Endotoxins

Health-based recommended occupational exposure limit

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Report of the Dutch expert committee on ocupational standards,
a committee of the Health Council of the Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

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Samenvatting en advieswaarde

1 Vraagstelling

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid leidt de Commissie WGD van de Gezondheidsraad gezondheidskundige advieswaarden af voor beroepsmatige blootstelling aan toxische stoffen in lucht op de werkplek. De aanbevelingen van de commissie vormen de eerste stap in een drietrapsprocedure die moet leiden tot wettelijke grenswaarden (MAC-waarden).

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan endotoxinen en presenteert zij een gezondheidskundige advieswaarde voor beroepsmatige blootstelling. Haar conclusies zijn gebaseerd op wetenschappelijke publicaties die vóór april 1998 zijn verschenen.

2 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 bactiën 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.

3 Monitoring

Milieumonitoring vindt plaats door waterige extracten, die uit luchtstof-monsters zijn verkregen, te onderzoeken met de Limulus Amebocyte Lysate (LAL) test. Er bestaan geen algemeen geaccepteerde standaarden voor de luchtbemonsterings- en extractieprocedures. De commissie beveelt voor de luchtbemonstering het gebruik van glasvezelfilters aan en voorts bewaring van de monsters bij een temperatuur lager dan 18 ºC onder nul, vermijding van herhaaldelijk bevriezen en ontdooien, extractie in pyrogeen-vrij water dat 0,05% van een detergens (Tween-20) bevat en, tenslotte, analyse met de kinetisch-chromogene of turbidimetrische LAL-test.

4 Huidige grenswaarden

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

5 Kinetiek

Er bestaan vrijwel geen gegevens over de absorptie en de distributie van ingeademde endotoxinen in het lichaam. Endotoxinen die, aan deeltjes gebonden, terechtkomen in de bovenste luchtwegen, worden via mucociliair transport verwijderd. Men neemt aan dat dieper doorgedrongen endotoxinen onschadelijk worden gemaakt door macrofagen en polymorfonucleaire leukocyten.

6 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 en proefdieronderzoek zijn aanwijzigingen 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.

Het vermoeden bestaat dat chronische inhalatoire blootstelling aan endotoxinen bij mensen leidt tot aspecifieke versterking van de immuunrespons op antigenen (adjuvans-effect). De juistheid van dit vermoeden is echter nog niet aangetoond. Er zijn geen gegevens die duiden op kankerverwekkende, mutagene of reproductie-toxische eigenschappen van endotoxinen.

7 Risico-evaluatie

De in de literatuur genoemde niveaus van inhalatoire blootstelling aan endotoxinen waarbij (juist) geen effect wordt waargenomen (NEL's) variëren van 9 tot 180 nanogram per kubieke meter lucht (ongeveer 90 tot 1800 EU*/m^3). Ze zijn hoofdzakelijk ontleend aan de uitkomsten van onderzoek met vrijwilligers. De NEL's die ontleend zijn aan resultaten van (arbeids)epidemiologisch onderzoek, zijn voor acute gezondheidseffecten niet essentieel anders dan voor chronische effecten.

De commissie neemt een NEL van 90 EU/m^3 als uitgangspunt voor het afleiden van een gezondheidskundige advieswaarde. Deze waarde geldt voor acute luchtweg-effecten en is gebaseerd op de uitkomsten van een goed opgezet experimenteel onderzoek waarin klachtenvrije vrijwilligers werden blootgesteld aan endotoxinen-bevattend katoenstof. Om rekening te houden met een mogelijk verhoogd risico voor bepaalde groepen, en om te verdisconteren dat chronische luchtweg-effecten zich bij lagere doses zouden kunnen voordoen dan acute effecten, kiest de commissie een veiligheidsfactor ter waarde van 2.

8 Gezondheidskundige advieswaarde

Voor blootstellingen aan endotoxinen in de lucht op de werkplek komt de commissie tot een gezondheidskundige advieswaarde van, afgerond, 50 EU/m^3, hetgeen

* Endotoxin Unit: dit is een maat voor de endotoxine activiteit in de LAL-test; 1 ng endotoxine EU is ongeveer gelijk aan 10 EU.

overeenkomt met ongeveer 5 ng/m^3. Deze advieswaarde heeft betrekking op de concentratie van inhaleerbare deeltjes waaraan personen beroepsmatig kunnen worden blootgesteld, gemiddeld over een achturige werkdag.

Executive Summary

1 Scope

At the request of the Minister of Social Affairs and Employment, the Dutch Expert Committee on Occupational Standards (DECOS) of the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentrations of toxic substances in air at the workplace. The recommendations of the committee constitute the first step in a three-step procedure that leads to legally binding limit values.

In the present report the committee discusses the consequences of occupational exposure to airborne endotoxin and presents a health-based occupational exposure limit. The committee's conclusions are based on scientific publications prior to April 1998.

2 Physical and chemical properties

Endotoxins are part of the outer membrane of gram-negative bacteria and are composed of proteins, lipids and lipopolysaccharides (LPS). LPS of gram-negative bacteria refer to a class of pure lipid carbohydrate molecules (free of protein and other cell wall components) that are responsible for most of the biologic properties characteristic of bacterial endotoxins. LPS are soluble in water. LPS-molecules are stable and composed of lipid and polysaccharide. The lipid moiety of LPS is termed 'lipid A' and is responsible for its toxic properties. The composition of lipid A, an

amphipathic and zwitterionic phosphoglycolipid, is remarkably constant among various bacterial species. The hydrophilic polysaccharide moiety is composed of O-specific side chains and core sugars and varies considerably between bacterial species.

In the environment airborne endotoxin is related to the occurrence of gram-negative bacteria. Animal faeces and bacteria-contaminated plant materials contribute most in organic dust-related endotoxin exposure. Occupational endotoxin exposure is therefore most prevalent in agricultural and related industries.

3 Monitoring

Environmental monitoring is usually performed by sampling airborne dust and subsequent analysis of aqueous extracts by using a *Limulus* Amebocyte Lysate (LAL) test. No generally accepted standard sampling and extraction procedures exist. The committee recommends personal or area samplers to collect inhalable dust using glass fibre filters, sample storage below -18 ºC with the avoidance of repeated freezing and thawing of samples, extraction in pyrogen free water with 0.05% of the detergent Tween-20, and analysis with a chromogenic kinetic or turbidimetric LAL assay.

4 Current limit values

At present no previous occupational exposure limit has been established in the Netherlands or other countries.

5 Kinetics

Hardly any data are available on the absorption and the distribution of endotoxins after inhalation. Particle associated endotoxin deposited in the upper airways is eliminated by mucociliary transport, endotoxin deposited in the deeper airways is assumed to be eliminated by macrophage and polymorphonuclear leucocyte phagocytosis.

6 Effects

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 dyspnea, headache and joint aches occurring a few hours after the exposure. Acute effects have been demonstrated in both experimental exposure studies with human volunteers and epidemiological studies in occupationally exposed workers. Bronchial obstructive responses associated with an increase in non-specific

bronchial reactivity were demonstrated in asthma and rhinitis patients exposed to pure LPS. Epidemiological and animal studies suggest that chronic endotoxin exposure may lead to chronic bronchitis and reduced lung function. Both acute and chronic effects are most likely induced through inflammatory responses in the lungs in which the alveolar macrophage plays a key role.

In the literature it is hypothesized that chronic inhalatory endotoxin exposure may increase non-specifically the immune response to antigens in man (adjuvant effect). There is, however, at present no direct proof that may support this hypothesis. There are no data indicating carcinogenic, mutagenic or reproduction effects due to endotoxin exposure

7 Hazard assessment

In the literature no effect levels (NELs) for inhalatory endotoxin exposure have been calculated ranging from 9 to 180 ng/m^3 (approximately 90 -1800 EU*/m^3) based mainly on experimental endotoxin exposure studies. Calculated NELs for chronic and acute respiratory effects based on epidemiological studies in occupationally exposed populations are comparable. Starting point for the establishment of a health-based recommended occupational exposure limit by the committee is the NEL of 90 EU/m^3 based on acute respiratory effects and obtained from a large and well designed experimental exposure study in which non-symptomatic subjects from the general population were exposed to endotoxin contaminated cotton dust. A safety factor of 2 was applied to compensate for increased risks for certain groups of workers and also taking into account that endotoxin may have chronic pulmonary effects at levels which may be lower than for acute respiratory effects.

8 Recommended occupational exposure limit

The committee recommends a health-based occupational exposure limit for airborne endotoxin of, rounded off, 50 EU/m^3, which is approximately 5 ng/m^3, based on personal inhalable dust exposure, measured as an eight hour time weighted average.

* Endotoxin Unit: measure for LAL-test activity; 1 ng endotoxin is approximately 10 EU.

Chapter 1

Scope

1.1 Background

In the Netherlands occupational exposure limits for chemical substances are set using a three-step procedure. In the first step a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the Minister of Social Affairs and Employment (Annex A). This evaluation should lead to a health-based recommended exposure limit for the concentration of the substance in air. Such an exposure limit cannot be derived if sufficient data are not available or if the toxic action cannot be evaluated using a threshold model. In the latter case an exposure-response relationship is recommended for use in regulatory standard setting.

In the next phase of the three-step procedure the Social and Economic Council advises the Minister on the feasibility of using the health-based value as a regulatory Occupational Exposure Limit (OEL) or recommends a different OEL. In the final step of the procedure the Minister of Social Affairs and Employment sets the official Occupational Exposure Limit.

1.2 Committee and procedure

The present document contains the assessment of DECOS, hereafter called the committee, of the health hazard of endotoxins. The members of the committee are listed in annex B. The first draft of this report was prepared by ir J Douwes and dr ir D

Heederik from the Department of Environmental Sciences, Environmental and Occupational Health Group of the Agricultural University, Wageningen, by contract with the Ministry of Social Affairs and Employment.

In 1997 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. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

Literature was retrieved from the on-line data bases Medline and Niohtic, starting from 1980. The final search has been carried out in December 1995. From the papers published between 1996 and April 1998 only the relevant ones have been included in the report.

Chapter 2

Identity, properties and monitoring

2.1 Identity

Endotoxins are integral components of the outer membrane of gram-negative bacteria and are composed of proteins, lipids, and lipopolysaccharides. The term ‘endotoxin’ refers to the toxin present on the bacterial cell wall which is often liberated as a result of cell lysis. Lipopolysaccharides (LPS) of gram-negative bacteria refer to a class of pure lipid carbohydrate molecules (free of protein and other cell wall components) that are held responsible for most of the biologic properties characteristic of bacterial endotoxins. LPS are amphiphilic macro molecules containing a lipid and a polysaccharide moiety. This group of macro molecules have been detected in such taxonomically remote groups of gram-negative bacteria as *Enterobacteriaceae, Pseudomonadaceae* and *Rhodospirillaceae* but have not been found in cell walls of gram-positive bacteria, mycobacteria or fungi (Mor79, Rie85). Eventhough most of the biologic effects can be reproduced by purified LPS it is not correct to assume that this term is always preferable to the term endotoxin in defining biologic responses to this bacterial product. The terms ‘endotoxins’ and ‘lipopolysaccharides’ are therefore often used interchangeably in the scientific literature (Mor79).

