  Swedish Work Environment Authority, Stockholm, Sweden, scientific secretary

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 Health Council and interests

Members of Health Council Committees – which also include the members of the Advisory Council on Health Research (RGO) since 1 February 2008 – are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public draft

A draft of the present report was released in 2009 for public review. The following persons and organizations have commented on the draft review:

- R.D. Zumwalde, National Institute for Occupational Safety and Health, Cincinatti, USA
- V. Gálvez Pérez, Instituto Nacional de Seguridad e Higiene en el Trabajo - Centro Nacional de Nuevas Tecnologías (INSHT-CNNT), Madrid, Spain
- Vereniging Smeerolieondernemingen Nederland
- Hoofdproductschap Akkerbouw, Productschap Akkerbouw, Diervoeders, Vee, Vlees en Eieren, Tuinbouw, Vereniging van Afvalbedrijven, Koninklijke vereniging voor afval-en reinigingsmanagment.

Annex **D**

Abbreviations

A	Aerosol
BAL	Bronchoalveolar lavage
sCD14	Soluble endotoxin receptor
mCD14	Membrane endotoxin receptor
C.I.	Confidence interval
CRP	C-reactive protein
ECP	Eosinophilic cationic protein
EU	Endotoxin Unit
FEF_{25-75}	Forced expiratory flow at 25 to 75% of lung volume (same as MMEF)
FEV_1	Forced expiratory volume in 1 second
ΔFEV_1	**(**Across-shift) change in FEV over an exposure period of several hours
FVC	Forced vital capacity
GM	Geometric mean
$IFN\gamma$	Interferon-gamma
IgE	Immunoglobulin E
IL	Interleukin
LBP	Lipid-binding protein
LPS	Lipopolysaccharide
LRTS	Lower respiratory tract symptoms

M	Mean
MD	Median
MMEF	Maximal midexpiration flow (average flow over middle half of FVC)
MEF_{25}	Maximum expiratory flow rate at 25% of vital capacity
MPO	Myeloperoxidase
MRC/ECCS	Medical Research Council/ European Community for Coal and Steel
NAL	Nasal lavage
NF-kB	Nuclear factor kB
NOS	Nitric oxide synthase
OEL	Occupational exposure limit
ODTS	Organic dust toxic syndrome
OR	Odds ratio
PC	Provocative concentration
PEF	Peak expiratory flow
PM_{10}	Particulate matter < 10 m in aerodynamic diameter
PMN	Polymorphonuclear leukocyte = neutrophil
P	Particles
R	Range of means per site
r^2	Correlation coefficient
RDF	Refuse-derived fuel
SD	Standard deviation
SEM	Standard error of the mean
SPT	Skin prick test
SMR	Standard mortality ratio
T-cell	Thymus cell derived
Th_1	T-helper type1
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor alpha
TWA	Time-weighted average

Organisations

EN	Committee Européen de Normalisation (European Committee on Standardisation)
DECOS	Dutch Expert Committee of Occupational Safety
DFG-Mak	Deutsche Forschungsgemeinschaft, Mak-werte Commission

IHOC	International Committee on Occupational Health
NEG	Nordic Expert Committee
NEN	Nederlands Normalisatie Instituut
OSHA	Occupational Safety and Health Association