KEYNOTE REVIEWS AAEM

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TOWARDS AN OCCUPATIONAL EXPOSURE LIMIT FOR ENDOTOXINS ?*

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Abstract: Endotoxins are part of the outer membrane of Gram-negative bacteria and are present ubiquitously in the environment. The lipopolysaccharide (LPS) part of the molecule is responsible for its toxic properties. Environmental monitoring is usually performed by sampling airborne dust and subsequent analysis of aqueous extracts by using a LAL assay. The kinetic version of this assay can measure concentrations as low as in the pg/m^3 range. A generally accepted protocol is not yet available. Endotoxin levels are related to the occurrence of Gram-negative bacteria. Animal faeces and bacteria contaminated plant materials contribute most to organic dust related endotoxin exposure. Endotoxin exposure is therefore most prevalent in agricultural and related industries. Acute health effects are dry cough and shortness of breath accompanied by a decrease in lung function, fever reaction and malaise, and sometimes dyspnea and headaches occurring a few hours after the start of the endotoxin exposure. Epidemiological and experimental studies suggest that chronic endotoxin exposure may lead to chronic bronchitis and reduced lung function. Depending of the relevant health effect, no effect levels range from 9 to 170 ng/m^3. The Dutch Expert Committee on Occupational Standards (DECOS) of the National Health Council proposed a health based recommended limit value of 50 Endotoxin Units/m^3 (4.5 ng/m^3) over an 8 hour exposure period.

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INTRODUCTION

At the request of the Minister of Social Affair and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for ambient air concentrations of toxic substances at the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS). They constitute the first, and scientific, stage in a three-stage procedure that leads to legally binding limit values.

In this paper part of the public draft DECOS report is presented that recommends a health-based occupational exposure limit [4]. The committee's conclusions are based on scientific publications prior to April 1996.

OCCURRENCE, PHYSICAL AND CHEMICAL PROPERTIES

Endotoxins are part of the outer membrane of Gram-negative bacteria and are composed of proteins, lipids and lipopolysaccharides. Lipopolysaccharides (LPS) of Gram-negative bacteria refer to a class of pure lipid

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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 stable water soluble molecules 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 to organic dust related endotoxin exposure. Occupational endotoxin exposure is therefore most prevalent in agricultural and related industries.

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 [e.g. 6]. DECOS recommends personal or area samplers to collect total or inhalable dust using glass fibre filters, sample storage below -18°C with the avoidance of repeated freezing and thawing of samples, extraction in pyrogen free water with 0.05% tween-20, and analysis with a chromogenic kinetic or turbidimetric LAL assay.

The LAL-assay was adapted as the standard assay for endotoxin detection by the American Food and Drug Association 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 ≈ 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 [1]. This has not been evaluated in experimental studies. Good correlations between endotoxin levels as measured with the LAL test and acute health effects exist [13].

Since different LAL 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. 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 researchers still publish endotoxin levels in nanograms rather than in Endotoxin Units.

CURRENT LIMIT VALUES

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

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 mucocilliary transport, while endotoxin deposited in the deeper airways is assumed to be eliminated by macrophage and polymorphonuclear leucocyte phagocytosis.

EFFECTS

Acute health effects after inhalation of endotoxin are dry cough and shortness of breath accompanied with a decrease in lung function, fever reactions and malaise, and sometimes dyspnea, headache and joint aches occurring a few hours after the exposure (see for a review by Rylander [13]). 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 nonspecific 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 [11].

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.

HAZARD ASSESSMENT

In the literature 'No Effect Levels' for inhalatory endotoxin exposure have been calculated ranging from 170 to 9 ng/m^3 (approximately 1700–90 EU/m^3) based mainly on experimental endotoxin exposure studies [2, 3, 12, 13]. Calculated 'No Effect Levels' for chronic and

acute respiratory effects based on epidemiological studies in occupationally exposed populations were comparable [5, 9, 10, 14, 16, 17]. Starting point for the establishment of a health based-recommended occupational exposure limit by the committee is the No Observed Adverse Effect Level (NOAEL) of 9 ng/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. Other studies showed similar effects but lacked statistical power to accurately estimate a 'No Effect Level'. 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 observed for acute respiratory effects [7, 8, 15].

RECOMMENDED OCCUPATIONAL EXPOSURE LIMIT

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit for airborne endotoxin of 50 Endotoxin Units/m^3 (approximately 4.5 ng/m^3) based on personal inhalable dust exposure measured as eight hour time weighted average in the most recent version of their draft report. This report can be commented by the scientific community. A final version of the report, including a final proposal for a health-based exposure limit is expected to be published in the autumn of 1997.

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