2.2 Physical and chemical properties

LPS are stable water-soluble molecules composed of lipid and polysaccharide. The lipid moiety of LPS is termed ‘lipid A’ and is responsible for the toxic properties of

LPS. The composition of lipid A, an amphipathic and zwitterionic phosphoglycolipid, deviates significantly from other lipids in biological membranes. Among various bacterial species the composition of lipid A is remarkably constant. The hydrophilic polysaccharide moiety is composed of O-specific side chains and core sugars. The composition of O-specific chains varies considerably between bacterial species. Each bacterial serotype synthesizes unique LPS at least varying in the composition of the O-specific chain. The composition of the core is relatively constant and is identical or closely related for large groups of bacteria (Mor79, Rie85). The two innermost saccharide units are usually composed of components only found in LPS, L-glycero-D-manno-heptose and 2-keto-3-deoxyoctonic acid. 2-keto-3-deoxyoctonic acid is ketosidically linked to the disaccharide of lipid A and seems always present in LPS. The lipophilic portion of LPS (lipid A) in for example enterobacteria is composed of a disaccharide (B-1,6 linked N-acetyl-D-glucosamine, phosphorylated) which normally carries four fatty acids, which may be branched. In other gram-negative species structural deviations may occur. The fatty acids are 3-hydroxy-substituted and the most common is 3-hydroxymyristic acid. These are not found in gram-positive bacteria or in fungi and have been used as chemical markers of gram-negative bacteria (Bei91).

In the environment airborne endotoxins are usually associated with dust particles or aqueous aerosol with a broad size distribution. Data, however, have shown that the smaller size aerosol fractions contain greater amounts of endotoxins per weight of aerosol.

2.3 Analytical methods

Absolute LPS measures have been described in the literature such as electrophorese techniques, gas chromatography - mass spectrometry (GCMS) and high performance liquid chromatography (HPLC) (ACG89, Ryl89b, Son90, Fox93). These methods, however, require elaborate LPS extraction procedures. Few comparative studies have been performed using different analytical methods. Variable results were found comparing GCMS with a functional assay (LAL-assay) (Son90, Wal93).

The *Limulus* amebocyte lysate (LAL) test is at present the most accepted assay for endotoxin measurements. This functional assay, which is highly sensitive, is based on the activation of a clotting enzyme (via factor C) present in the lysate of hemolymph of the *Limulus polyphemus* (horseshoe crab). The test is normally performed in microtiterplates. The LAL-assay is adapted as the standard assay for endotoxin detection by the American Food and Drug Administration in 1980. The more recent chromogenic kinetic versions of the LAL-assay are very sensitive and have a broad measurement range (0.01 - 100 Endotoxin Units (EU)/mL $\approx$ 1 pg/mL - 10 ng/mL). The

detection limit for airborne endotoxin measurements is approximately 0.05 EU/m^3 (5 pg/m^3) for this method. The LAL-method does not represent an absolute LPS measure but measures the portion of endotoxins that are biologically available to the assay. This biologically active portion measured with the LAL assay is expected to represent a relative toxicity measure. The variation in sensitivity of the LAL-assay for different substrates would correspond in general with the variation in biological response in mammals (ACG89). This has not been evaluated in experimental studies. Good correlations between endotoxin levels as measured with the LAL test and acute health/toxic effects have been observed (Ryl94a).

Since different test batches may give different results an internal standard must be used. The Food and Drug Administration (FDA) uses a reference standard endotoxin (RSE):E. coli-5 (EC-5) as part of their standardization procedures. Endotoxin samples need to be referenced to the RSE:EC-5. Large differences in both the hydrophilic and to a lesser extent the lipid A moiety between endotoxins of different species or strains make a comparison on weight basis almost meaningless (Coo85). The RSE:EC-5 based on purified LPS from *E. coli* is therefore expressed in Endotoxin Units (EU) which is a measure for LAL test activity. Since the RSE is too expensive and also exhaustible one has chosen to use a control standard endotoxin (CSE), which is standardized to the RSE. The CSE is in general based on *E. coli* and sometimes on *S. abortus equi*. The CSE is normally included in commercial LAL tests. Nevertheless most studies publish endotoxin levels in nanograms rather than in Endotoxin Units.

Results may not be valid under different conditions because other constituents present in the sample may interfere with the LAL assay causing inhibition or enhancement of the test and aggregation and adsorption of endotoxins resulting in under- or overestimation of the endotoxin concentration (Hol93a). Techniques such as spiking with known quantities of purified endotoxin and analysis of dilution series of the same sample, have been described to deal with these interferences (Whi88, Mil90, Mil92, Hol93a). Until recently the LAL-assay was considered to be very specific for LPS from gram-negative bacteria. However, peptidoglycans (constituents of gram-positive bacteria), glucans (mould and plant constituents) and some other polysaccharides also react via another pathway (factor G instead of factor C) with the proclotting enzyme in the LAL enzyme reagent, and this may result in false positive results (Mor81, Tan91, Mik82). Most of these LAL reactive polysaccharides, however, are much less potent in their LAL activation than bacterial endotoxins. Interference by LAL reactive polysaccharides may differ for different LAL tests from different LAL producers depending on the LAL processment procedures. With the LAL test only free, cell wall dissociated, endotoxins are detectable (Ryl89b, Son90), while inhalation experiments with animals have shown that cell bound endotoxins (whole cells) have similar or even increased toxicity compared to free endotoxin (Dun86, Bur90).

In spite of the above mentioned, it is generally accepted that in most situations the endotoxin activity can be accurately measured using the LAL assay. At present an increasing number of applications of the LAL test have been described, including detection of endotoxins in pharmaceutical preparations, food, radioisotopes, water supplies, and medical devices, as well as in various body fluids such as blood, cerebrospinal fluid, and urine (Lev85). Over the last decade the LAL assay has become widely used for the quantification of endotoxins in both occupational and general environments.

2.3.1 *Environmental monitoring*

Environmental monitoring of endotoxins is usually performed by sampling airborne dust and a subsequent aqueous extraction. Dust is sampled on filters using pumps to draw air through the filters. Several types of filters are commonly used for endotoxin sampling: cellulose, polyvinylchloride (PVC), glass fibre, teflon, polycarbonate. For extraction no standard method exists. Most laboratories use pyrogen-free water or buffers like Tris or phosphate triethylamine pH 7.5 with or without detergents such as Tween-20, Tween-80, Triton-X-100 and saponin. The use of buffers and dispersing agents may be beneficial in the LAL-assay in case of deviation from the optimal pH (6.5) or increased ion strength of the extract. The most common way of extraction is rocking or sonication of filters in extraction media, or a combination of both. A few studies have been published on optimization of filter choice, filter extraction methods, extraction buffers and choice of glassware (Ole89, Mil90, ACG89, Gor92, Nov86, Dou95), but a generally accepted protocol is not yet available. Results of some of these studies suggest that substantial differences in endotoxin exposure assessment may be expected between research groups as a consequence of the use of different protocols for endotoxin sampling and extraction (Gor92, Dou95). Most laboratories store their extracts frozen at a temperature of -20^{o} C in either glass or plastic container materials. Little attention, however, has been addressed to the influence of storage conditions on the stability of endotoxins in dust extracts. One study reports a dramatic effect on the detectable endotoxin level, ranging from 20-90% reduction after 1 to 9 repeated freeze (-20^{o} C) thaw cycles (Dou95). The same study, however, indicated that storage duration of samples at -20^{o} C did not significantly influence the endotoxin concentration.

2.3.2 *Recommendations*

The committee recommends the following procedures for collection, storage, extraction and analysis of airborne dust samples for endotoxins.

- Sample collection: personal or area samplers designed to collect inhalable dust using glass fibre filters. For comparison with the OEL it is recommended to use personal sampling while in general, personal exposure characterizes the individual's exposure better than area sampling. Area sampling can be used to identify sources or areas of exposure.
- Sample storage: aseptically and stored below -18° C; repeated freezing and thawing of samples should be avoided.
- Sample extraction: pyrogen free water with 0.05 % Tween-20 with vigorous rocking for 1 hour followed by centrifugation at 1000 x G.
- Extract storage: aseptically and stored below -18° C; repeated freezing and thawing of samples should be avoided.
- Analysis: chromogenic kinetic or turbidimetric kinetic LAL assay.

All glassware used during extraction, storage and analyses should be rendered sterile by heating at 190° C for four hours.

Chapter 3

Sources

3.1 Natural occurrence

Because of their ubiquitous nature, bacterial endotoxins are found in various environments. Endotoxin levels are related to the occurrence of gram-negative bacteria which at their turn are influenced by environmental conditions such as substrate availability, temperature, humidity etc. As a consequence endotoxin levels may vary much in soil, air and water. For occupational diseases only airborne endotoxins seem relevant. Endotoxins become airborne during manufacturing or handling of organic materials. Animal faeces and bacteria-contaminated plant materials contribute most in organic dust related endotoxin exposure. Endotoxin exposure is therefore most prevalent in agricultural and related industries as for example pig, chicken, cow and horse farming, grain elevators, cotton industry, potato processing industry, poultry slaughter houses, flax processing industry, animal feed industry, water sewage treatment and sewage composting plants, garbage handling facilities, organic waste composting facilities, wood chip composting and timber storing facilities. Air humidifiers in buildings and recycled industrial process waters may also be an important source of airborne endotoxin exposure. In addition, industrial oils and emulsions may also contain substantial amounts of endotoxins as a result of contamination with gram-negative bacteria. With exception of the latter, endotoxin exposure seems mainly associated with organic dust exposure. Organic dust is broadly defined and represents dust with a very heterogeneous composition (Ryl94a) and constituents of organic dust can vary from plant matter, to molds and spores,

mycotoxins, bacteria, their biochemical components (such as endotoxins), and excretions, enzymes and animal matter including parts of insects, rodents and birds and their excreta (Bol95). In the past decades endotoxins have been recognized to be a very important biologically active component in most organic dusts.

3.2 Man-made sources

There are no man-made sources of endotoxins.

Chapter 4

Environmental levels and human exposure

4.1 Environmental levels

4.1.1 Water

One publication reported on endotoxin levels in lake and tap water. More than 100 people in one community in Finland experienced respiratory health problems (‘bathwater fever’) after inhaling aqueous aerosol from an endotoxin contaminated drinking water source (Mui80). The drinking water source was a small lake heavily contaminated with gram-negative bacteria. Analysis of tap and lake water revealed endotoxin concentrations ranging from 0.2 to 1.0 µg/mL. These concentrations are exceptionally high and situations like that are not likely to occur in the Netherlands. Actual endotoxin levels in tap water in the Netherlands have not been published in the peer reviewed literature.

4.1.2 Food

Oral intake of large amounts of endotoxins via contaminated pig feed did not cause clinical symptoms in pigs, though the liver was to some extent affected (Hol93b). Another study in pigs, however, concluded that a combined oral and airborne endotoxin challenge induced hematological and cytological changes that might have contributed to the development of respiratory disorders (Hol94). Actual endotoxin

levels in human diet are not known. It should be realized that endotoxins are excreted via faeces originating from gram-negative bacteria in the intestines.

4.1.3 Air

Relevant airborne endotoxin exposures are mainly limited to occupational environments and may occasionally occur in outside situations in the vicinity of agricultural and related industries. Endotoxin levels in various occupational environments are presented in table 1 (4.2.2). In this table Dutch endotoxin data were complemented with international endotoxin exposure data. These data indicate that differences in airborne endotoxin levels are found between various industries. Airborne endotoxin levels also vary between departments and job title. Exposure levels in industrial units within the same industry may also vary substantially.

4.2 Human exposure

4.2.1 General population

The general population may become exposed to endotoxins when living in the vicinity of industries that emit organic dust in the environment. Limited data are available on this subject. One small exposure study performed in the vicinity of pig farms and another one near a grass drying mill revealed no increased endotoxin exposure (non published results, Epidemiology and Public Health, Agricultural University Wageningen, The Netherlands). Another study performed in the Netherlands measured endotoxin in the outdoor air in the vicinity of a soy processing facility and in living areas located near the soy plant. Whereas increased levels of airborne endotoxin were found in the outdoor air on the premises of the soy plant (geometric mean, 42 EU/m^3), no increased levels were found in the living areas immediately surrounding the soy processing facility (Str95).

Substantial amounts of bacterial endotoxins can be found in house dust. Mean endotoxin levels of 2.59 ng/mg housedust have been described by Michel *et al.* (Mic91a). They documented that the clinical severity of asthma in patients was positively correlated with the endotoxin concentrations found in the dust. At present, information about airborne endotoxin levels in the domestic environment is lacking, and it is not yet possible to make reliable estimates of the dose that can be inhaled by subjects living in homes with elevated levels of endotoxin in the house dust.

4.2.2 *Working population*

The role of endotoxin exposure in the development of work-related adverse pulmonary effects is studied intensively the last decade. A number of exposure studies showed elevated endotoxin exposure in a large variety of occupational environments. The occupational population at risk for endotoxin exposure in the Netherlands is estimated to be several hundreds of thousands among which at least 100,000 farm operators, farm workers and their families and workers in closely related industries (Hee88) as for example the animal feed industry, vegetable fibre processing industries, wood industry, industries for manure, compost and spawn processment for mushroom cultivation. The mean personal exposure data as presented in table 1 indicate that these agricultural and closely related occupations have the highest endotoxin exposure. Workers involved in waste disposal, sewage treatment and subsequent composting of organic waste and sewage is another important occupational group at risk. Other groups of workers at risk are: people working in buildings with contaminated air conditioners or humidifiers (due to poor maintenance), people working in industries where bacteria contaminated machining fluids become airborne in the process, and workers involved in industrial biotechnology processes. Exposure data in table 1 are presented as mean values, the actual endotoxin exposure levels however, both ambient and personal, may occasionally exceed these data to a large extent.

Table 1 Dust and endotoxin exposure in various occupational environments both in The Netherlands and abroad. Exposures are presented as ranges in mean levels (both arithmetic and geometric means have been used) per department or jobtitle within one industry, else the exposure is given as the mean of all measurements in the industry.

type of industry	dust fraction	n	mean dust conc (mg/m^3)	n	mean endotoxin conc (ng/m^3)
grain elevator and animal feed industry					
DeLucca *et al*. (1984)	respirable[c]	69	<0.3	69	0 - 0.74
Smid (1992a)[b]	inhalable[c]	530	0.8 - 9.8	530	1.2 - 28.5
	inhalable[a]	79	0.8	79	1.9
water sewage treatment plant					
Melbostad *et al*. (1994)	?[c]	24	?	23	30
Westveer *et al*. (1993)[b]	inhalable[c]	52	<0.3	52	1[d]
	inhalable[a]	41	<0.3	48	0.08 - 8.1[d]
air humidifiers in buildings					
Flaherty (1984)	-	-	-	-	qualitative identification
Kateman (1990)[b]	inhalable[c]	15	0.5 - 0.6	6	0.064 - 0.018
pig farmers					
Clark *et al.* (1983)	total[a]	18	1.8 - 5.2	18	40 - 280
Attwood *et al*. (1987)[b]	total[a]	170	2.8 - 4.9	166	120 - 128
	D_{50} ≤8.5 μm[a]	171	0.9 - 1.5	166	105 - 115
Donham *et al.* (1989)	total[c]	57	6.8	57	240
	respirable[c]	57	0.34	57	230
Preller *et al*. (1995)[b]	inhalable[c]	360	2.4	350	92
chicken farmers					
Clark *et al.* (1983)	total[a]	7	1.0 - 3.7	7	120 - 500
Thelin *et al*. (1984)	?[c]	25	5.8 - 28.1	25	130 - 1090
Veld (1986)[b]	D_{50}<8.5μm[c]	23	0.8 - 4.9	19	145 - 871
	total[a]	24	2.5 - 13.1	22	225 - 1340
Jones *et al*. (1984)	total[a]	9	approx. 2 - 10	7	24 - 59
	respirable[a]	9	approx. 0.08 - 0.5	7	3.8 - 9.8
poultry slaughter houses					
Lenhart *et al.* (1990)	inhalable[a]	17	20.2	17	250
	respirable[a]	19	1.75	19	13
Hagmar *et al*. (1990)	total[c]	24	3.1 - 7.7	24	40 - 780

Table 1 Continued.

cotton industry					
Rylander & Morey (1982)	respirable[a]	-	-	36	20 - 370
Kennedy *et al*. (1987)	PM<15μma	130	0.59 - 1.17	62	2 - 530
potato processing industry					
Zock *et al*. (1995)[b]	inhalable[c]	211	0.4 - 21.1	195	9 - 102[d]
	inhalable[a]	81	0.2 - 19.3	68	1 - 4000[d]
garbage handling and composting facilities					
Amelsfoort *et al*. (1994)[b]	inhalable[c]	25	0.5 - 25.7	28	3 - 131
Sisgaard *et al.* (1994)	total[a]	63	0.62 - 0.74	63	0.8 - 2.5
sugar beet processing industry					
Forster *et al*. (1989)	?[a]	?	1.4 - 3.5	?	2.5 - 32
biotechnology industry					
Palchak *et al*. (1988), controlled exposure	?	59	?	59	0.33 - 1.39
without controlled exposure	?	34	?	34	162.85
breweries					
Carvalheiro *et al*. (1994)	?[a]	?	?	?	60 - 927

[a] area sampling

[b] Dutch situation

[c] personal sampling

[d] nanograms were calculated from Endotoxin Units by dividing with a factor 10

Chapter 5

Kinetics

5.1 Absorption

It is known that free endotoxins can form complexes with serum proteins resulting in a modification of the biological activity of endotoxins (Mor85).

Of most importance to occupational exposure are the activities of endotoxins in the lung. Airborne aerosols or dust particles containing bacteria, bacterial fragments and bacterial components such as endotoxins, as might be found in occupational environments, are of a size that can deposit at each level of the respiratory tree. Whole bacteria have particle sizes of 1-3 μm and fragments of gram-negative bacteria range down to molecular aggregates. Such particles, if deposited in the trachea and large bronchi, are eliminated by mucociliary transport (Jac89). Smaller particles deposit in the deeper airways (small bronchi, bronchioli, alveoli) where endotoxin can generate its inflammatory reactions. During the inflammation process, the paracellular and transcellular permeability of the epithelium may alter, allowing endotoxins or other toxins in organic dust to cross this barrier. Inactive cell-bound endotoxins, deposited in different parts of the lung can be liberated and become biologically active by several mechanisms (Mor85): during lysis of bacteria by antibiotics or complement, during phagocytosis of bacteria by macrophages and polymorphonuclear leucocytes (PMN), which results in both free endotoxins and phagocyte-bound endotoxins with similar or even increased toxicity (Dun86), and during reproduction of bacteria.

5.2 Distribution

Endotoxin distribution experiments in mice showed that radiolabeled LPS intravenously injected in a lethal dose is distributed fast over extravascular spaces with a half-life of approximately 10 hours. The majority of the LPS was deposited in the liver (approx. 25%). Much less endotoxin was taken up by the spleen (approx. 1%) and lymph nodes (approx. 0.5%) (Mor85). This study did not indicate where the rest of the LPS was deposited. The committee is not aware of any data on the exact distribution of endotoxins after inhalation.

5.3 Elimination

No data are available on the exact elimination mechanisms but most of the endotoxin that is deposited in the lung is assumed to be eliminated by macrophage and polymorphonuclear leucocyte phagocytosis (Jac89).

5.4 Possibilities for biological monitoring

LPS can be measured in serum and plasma samples from patients with a septic shock due to infection by gram-negative bacteria (Lev85). No studies have been performed in which endotoxin levels in plasma and serum samples were determined after occupational (inhalatory) endotoxin exposure.

Chapter 6

Effects

Thomas' (Tho74) suggestions that endotoxins are 'read by our tissues as the very worst of bad news' and that in response to these molecules 'we are likely to turn on every defense at our disposal', elaborate beautifully the toxic potential of these macromolecules (Mor79).

6.1 Animal experiments

Human exposure studies, both experimental and epidemiological, provide sufficient knowledge to describe health effects of endotoxin exposure in man. Besides, humans are one of the most sensitive organisms for endotoxin exposure (Wol73). Therefore, only relevant animal experiments, describing acute and chronic pulmonary effects of endotoxin exposure, that support observations in man are included. These studies are discussed in paragraph 6.2 in connection with the human data.

There are no animal experiments described in the literature that indicate carcinogenic, mutagenic or reproduction effects due to endotoxin exposure.

6.2 Observations in man

Pernis *et al.* (Per61) and Cavagna *et al.* (Cav69) were probably the first to recognize the role of endotoxins in the so called 'monday morning malaise' among cotton workers. This early stage of byssinosis, an occupational lung disorder, is characterized by a distinctive temporal occurrence of chest tightness accompanied by malaise, which

is most notably during the first work shift of the week after an exposure free weekend. Byssinosis is commonly observed in textile workers and is caused by inhalation of cotton, flax, or hemp dust (Cas84). Many reports have suggested an important role for endotoxins and gram-negative bacteria in the etiology of byssinosis (Cin77, Cas84, Hag84, Ryl85a).

As new environments with organic dust exposure were found, the lung diseases observed were named after the particular environment in which they were detected. Diseases as for example mill fever, grain fever and humidified fever are all based on an acute inflammation with similar resulting pathology (toxic pneumonitis) (Ryl94b). Endotoxin is regarded as a very important component in the etiology of these diseases but is thought not to be the only factor. The terms 'organic dust toxic syndrome' and 'inhalation fever' are also often used when referred to these occupational diseases with similar pathology.

6.2.1 *Acute toxicity*

Clinical response

Clinical symptoms after intravenous administration of endotoxin in human volunteers consisted of joint aches and fever, shivering and other influenza-like symptoms. In addition, leucocytosis was observed with a negative chest X-ray (Mer75). Symptoms disappeared the next day and repeated exposure yielded similar but less intense symptoms. Obviously, multiple exposures may lead to some kind of tolerance. Greisman and Hornick (Gre69) described an endotoxin tolerance that lasted 5 weeks after a long-term exposure. Wolff (Wol73) stated that man is one of the most sensitive organisms for endotoxin exposure. Experiments showed that 90 to 120 minutes after intravenous administrations of endotoxin in volunteers, fever reactions occurred which lasted 3 to 4 hours. A maximum elevation in body temperature of 1.9^{o} C was demonstrated using 2 ng endotoxin derived from *Salmonella abortus equi* per kg body weight. To yield a similar reaction using *S. typhosa* endotoxin, 5 ng/kg was needed. In addition, Wolff showed that granulocytosis could be demonstrated at lower endotoxin concentrations than needed for the fever reactions to occur. Elin *et al.* (Eli81) were able to demonstrate fever reactions in man after intravenous administration of endotoxin concentrations as low as 0.1 to 0.5 ng/kg body weight using *E. coli* endotoxin.

Data on inhalation experiments on human volunteers are also available. Pernis *et al.* (Per61) themselves inhaled endotoxin aerosols derived from *E. coli* in doses of 5, 10 and 20 µg. Endotoxin was dissolved in saline solution. Acute effects were dry cough

and shortness of breath accompanied with a decrease in FEV_1 (spirometric lung function parameter: forced expiratory volume in 1 second). Inhalation of *S. abortus equi* endotoxin in a dose of 20 μg resulted in similar symptoms accompanied with light fever reactions and malaise. Cavagna *et al.* (Cav69) demonstrated a more than 10% decrease in FEV_1 in two of eight volunteers that were exposed to a dose of 80 μg *E. coli* endotoxin. Jamison and Lowry (Jam86) demonstrated a 11% decrease in lung diffusion capacity in volunteers (n=6) exposed to a dose of 12 μg *Enterobacter agglomerans* endotoxin. In this study they did not demonstrate changes in lung function parameters such as forced vital capacity (FVC), FEV_1 and maximum expiratory flow rate at 50% of vital capacity (MEF_{50}). Muittari *et al.* (Mui80) also showed a decrease in lung diffusion capacity in four volunteers after a four hours exposure to a dose of 0.8 to 4 μg endotoxin (approx. 0.01 μg/kg body weight). They demonstrated a very strong reaction in a bronchial hyperreactive person. This increased sensitivity of individuals with bronchial hyperreactivity was confirmed by Van der Zwan *et al.* (Zwa82) in a study among 19 chronic non-specific lung diseases (CNSLD)-patients and by Michel *et al.* in 8 asthma patients (Mic89), and later in another 12 asthma and 4 perennial rhinitis patients (Mic92a). In Michel's studies, bronchial obstructive responses associated with an increase in non-specific bronchial reactivity were demonstrated in asthma and rhinitis patients at a dose of 20 μg LPS, while at this level no bronchoconstriction was observed in healthy subjects. No significant response was observed in the asthmatic and rhinitis patients at a dose of 0.2 and 2 μg LPS. Healthy subjects responded at a dose level of 200 μg LPS. The threshold for these effects is probably smaller than 20 μg but could not be determined (Mic89). The LPS induced bronchial obstruction demonstrated in asthma patients was shown to be associated with an inflammatory process (Mic92b) rather than with atopy (Mic92a). Rylander (Ryl86) demonstrated decreased FEV_1 values and decreased diffusion capacity in man after provocation with pure LPS. The provocation dose was not mentioned.

Above cited studies were in general performed using pure LPS and a limited numbers of participants. From these data it is not possible to establish a dose-response relationship between endotoxin exposure and acute effects. For this reason it is not possible to estimate a no effect level (NEL) from these data.

More insight in a possible NEL based on acute effects was given by two experimental studies.

Rylander and co-authors (Ryl85b) published a study in which 15 cotton mill workers (male and female of whom 8 persons had a history of byssinosis) were exposed for four hours (on mondays) in an experimental cardroom to cotton dust containing gram-negative bacteria (mainly *Enterobacter agglomerans*) and their

endotoxins. Cotton came from different geographic locations. During the experiment dust and endotoxin levels were determined. FEV_1 and the number of neutrophils before and after work were measured and the prevalence of symptoms of byssinosis was recorded. Personal dust and endotoxin concentrations ranged from 0.09 to 3.97 mg/m^3 and 0.07 to 5.62 μg/m^3, respectively. Poor correlation was found between dust and endotoxin levels. Endotoxin exposure was significantly related to FEV_1 decrease over the exposure period when adjusted for the amount of dust ($r=-0.56$, $p<0.05$). Dust level was not related to change in FEV_1 when adjusted for endotoxin exposure. The authors calculated an endotoxin concentration of 33 ng/m^3 at which average FEV_1 changes were zero using individual FEV_1 changes and ambient endotoxin concentrations in a regression analysis ($r=-0.56$, $p<0.05$). A correlation was found between personal changes in the number of neutrophils and personal endotoxin exposure ($r=0.26$, $p=0.05$). Furthermore, there was a dose-response relationship between endotoxin levels and the number of subjects with symptoms of byssinosis ($r=0.81$, $p<0.001$). A 'no response level' for this health effect was not calculated. At the highest endotoxin levels, workers without previous symptoms of byssinosis also experienced fever, chest tightness, and breathing difficulties at the end of the shift.

To define the relation between exposure to endotoxins and the airway response to inhaled cotton dust, Castellan and co-workers (Cas87) pooled and analyzed data from several experimental studies. The pooled data set involved a total of 108 separate sessions of exposure to dust and 32 different cottons. Each dust exposure session involved exposing a group of 24 to 35 pre-screened healthy subjects (n=33 to 61) from the general public to dust from one of the cottons for six hours in an experimental cardroom. Volunteers were selected on the absence of airway symptoms or disorders as for example asthma, bronchitis and dyspnea, and a FEV_1 of more than 80% of the predicted value. The remaining subjects were further screened on the basis of ventilatory responses during two exposures to cotton dust (1 mg/m^3 dust, 100 ng/m^3 endotoxin) and excluded when a FEV_1 decrease greater than 30% was determined. From the remaining 'healthy' group those subjects were included with a mean decrease in FEV_1 of at least 5%. The expected variation in healthy non-exposed individuals is 3 to 4%. (ATS 1979). Apart from the 108 exposure sessions, 66 sessions of exposure to clean air were included in this study. In this study the average concentration of airborne dust and endotoxins (from 108 exposure sessions) were not strongly correlated and ranged from 0.12 to 0.55 mg/m^3 and 6 to 779 ng/m^3, respectively. The group mean percentage change in FEV_1 over the exposure shift ranged from +0.5 to -9.1 %. The 95% confidence interval for the overall mean percentage change in FEV_1 for the exposure to clean air was -0.16 to +0.20 %, justifying statistical comparison of the mean response in each dust-exposure session with zero. When data from the 108 exposure sessions were pooled, the dust concentration was not correlated with the

group mean percentage change in FEV_1. In contrast, a clear exposure-response relation was observed between endotoxin concentration and group mean percentage change in FEV_1 ($r=-0.85$; $p<0.0001$). The linear regression model calculated from these data yielded a zero percentage change in FEV_1 at 9 ng/m^3. An 'effective concentration' required for 50% of the exposed population to achieve a 5 % or larger decrease in FEV_1 (EC_{50}) was calculated to be approximately 100 ng/m^3. EC_{50} for a 10% decrease in FEV_1 was suggested to be 1 μg/m^3.

The fact that the calculated threshold level in Rylander's study (33 ng/m^3) was higher than the one calculated in Castellan's study (9 ng/m^3), was consistent with the fact that the exposure sessions in Castellan's study were two hours longer than those in the study by Rylander *et al.* In addition, in Castellan's study the sensitivity was enhanced for assessment of acute airway responses by selecting subjects of a healthy population (with absence of airway symptoms) on the basis of airway responsiveness testing during preliminary screening. Furthermore, the population in Rylander's study consisted of cotton mill workers who had been occupationally exposed to the same agent for years. Long-term exposure may have caused tolerance for acute effects such as described by Greisman and Hornick (Gre69), obscuring the actual dose-response relationship. The dose-response relationship described in Rylander's study may also have been obscured by a possible 'healthy workers effect'. Both studies strongly support the hypothesis that inhaled endotoxin has a causative role in the acute pulmonary response in a dose-dependent way. It cannot be ruled out, however, that other constituents of cotton dust may also be of importance in the development of acute pulmonary effects. This was indicated by a study in which changes in lung function were demonstrated (MEF_{40}) when subjects were exposed with an endotoxin-free eluate of cotton dust (Buc86). In addition, Haglind and Rylander (Hag84) calculated thresholds for zero FEV_1 reaction of 170 ng/m^3 endotoxin from cotton dust exposure experiments for previously non-exposed non-smoking subjects (n=13) using washed cotton. The endotoxin exposure ranged from 0.08 to 12 μg/m^3. The washing procedure could have removed other components from the cotton, important in the development of acute pulmonary effects, thus resulting in a higher threshold than found in the other studies. Jamison and Lowry (Jam86), for instance, were not able to show lung function changes when subjects were provoked with endotoxin from *Enterobacter agglomerans* isolated from cotton dust using a dose of 5 and 12 μg.

In Haglind and Rylander's study (Hag84) it was demonstrated that a dose related decrease in FEV_1 was more pronounced in smoking cotton mill workers resulting in a calculated threshold of 80 ng/m^3 endotoxin vs. 170 ng/m^3 for non-smokers, suggesting an increased risk for smokers. The number of smoking subjects involved, however, was very small (n=4).

Endotoxin-related acute lung function changes as reported in the above summarized experimental studies have been confirmed in a number of field studies.

Donham and co-workers (Don89) found a relationship between endotoxin exposure and a cross-shift decrement of FEV_1 and MEF_{25} in non-smoking swine confinement workers (n=41). The mean 2 to 8 hour endotoxin exposure, characterized by area sampling of total dust, was 180 ng/m^3. A NEL of 180 ng/m^3 was estimated.

Smid and coworkers (Smi94) showed cross-shift endotoxin exposure-response trends for maximum mid-expiratory flow (MMEF) and MEF_{50} in workers in the animal feed industry (n=119). For the same lung function variables, and for FEV_1 and MEF_{25} a significant across-week change was also detected. The mean 8-hour personal inspirable endotoxin exposure was 29.3 ng/m^3 which was far below the NEL estimated in the swine confinement study. In this latter study, however, circadian rhythms were not taken into account as was done in the animal feed study, the exposure was characterized by area samples instead of personal samples, and shift duration varied from 2 to 8 hours compared to 8 hours in the animal feed study.

Sigsgaard *et al.* (Sig92) studied the relation between respiratory disorders and dust and endotoxin exposure in 253 cotton mill workers. Baseline and cross-shift lung function, symptoms and personal respirable dust were monitored. Mean 7-hour exposures ranged from 0.17 to 0.58 mg/m^3 for respirable dust and from 9 to 126 ng/m^3 (n = 21) for respirable endotoxins. A dose-response relationship between endotoxin exposure, based on three exposure categories according to current workplace, and byssinosis was found. Byssinosis was defined as chest tightness mostly on the first day of the working week. No such relationship was found with respirable dust. No statistical significant association was found for endotoxin exposure and acute work shift change in FEV_1, whereas respirable dust exposure did show a relationship with cross shift FEV_1-change. This study also showed that a significant fall in baseline FEV_1 and FVC was associated with cumulative endotoxin exposure but not with exposure to respirable dust. Cumulative exposure was calculated as the product of working years and the actual endotoxin exposure.

Milton *et al.* (Mil95, Mil96) reported a study on 130 workers in a fiberglass factory where the spray water contained endotoxins. Symptoms of fever, joint pains and other influenza-like symptoms were reported by persons exposed to higher levels of endotoxins than for persons having respiratory symptoms (Mil95). There was an endotoxin exposure-response relationship with cross-shift changes in PEF (peak expiratory flow) and PEF changes from pre-shift to arising the next day, 16 to 20 hours after the exposure (Mil96). In this study, self-reported PEF was obtained from 37 workers for a total of 181 days off work and 187 days at work. Concurrent personal exposure monitoring was performed on all working days and pre and post-shift spirometry was obtained on at least two days. An effect on cross-shift changes in FEV_1

was also suggested but was not as strong as that demonstrated for PEF (due to the study design). The fact that number of years since the start of frequent work in the area with the highest endotoxin exposure was associated with increased amplitude of PEF also suggests a long-term effect of endotoxin exposure. Area samples showed endotoxin levels in high exposed areas that ranged from 885 to 3,620 ng/m^3, but occasional values up to 27.8 μg/m^3 were also found. Personal endotoxin levels ranged from 0.4 to 759 ng/m^3.

The data from this study provided evidence that acute effects on lung function result from endotoxin exposures as low as 4 to 15 ng/m^3 (minimum and maximum of medium exposure group; geometric mean = 8.4 ng/m^3). It is important to recognize that the fiberglass wool study is the only field study showing endotoxin effects in an environment with no organic dust exposure. Interestingly, the lowest-observed-effect level (LOEL) found in this study (8.4 ng/m^3) agreed closely with thresholds for acute response determined in the cotton dust challenge studies described earlier (Cas87, Ryl85b).

Sama and co-workers (Sam94), studied 386 automotive workers exposed to machining fluids (mineral oil). They found no association between endotoxin exposure and over-shift FEV_1 decrement (≥ 4%). The mean endotoxin exposure was approximately 0.87 ng/m^3. This study suggests that acute effects may not appear at these low endotoxin levels.

A recently conducted study in the potato processing industry showed that an average exposure level above 50 EU/m^3, with individual measurements as high as 284 EU/m^3, was associated with across-shift lung function changes in FEV_1 and MMEF (Zoc98).

Physiological response

The clinical responses such as fever, chills and malaise accompanied by a dry cough, and sometimes dyspnea and headache occurring a few hours after endotoxin exposure, are caused by acute inflammation in the lung (Ryl94c). The cell mechanisms behind the inflammatory response are now fairly well understood and are based both on animal studies and experimental studies in man. The following text will give a brief overview of these inflammation processes in the lung.

In the distal portions of the lung (small bronchi, bronchioles and alveoli), the first cell to respond to endotoxin assault is the alveolar macrophage. Endotoxins either soluble or associated with particulate matter, will activate the macrophage causing the cell to produce a host of mediators including:

- lysosomal enzymes, initially directed towards the defense against bacteria (or other viable agents)

- chemotactic factors for PMNs
- membrane-derived factors such as arachidonic acids (AA) metabolites (e.g., prostaglandins, thromboxanes, leukotrienes and platelet activating factor (PAF))
- interleukin-1
- tumor necrosis factor (TNF)
- colony stimulating factor (CSF) (Jac89) (Mic95).

These mediators are generated very rapidly after exposure, in the order of a few hours and the half-life of these mediators is short, in the order of a few days (Ryl93a, Mic95). Cell activation as discussed above can take place in previously unexposed cells. Thus, one can characterize the reaction as toxic, to differentiate it from the sensitization reaction where a previous exposure has rendered the cell more sensitive or where the presence of antibodies, formed during previous exposure, is a prerequisite for the reaction to occur (Ryl94c). Endotoxins can also activate both the classical and alternative pathways of the complement system. This was demonstrated *in vitro* with human and animal serum (Mor77, Mor78) and *in vivo* (Hor84). Components of the complement system can be transported into the lung from the serum or be synthesized by pulmonary cells, such as the alveolar macrophage (Alp84).

The inflammatory mediators serve as chemo-attractants and activators of other cells. They recruit blood cells, PMNs and platelets to the interstitium and may stimulate resident cells of the airways, such as mast cells, to release mediators in a secondary response.

While endotoxins have been shown to have no direct effect on airway epithelium, mediators released from macrophage or activated serum complement may alter both the paracellular and transcellular permeability of the epithelium, thereby allowing endotoxins or other toxins in organic dust to cross this barrier and exert subepithelial effects. Furthermore, the epithelium itself may respond to this secondary stimulation by producing its own specific mediators (Jac89).

In a secondary response PMNs recruited to the airways will respond to other macrophage derived factors and to endotoxins either directly or indirectly via activated complement. Activated PMNs in turn release a host of factors which in their proper context defend the host. However, with large doses or continuous insult, PMNs have the potential to damage host tissues. In addition to releasing lytic enzymes and oxidative products, PMNs will release factors that influence other cells (e.g., platelets and endothelial cells) and cause specific reactions such as vascular or airways smooth muscle contraction. Platelets recruited to the air-blood barrier are activated by PAF, an AA metabolite. Once activated, platelets, by release of cellular metabolites can effect other cell systems and structures (endothelium, smooth muscle) which in turn become activated (Jac89).

Although it is known that endotoxins can activate complement *in vivo* and *in vitro* and there is evidence that components of the complement system play a role in endotoxin induced acute lung injury, animal experiments suggested that the complement system does not play a major role in the pulmonary response to inhaled endotoxins (Gor94).

6.2.2 *Long-term exposure*

Clearly, endotoxins have a major impact on the biology of the lung. At background levels of exposure, the above mentioned responses protect the host by inactivating the endotoxins and adapting physiology to handle the insult.

Repeated exposure to endotoxin levels higher than background level may lead to short-term tolerance of the exposed subjects resulting in less intense acute symptoms (Mer75, Gre69). However, long-term repeated exposure to levels of endotoxins not ordinarily encountered may overwhelm the body's capacity to detoxify or eliminate the endotoxins effectively and result in chronic pulmonary disease (Jac89).

It seems reasonable to presume that toxic pneumonitis and chronic bronchitis represent extremes on a scale of inflammation in the airways and the lungs, the first being acute and involving a number of tissue layers, and the second being chronic and confined to the epithelium (Ryl94c). Epidemiological and animal studies suggest that chronic exposure to endotoxin present in occupational organic dusts may indeed, most likely via chronic inflammation, lead to chronic bronchitis and reduced lung function (Ken87, Smi92b, Ell84, Don89).

The pathology of chronic bronchitis is well known: excessive growth of mucous glands and sub-epithelial glands with a mucous that is more viscous than normal. As a result of these changes, mechanical clearance of the airways, as well as their defense towards inhaled microorganisms, may be decreased. Data from animal studies suggest that these changes are also caused by inflammatory mediators where activated T-cells and eosinophils play a dominant role. There is less knowledge on which mediators are important and how the different cell systems interact (Ryl94c).

Based on the results of an experimental animal study it was hypothesized that workers continuously exposed to inhalatory endotoxins that are secondarily exposed to other pulmonary insults such as surgical manipulation, intubation, anaesthesia, hypoxia, burns or even recent exposure to endotoxins may be at increased risk for developing 'acute respiratory distress syndrome' (ARDS) (Bur90).

It is assumed that endotoxins do not play a role in IgE-mediated processes such as atopy. Endotoxins, however, may have an adjuvant activity in the development of allergic reactions such as for instance allergic alveolitis (Edw81). Adjuvants are agents that non-specifically increase the specific immune response directed to antigens and it

is known from animal experiments that LPS possess adjuvant activity (Mor79, Mor87, Wei83). It is at present not clear whether long term inhalation exposure of endotoxins may lead to an increased risk of sensitization in man to both occupational and more common allergens. However, experiments *in vitro* (Cle90a, Cle90b, Cle91, Nor86) and *in vivo* (Mic91b) showed further evidence that a certain role of endotoxins in IgE-mediated immunological processes seems likely.

6.2.3 *Epidemiological studies*

Apart from the acute effects of airborne endotoxin exposure several large epidemiological studies in the cotton industry (Ken87), the grain processing and animal feed industry (Smi92b) and the swine confinement industry (Vog98) give indications for endotoxin related chronic respiratory disorders.

Kennedy and co-authors studied the relationship between endotoxin and dust exposure and lung disease in 443 cotton workers and 439 control subjects from a silk mill. A respiratory questionnaire was administered and pre- and postshift FVC and FEV_1 were determined for each worker. Multiple area samples were analyzed for total elutriated dust concentration (0.15 - 2.5 mg/m^3) and endotoxins (2 - 550 ng/m^3). The cotton worker population was stratified by current and cumulative dust or endotoxin exposure. Groups were then compared for FEV_1, FVC, FEV_1/FVC%, percentage change in FEV_1 over the shift, and prevalences of chronic bronchitis and byssinosis. All analyses were adjusted for confounders such as age, height and smoking habits. No dose-response relationships were demonstrated comparing dust concentration to any pulmonary function or symptom variable. A dose-response trend was seen with the current endotoxin level and FEV_1, change in FEV_1 over the shift, and the prevalence of byssinosis and chronic bronchitis, 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 exposition was statistically significant for FEV_1 and was calculated to be -0.242 to -0.778 L per $\mu g/m^3$. The relation with chronic bronchitis was also significant and the calculated odds ratio was 1.7 to 2.2. No significant relations were found for change in FEV_1 over the shift or byssinosis prevalence. Dose-response relationships calculated from cumulative exposure were not as clear as those for current exposure. Cumulative exposure was estimated for each worker by assigning an exposure level to each working area and summing the products of exposure level times years employed in that area for all areas reported by the worker. The authors assumed that past exposure levels were similar to current ones. This assumption may not have been correct for many reasons and may obscure the assessment of exposure-response relationships with cumulative exposure. In contrast to the experimental studies (Cas87, Ryl85b), this study failed to show a statistically

significant correlation between current exposure and acute effects such as a cross-shift change in FEV_1 and byssinosis (a trend, however, could be observed). The most important result, however, was the authors' observation of a dose-response relationship between endotoxins and chronic lung impairment (decrease in baseline FEV_1 lung function, increased chronic bronchitis prevalence). In addition, the authors attempted to asses the presence of a threshold level of endotoxin exposure by comparing the silk workers with the cotton workers who had always worked in an area with 'low endotoxin' levels (less than 20 ng/m^3). They found no difference in baseline spirometry, but the increased prevalence of byssinosis and chronic bronchitis and the augmented cross-shift change in FEV_1 suggested, according to the authors, that even exposure to endotoxins at 1 to 20 ng/m^3 constitutes an 'adverse respiratory health effect' as defined by the American Thoracic Society guidelines (ATS85).

Smid and co-authors (Smi92b) studied the relationship between endotoxin and dust exposure and lung disease in a cross-sectional study among 315 animal feed workers working in 14 animal feed mills in the Netherlands. The study comprised monitoring dust and endotoxin exposure, spirometric lung function measurements (FVC, FEV_1, MMEF, and flow-volume parameters) and a questionnaire for respiratory symptoms. The average 8-h personal inhalable dust exposure was 9 mg/m^3 grain dust (range, 0.2 - 150 mg/m^3) and 25 ng/m^3 endotoxins (range, 0.2 - 470 ng/m^3) based on 530 personal dust measurements. On the basis of these measurements and the occupational history of the workers, the number of years 'worked in dust' and an estimate of the cumulative dust and endotoxin exposure were calculated. An external control group was selected without exposure to agents that may effect 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 trends between different endotoxin exposure categories appeared to be greater than those between dust categories. The stronger relationship for endotoxins was also indicated by similar or lower p values than those for dust exposure. This indication is even stronger when one considers that the precision of the endotoxin exposure measurements was considerably lower than the precision of dust measurements. The dose-response relation for current endotoxin exposure and FEV_1 was calculated to be -4.91 L per µg/m^3. No clear differences in symptom prevalences existed between different exposure groups for both current dust and current endotoxin exposure. In this study estimated cumulative exposure of both dust and endotoxins was significantly related to lung function impairment. No

consistent relationship existed between cumulative exposure levels and respiratory symptoms. The number of years employed in production of animal feed, however, did relate to respiratory symptoms. It was indicated that for chronic cough, chronic phlegm, and ever and frequent wheezing elevated prevalences were found in two middle exposed groups, whereas the group exposed longest reported less symptoms. The latter was probably caused by a 'healthy workers effect'. Because of the sizes of the groups, none of the odds ratios was statistically significant. The main difference between current and historic exposure in their relation with lung function in this study, was a more pronounced FVC decrease for current exposure than for historic exposure.

Smid later suggested a threshold level between 3 and 7.5 ng/m^3 based on the animal feed studies (Smi93). Both acute and chronic lung function effects were demonstrated in the intermediate exposure group (40 ng/m^3) as compared to the low exposure group (<15 ng/m^3). The upper limit of the lower exposure group was chosen as the lowest observed effect level (LOEL). It was estimated from regression models that 40 years of exposure to 15 ng/m^3 may lead to a decrease in FEV_1 of approximately 200 mL (approx. 5%) (Smi92b). For MEF_{75}, the effect would be 1200 mL (approx. 16%). This suggests that a real NEL would be below 15 ng/m^3. Taking into account selection and attenuation leading to downward bias, the author applied a safety factor on the LOEL and proposed a tentative threshold level between 3 and 7.5 ng/m^3.

A study in the swine confinement industry showed endotoxin related excess lung function decline in 171 pig farmers over a three year period. The mean decline in FEV_1 was 73 mL/yr, and a doubling of the endotoxin exposure was associated with an additional decline in FEV of 19 mL/yr (Vog98).

Two somewhat smaller epidemiological investigations also showed endotoxin related chronic respiratory effects.

Heederik *et al.* (Hee91) studied the relation between endotoxins and lung function among 183 farmers working in pig farms. Dust and endotoxin levels were determined by area sampling and the concentrations were 4.01 mg/m^3 and 130 ng/m^3, respectively. In 62 farms exposure measurements were taken in more than one stable. Base line lung function and respiratory symptoms were monitored in all farmers. The endotoxin concentration in the stables was negatively related to most lung function variables, but only for the subgroup of 62 farmers where the exposure measurements were more extensive, a statistically significant relationship was found between endotoxin exposure and FEV_1 which was calculated to be -2.08 L per μg/m^3. A borderline statistically significant and negative relationship was found between endotoxin concentration and FVC. No consistent relationship between lung function variables and dust exposure was found. A positive relationship for all farmers was found for

endotoxin exposure and respiratory symptoms experienced during or shortly after work but not for dust and symptoms.

In a study on 34 cotton mill workers, Rylander and Bergstrom (Ryl93b) studied airway responsiveness and the amount of airborne endotoxins at different work sites in the mill. Airport baggage loaders were used as controls. Endotoxin and dust levels were monitored by area sampling at various work sites in the mill. Two samples were taken at each site; one before and one after testing the subjects. The mean 3 to 4 hour endotoxin exposure ranged from 20 to 320 ng/m^3. Base line bronchial reactivity was measured prior to work, performing methacholine challenge tests. A dose-dependent relationship was found between FEV_1 decrease after methacholine administration (dose: 1.25 mg) and airborne endotoxin levels but not for dust. The authors concluded that endotoxins were associated with chronic increased bronchial reactivity in these workers.

A finding of major importance in the field studies is that chronic lung function effects were more clearly related to endotoxin exposure than to dust exposure suggesting an important role for endotoxins in the etiology of occupation related chronic lung diseases. It is, however, at present not clear whether this presumed endotoxin effect is dependent on other toxins such as for instance gossopyl and tannins present in cotton dust, or other microbial components such as glucans (mold components) and peptidoglycans (components of gram-positive bacteria) present in grain dust. Most organic dusts are heterogeneous mixtures of a great variety of components hampering the assessment of clear exposure response relations for endotoxins in epidemiologic studies such as described above. The differences in exposure-effect estimates between the animal feed worker study and the cotton worker study (10-20 fold steeper in the animal feed worker study) may be partly due to differences in composition of dust. Several other reasons for these differences might exist such as differences in sampling techniques and laboratory analysis, non linear exposure-response relationships, and variation in endotoxin toxicity. Furthermore, the exposure measurements in Smid's study were clearly more extensive than in Kennedy's study, suggesting a possible underestimation of the exposure-response relationship in the cotton-workers-study because of random errors in exposure. The exposure-response relationship between endotoxin exposure and FEV_1 decline estimated by Heederik *et al.* (Hee91) was in agreement with what was found by Smid *et al.* (-2.08 L and -4.91 L per μg/m^3 respectively). Exposure assessment and endotoxin analysis were highly comparable in both studies. Because of above mentioned reasons it is very difficult to determine a NEL from these epidemiologic studies. Important however in the establishment of an OEL for endotoxins, are the observations in these studies that chronic lung function impairment is demonstrated to be related with endotoxin exposure at similar levels as

described for acute effects in experimental studies. The exposure limits suggested in both studies, which are both less than 20 ng/m^3 are in remarkable agreement with the experimental results discussed in 6.2.1.

6.3 Summary

Endotoxins have been recognized as very important components in the etiology of occupational lung diseases caused by inhalatory exposure of organic dust. Intravenous administration of LPS in man elicited clinical symptoms consisting of joint aches and fever, shivering, other influenza-like symptoms and leucocytosis. Symptoms disappeared the next day. Inhalation experiments with LPS showed the following acute clinical effects in human volunteers: fever, shivering, joint aches, influenza like symptoms (malaise), dry cough, dyspnea, chest tightness and leucocytosis. Exposed subjects also showed a dose-related acute lung function decrease (FVC, FEV_1 and flow-volume variables), acute decreased lung diffusion capacity and acute bronchial obstruction. Acute lung function effects and respiratory symptoms found in experimental studies where subjects were exposed to cotton dust were confirmed by several field studies, conducted in swine confinement workers, animal feed workers, fiberglass factory workers and cotton mill workers. Two large epidemiological studies, one conducted in the cotton industry and one in the animal feed industry, reported dose-related chronic effects such as decreased FEV_1, FVC, flow-volume variables and respiratory symptoms. Two other, somewhat smaller studies, also showed endotoxin related chronic respiratory effects in swine confinement workers and cotton mill workers.

In both the experimental and the field studies NELs were calculated based on pulmonary effects. These calculated NELs and suggested threshold levels are summarized in table 2.

In the literature it is hypothesized that chronic inhalatory endotoxin exposure may increase non-specifically the immune response to antigens in man (adjuvant effect). There is, however, at present no direct proof that supports this hypothesis.

Table 2 Calculated and suggested no effect and threshold levels for occupational endotoxin exposure presented in the literature published in the period 1980-1995.

source	calculated no effect level based on FEV_1 decreases	suggested threshold level	study design	population	exposure duration in hours	dust origin
Haglind 1984	170 ng/m^3 [a]	-	experimental exposure, acute effects	- non smoking non selected subjects (university students)	?	cotton
	80 ng/m^3 [a]			- smoking cotton mill workers		
Rylander 1985b	33 ng/m^3 [a]	-	experimental exposure, acute effects	cotton mill workers	4	cotton
Castellan 1987	9 ng/m^3 [a]	-	experimental exposure, acute effects	non symptomatic subjects from general population	6	cotton
Palchak 1988	-	30 ng/m^3	literature review	-		-
Milton 1989	-	0.3-3 ng/m^3	literature review	-		-
Kennedy 1987	<1-20 ng/m^3 [b]	-	epidemiological, acute and chronic effects	cotton mill workers	approx. 8	cotton
Donham 1989	180 ng/m^3 [a]	-	epidemiological, acute effects	non smoking swine confinement workers	2-8	pig faeces and feed
Smid 1992, 1993	<15 ng/m^3 [a]	3-7.5 ng/m^3	epidemiological, acute and chronic effects	animal feed workers	approx. 8	grain
Milton 1995, 1996	<8.4 ng/m^3 [c]		epidemiological, acute and chronic effects	glass wool manufacturing workers	4	recycled wash water

[a] based on decline in FEV_1

[b] based on increased chronic bronchitis and byssinosis, and acute decline in FEV_1

[c] based on acute decline in PEF

Chapter 7

Existing guidelines, standards and evaluations

Only one previous evaluation on occupational endotoxin exposure was conducted by an international organization. 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 (Ryl89b). As an example, the report states that organic dust toxic syndrome (ODTS) is elicited at a level of 1000 to 2000 ng/m^3, while acute bronchoconstriction occurs at levels of 100 to 200 ng/m^3, and mucous membrane irritation (MMI) at levels of 20 to 50 ng/m^3. The report states that these levels may be lower for sensitive subjects.

Chapter 8

Hazard assessment

8.1 Assessment of health hazard

Airborne endotoxin exposure, such as may occur in certain occupational settings, has convincingly been shown to generate biological and clinical effects in man. The lungs appear to be the main target organ in which these adverse effects occur. As mentioned in chapter 7 no exposure limits for endotoxin exposure were determined by official bodies, but some research groups have calculated no effect levels (NELs) or suggested threshold levels for airborne endotoxin exposure, based on acute and chronic pulmonary effects in man. Table 2 presents the different NELs and thresholds published during the last decade. More specific information regarding these studies is given in chapter 6.

The studies summarized in table 2 are all based on respiratory effects measured by acute or chronic decrease in FEV_1 which appears to be the most important and most sensitive health effect. At present, it is not clear whether other lung function parameters (e.g. FVC, MEF, MMEF, diffusion capacity) or clinical parameters or symptoms (e.g. leucocytosis, fever, cough, bronchial hyperreactivity) might be more sensitive indicators of adverse effects of inhalatory endotoxin exposure. A decrease in FEV_1, however, is known to be the parameter most consistently affected by endotoxin exposure. In addition, small decrements (100-200 mL) in FEV_1 were proven to be a sensitive indicator of respiratory impairment (chronic non-specific lung diseases, CNSLD) and mortality (Ann86, Ebi89, Hee92) Therefore, the committee will base its

recommendations for an occupational exposure limit (OEL) for endotoxins on a decrease in FEV_1.

All NELs summarized in table 2 were calculated using linear statistical models and are comparable with the exception of the result of one experimental study which yielded a much higher value, viz. 170 ng/m^3 for non-smokers and 80 ng/m^3 for smokers (Hag84) as compared to 9 to 33 ng/m^3 in the other studies. Washing procedures of the cotton used in Haglind's exposure study might be an explanation of this great difference. Furthermore, the number of subjects used in this study was very small (13) compared to that in the other studies. The differences between the other studies can probably be explained by different exposure times (4, 6 or 8 hours) and by differences in population selection, dust sampling, LAL-methods to quantify endotoxins and endotoxin origin (different gram-negative bacteria).

The calculated NELs have serious limitations. In the first place the linear statistical models on which they are based are not expected to represent precise estimates of the exposure-response relation at either very high or very low doses. Sigmoidal semi-log plots similar to many typical biologic dose-response relations are more likely to represent the real dose-response relation for endotoxins. Assuming the dose-response relation to be linear may result in over- or underestimation of the actual NEL.

Secondly, all NELs are based on studies where subjects were exposed to non-purified endotoxins. Other factors, present in the complex mixture of components that grain or cotton dust is composed of, may have influenced the observed dose-response relationship. It is unlikely that endotoxins are merely a marker for effects induced by other components, as experiments using purified LPS revealed similar effects.

Third, exposure experiments were conducted on healthy persons. It is known from other studies that particular groups, such as subjects with airway diseases, have an increased risk to develop pulmonary effects after endotoxin exposure. Exposure experiments with healthy persons may give no information on the dose-response relationship for these critical groups.

Fourth, the NEL may be dependent on the type of endotoxin as it is known that there are differences in toxic potency between endotoxins derived from different bacterial species. Furthermore, all endotoxin concentrations were expressed in ng/m^3 instead of Endotoxin Units/m^3 in these studies and no standard laboratory methods for endotoxin sampling, extraction and analysis were available. Expressing the OEL in EU/m^3 is an absolute necessity because there are large differences in the hydrophilic and the lipid A moiety between endotoxins of different species or strains (chapter 2.3). In order to express the NEL in EU/m^3, the values can be multiplied by a factor 10 which is, however, a rough approximation of the real conversion factor which can range from approximately 7 to 17.

The committee suggests that the OEL for endotoxins should be based on the avoidance of 'acute and chronic airway effects'. The limitations of the calculated NELs require a conservative interpretation. This means that these NELs should be interpreted as if endotoxin is the only causal factor in the development of acute and chronic airway effects. The calculated NELs summarized in table 2 range from 9 to 80 ng/m^3. The best studies both in terms of study design and statistical precision were Castellan's experimental study (Cas87) and Smid's epidemiological study (Smi92b), revealing calculated NELs of 90 and less than 150 EU/m^3 based on respectively acute and chronic pulmonary effects*. The other studies showed similar effects but lacked statistical power to accurately estimate a NEL. Smid and co-authors showed that long-term exposure to 150 EU/m^3 may still lead to chronic pulmonary effects (decrease in FEV_1 and flow volume variables) suggesting a NEL that may be considerably lower. As mentioned before small decrements in FEV_1 (100-200 mL) are proven to be a sensitive indicator of respiratory impairment and mortality. Assuming that a 100 mL decrease in FEV_1 over a 40 years working period, which is on average equivalent to a 9% excess decrease (Qua93), is a relevant effect, the committee calculated, using the effect estimate for FEV_1 response and cumulative endotoxin exposure obtained by Smid *et al.* (-0.34 L/(year.µg/m^3), Smi92b), that this effect occurs at an exposure level of 74 EU/m^3.

Based on these calculations, an OEL below 74 EU/m^3 should be recommended to provide a safe level below which no relevant chronic respiratory effects should occur for subjects working up to 40 years in an exposed environment. This value of 74 EU/m^3 is in close agreement with the 90 EU/m^3 NEL calculated by Castellan *et al.* (Cas87) for acute FEV_1 decline. However, selection effects and statistical attenuation may have biased the exposure-response relationship observed in the epidemiological study. The latter value (90 EU/m^3) is, therefore, likely to represent the most accurate and precise estimate of all proposed no effect values. In addition, this study was conducted using not previously exposed subjects from the general population rather than long-term exposed workers as were used in all other studies. Calculated dose-response relationships and related NELs may, apart from selection effects and statistical attenuation, also have been obscured by possible tolerance in working populations that were used in the other studies. Therefore, the committee bases its derivation of an OEL on the 90 EU/m^3 from Castellan's study. Taking into account that this NEL was based on 'healthy' subjects and 6 hour exposures, a safety factor should be applied to account for increased risks of certain groups of workers (see 8.2) and for 8 hour exposures. Also taking into account that endotoxin may have chronic pulmonary effects at levels which may be lower than for acute pulmonary effects,

* For these studies and all others a conversion factor of 10 (1 ng = 10 EU) for conversion of nanograms to Endotoxin Units is applied.

which can not be ruled out by the epidemiological studies focusing on chronic effects (Ken87, Smi92b, Hee91, Ryl93b), the committee decided to apply a safety factor of 2. The committee is aware of data indicating that subjects with an atopic or allergic status might be more susceptible to endotoxin exposure than other workers (Pre95, Mic92b). However, these studies have some shortcomings like very high exposure levels (more than 1000 EU/m^3) and the lack of a control group of non-asthmatic persons. It is expected that at exposure levels around the proposed standard differences in response between atopics and non-atopics or asthmatics and non-asthmatics will be relatively small. Therefore, the committee is of the opinion that a safety factor larger than 2 is not justified.

The committee recommends a health based occupational exposure limit of 45 EU/m^3 which is rounded off to 50 EU/m^3 (approximately equal to 5 ng/m^3).

8.2 Groups at extra risk

Groups suffering from chronic non-specific lung diseases (CNSLD), and more specific, groups suffering from asthma and bronchitis 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.

8.3 Health-based recommended occupational exposure limit

The committee recommends a health-based occupational exposure limit for endotoxin of 50 EU/m^3, which is approximately 5 ng/m^3 using a conversion factor of 10, based on personal inhalable dust exposure, measured as an eight hour time weighted average.

Chapter 9

Recommendations for research

Future research conducting exposure experiments in man using pure LPS to establish both dose-response relationships for acute pulmonary effects and NELs for airborne endotoxin (LPS) exposure is recommended. This research should focus at low level exposures (e.g. 10 - 300 EU), preferably using several endotoxin types from different bacterial strains and mixes of these different endotoxin types. In addition, large longitudinal epidemiological studies are recommended in order to establish more insight in both chronic effects caused by long-term airborne endotoxin exposure and the underlying processes of these effects. Chronic effects should focus on respiratory effects and a possible adjuvant effect which may non-specifically potentiate the immune response to antigens (including allergens) in man. This adjuvant effect may increase the risk to develop (respiratory) allergies. In addition, acute respiratory effects can be studied by performing overshift lung function tests. This research should be conducted in one or more industries knowing to represent a broad range in individual endotoxin exposures.

Furthermore, an international Round Robin test for endotoxin analyses and prior extraction of dust samples should be conducted, in order to gain insight in possible differences in endotoxin exposure assessment between different research groups using different protocols for endotoxin measurements. Such a study should result in recommendations for rigorous standardization of the endotoxin measurement method used in occupational hygiene including sampling, extraction and analyses methods.

For the committee,
Rijswijk, 1 September 1998

dr CA Bouwman,
scientific secretary

prof. dr VJ Feron,
chairman

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A Request for advice

B The committee

C Comments on the public review draft

D Abbreviations

E DECOS-documents

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 committee

- VJ Feron, *chairman*
  professor of toxicology; TNO Nutrition and Food Research Institute, Zeist
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- DJJ Heederik
  epidemiologist; Agricultural University, Wageningen
- PTh Henderson
  professor of toxicology; University Limburg, Maastricht
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- G de Jong
  occupational physician; Shell International Petroleum Maatschappij, The Hague
- G de Mik
  toxicologist; National Institute of Public Health and the Environment, Bilthoven
- J Molier-Bloot
  occupational physician; BMD Akers bv, Amsterdam
- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, Rijswijk

- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- HG Verschuuren
  toxicologist; DOW Europe, Horgen (Switzerland) (retired)
- AAE Wibowo
  toxicologist; Coronel Institute, Amsterdam
- F de Wit
  occupational physician; Labour Inspectorate, Arnhem
- CA Bouwman, *scientific secretary*
  Health Council of the Netherlands, Rijswijk
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Rijswijk

The first draft of the present advisory report was prepared by ir J Douwes and dr ir DJJ Heederik, from the Department of Environmental Sciences, Environmental and Occupational Health Group of the Agricultural University of Wageningen, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance was provided by E Vandenbussche-Parméus.
Lay-out: J van Kan.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 1997 for public review. The following organisations and persons have commented on the draft document:

- dr EC Rietveld
  Akzo Nobel, Diosynth bv, The Netherlands
- ir TMM Coenen
  Gist Brocades bv, The Netherlands
- ir Nic CA van den Brink
  Vereniging van Afvalverwerkers, The Netherlands

Annex **D**

Abbreviations

bp	boiling point
EC_{50}	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
HBR-OEL	health based recommended occupational exposure limit
h	hour
IC_{50}	concentration at wAbbreviationshich inhibition of a certain function is found up to 50% of the control value
LC_{50}	lethal concentration for 50% of the exposed animals
LC_{lo}	lowest lethal concentration
LD_{50}	lethal dose for 50% of the exposed animals
LD_{lo}	lowest lethal dose
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAEL	minimal adverse effect level
MAK	Maximale Arbeitsplatz Konzentration
MOAEL	minimal observed adverse effect level
MTD	maximum tolerated dose
NAEL	no adverse effect level
NEL	no effect level
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
PEL	permissible exposure limit
ppb	parts per billion (v/v)10^{-9}
ppm	parts per million (v/v)10^{-6}
RD_{50}	concentration at which a 50% decrease of respiratory rate is observed
REL	recommended exposure limit

STEL	short term exposure limit
tgg	tijd gewogen gemiddelde
TLV	threshold limit value
TWA	time weighted average
V_{max}	maximal reaction velocity of an enzyme

Organisations

ACGIH	American Conference of Governmental Industrial Hygienists
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Standards
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
INRS	Institut National de Recherche et de Sécurité (France)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Association (USA)
RTECS	Registry of Toxic Effects of Chemical Substances
SER	Social and Economic Council (Sociaal-Economische Raad NL)
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organisation

Toxicological terms

bid	*bis in diem* (twice per day)
bw	body weight
CARA	chronic non-specific respiratory diseases
CHD	coronary heart disease
CNS	central nervous system
ECG	electrocardiogram
EEG	electro encephalogram
FCA	Freunds Complete Adjuvans
FEV	forced expiratory volume
FSH	follicle stimulating hormone
GD	gestation day(s)
GPMT	guinea pig maximisation test
GSH	glutathione
HLiA	hamster liver activated
IHD	ischaemic heart disease
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratracheal
iv	intravenous
LH	lutheinising hormone
MAC	minimal alveolar concentration

MFO	mixed function oxidase
NA	not activated
PNS	peripheral nervous system
po	*per os* (= oral)
RBC	red blood cells
RLiA	rat liver activated
SCE	sister chromatid exchange
sc	subcutaneous
UDS	unscheduled DNA-synthesis

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	relative risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Analytical methods

AAS	atomic absorption spectroscopy
BEEL	biological equivalent exposure limit
BEI	biological exposure index
BEM	biological effect monitoring
BM	biological monitoring
ECD	electron capture detector
EM	environmental monitoring
FID	flame ionisation detector
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
HPLC	high performance liquid chromatography
IR	infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAS	personal air sampling
TLC	thin layer chromatography
UV	ultraviolet

Additional abbreviations in the present report

AA	arachadonic acid
CSE	control standard endotoxin
CNSLD	chronic non-specific lung-diseases
EU	endotoxin units
FVC	forced vital capacity
LAL	limulus amebocyte lysate
LPS	lipopolysaccharides
MEF	maximum expiratory flow
MMEF	maximum mid-expiratory flow

MMI	mucous membrane irritation
ODTS	organic dust toxic syndrome
PAF	platelet activating factor
PEF	peak expiratory flow
PMN	polymorphonuclear leucocytes

Annex **E**

DECOS-documents

DECOS has produced documents on the following substances.

To be ordered from the Health Council of the Netherlands:

Acetone cyanohydrin	1995/05WGD
p-Aramid fibres	1997/07WGD
Bisphenol A and its diglycidylether	1996/02WGD
Butanol (1,2- and t-)	1994/10
Cadmium and inorganic cadmium compounds	1995/04WGD
Calculating cancer risk	1995/06WGD
Carbon disulphide	1994/08
Chlorine dioxide	1995/07WGD
Chromium and its inorganic compounds	1998/01WGD
1,2-Dichloroethane	1997/01WGD
Diphenylamine	1997/05WGD
1,2-Ethanediamine	1996/03WGD
Ethyleneglycol ethers	1996/01WGD
Formamide and dimethylformamide	1995/08WGD
Hydrazinoethanol, phenylhydrazine, isoniazid, maleic hydrazide	1997/03WGD
Isopropyl acetate	1997/04WGD
Man made mineral fibers	1995/02WGD
Methyl Methacrylate	1994/09
Methacrylates. Ethyl methacrylate, n-butyl methacrylate and isobutyl methacrylate	1994/11
Methyl-t-butylether	1994/23
Methyl chloride	1995/01WGD
Pentaerythritol	1997/06WGD
Phenol	1996/04WGD

Piperidine	1997/08WGD
Propanol (1- and 2-)	1994/24
Propylene oxide	1997/02WGD
Quartz	1998/02WGD
Thrichloroethane (-1,1,1)	1995/03WGD
Trichloropropane (1,2,3-)	1994/25

The following documents, that were published before 1994, can be ordered from the Sdu Uitgeverij Den Haag.

Acetaldehyde	RA 6/92
Acrylaten	RA 13/87
Aflatoxine B1, B2, G1 en G2	RA 6/87
Allylglycidylether	RA 1/92
Amyl acetate	RA 4/90
Aniline	RA 2/89
Anorganisch Lood	RA 2/80
Anorganische Kwikzouten	RA 3/82
Arc welding fume particles not containing chromium and nikkel	RA 1/93
Arseenverbindingen (anorganische)	RA 2/84
Asbest	RA 1/84
Asbest, Evaluatie van risico op kanker bij beroepshalve blootstelling aan (aanvullend op RA 1/84)	RA 9/89
Benzeen	RA 5/89
Beryllium and beryllium compounds	RA 4/88
Blootstelling, Gezondheidskundige aspecten van het begrip en van het meten/schatten ervan	RA 8/90
Butadiene (1,3-)	RA 5/90
Cadmium	RA 5/80
Caprolactam	RA 4/84
Carbon monoxide	RA 7/92
Carbonylfluoride and PTFE pyrolysis products	RA 3/88
Carcinogene stoffen	RA 3/80
Chloor	RA 6/80
Chloroform	RA 7/87
ß-Chloroprene	RA 4/93
Chroom en chroomverbindingen	RA 6/85
Cyclohexane	RA 15/90
Cyclohexanol	RA 3/90
Cyclohexanone	RA 9/93
Dibroomethaan	RA 5/87
Dichloorethaan (1,1-)	RA 8/87
Diisocyanates	RA 3/91
Dimethyl- en diethylsulfaat	RA 12/90
Dimethylamine	RA 10/90
Dimethylbutane (2,2- & 2,3-)	RA 7/93
Dimethylhydrazine	RA 2/87

Dinitro-*ortho*-cresol (4,6-)	RA 4/87
Dioxaan (1,4-)	RA 1/87
Epichloorhydrine	RA 1/86
Ethylacetate	RA 10/91
Ethylacrylate	RA 6/90
Ethyl Methanesulphonate (EMS)	RA 4/89
Ethylamine	RA 7/90
Ethylbenzene	RA 9/91
Ethyleenoxide	RA 6/89
Fenylhydrazine	RA 2/87
Fluorcarbons (except FC11)	RA 15/87
Fluorine compounds (inorganic)	RA 1/89
Fluorine	RA 1/89
Formaldehyde	RA 3/87
Fosfine	RA 1/80
Fijn hinderlijk stof; gezondheidskundige aspecten van bijlage 3 bij de Nationale MAC-lijst 1989	RA 9/90
Gasoline	RA 3/92
Heptaan (n-)	RA 1/81
Heptane (n-)	RA 6/93
Hexaan (n-)	RA 11/87
Hexachlorobenzene	RA 2/88
Hexanone (2-)	RA 2/90
Hydrazine	RA 2/87
Hydrogenfluoride	RA 1/89
Hydroxyethylhydrazine	RA 12/87
Isopropylglycidylether	RA 1/92
Isopropoxyethanol (2-)	RA 2/87
Koolmonoxide (Carbon monoxide)	RA 2/79 (7/92)
Kwikalkylverbindingen - Korte keten	RA 5/82
Kwikverbindingen (Organische)	RA 4/82
Lachgas (Nitrous oxide)	RA 2/85 (2/92)
Lasrook (Arc welding fume.....nickel)	RA 1/93
Mangaan	RA 1/82
Metallisch Kwik	RA 5/81
1-Methoxypropanol-2	RA 5/93
2-Methoxypropanol-1	RA 5/93
1-Methoxypropylacetate-2	RA 5/93
2-Methoxypropylacetate-1	RA 5/93
Methylacrylate	RA 1/90
Methyleenchloride (Methylene chloride)	RA 1/83 (8/92)
Methyl ethyl ketone	RA 16/90
Methyl isobutyl ketone	RA 4/91
Methyl Methanesulphonate (MMS)	RA 4/89
Methylbromine	RA 13/90
Methylpentane (2- & 3-)	RA 7/93
Monochloorethaan	RA 2/82
Monoketones (7/8 carbon chain aliphatic)	RA 14/90

Nikkel en nikkelverbindingen	RA 3/85
Nitropropaan (2-)	RA 1/85
Nitrous oxide	RA 2/92
Ozone	RA 4/92
para-Dichloorbenzeen	RA 1/88
Pentaan	RA 2/81
Phthalate esters	RA 8/93
Phthalic anhydride	RA 3/89
Piperazine	RA 7/91
Polyvinyl chloride (PVC) dust	RA 2/93
Propoxyethanol (2-)	RA 12/87
Propoxyethylacetate (2-)	RA 12/87
Pyridine	RA 3/93
Selenium en -verbindingen	RA 7/89
Silicon dioxide, crystalline forms of	RA 5/92
Stikstofdioxide (Nitrogen dioxide)	RA 5/85
Styreen	RA 8/89
Talc dusts	RA 6/91
Tetrahydrofuran	RA 1/91
Thiourea	RA 11/90
Tolueen diisocyanaat	RA 4/80
Tolueen	RA 2/91
Trichloorethaan (1, 1, 1-)	RA 3/81
Trichloorethyleen	RA 3/83
Trichlorofluoromethane	RA 14/87
Triethylamine	RA 2/83
Trimethylamine	RA 9/87
Vanadium metaal en anorganische verbindingen	RA 10/87
Wood dust	RA 8/91
Xylene	RA 5/91
Zwaveldioxide (sulphur dioxide)	RA 4/85