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Endotoxin in metal working fluid (MWF) mist

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Endotoxin in metal working fluid (MWF) mist

Dr Helena Senior, Dr Christopher Barber and **Dr Gareth Evans**
Health and Safety Laboratory
Harpur Hill
Buxton
Derbyshire SK17 9JN

The risks to respiratory health from exposure to bacterial endotoxins are well established. The aims of this research were to:

- Review the evidence used to develop the health based recommended occupational exposure limit (HBROEL) for endotoxin of 90 endotoxin units per cubic metre (EU/m3) over an 8-hour period proposed by the Health Council for the Netherlands (DECOS); and to assess its relevance as a 'benchmark' to assess risks to respiratory health caused by endotoxin in metal working fluid mists.

- Assess whether the published evidence on endotoxin concentration in metal working fluids provides sufficient evidence that concentrations in mist are sufficient to cause harm to human health

The research concluded that there was a large discrepancy between concentrations of endotoxin and viable bacteria in mist compared to the concentrations in bulk fluid with airborne endotoxin levels generally falling close to or beneath the DECOS recommended level , whilst sump levels generally exceeded these by 100 to 1000 fold. Levels of viable bacteria captured in air were low compared to the levels in the sumps.

Further research is required to determine whether the discrepancy between bulk endotoxin and airborne levels is real or whether this is due to the impact of sampling or analytical methodology

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HSE Books

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ABBREVIATIONS

CFU	Colony forming unit
COPD	Chronic obstructive pulmonary disease
DECOS	Dutch Expert Committee on Occupational Safety
EU	Endotoxin units
FEV1	Forced expiratory volume in 1 second
GC-MS	Gas chromatography mass spectroscopy
HBROEL	Health based recommended occupational exposure limit
HSE	Health & Safety Executive
HSL	Health & Safety Laboratory
KLARE	Kinetic Limulus assay with resistant parallel line estimation
LAL	Limulus amoebocyte lysis assay
LEV	Local exhaust ventilation
LPS	Lipopolysaccharide
m^3	Cubic metre
MAT	Monocyte activation test
mg	Milligram
ml	Millilitre
MWF	Metalworking fluid
NIOSH	National Institute for Occupational Safety and Health
OEL	Occupational exposure limit
OSHA	Occupational Safety and Health Administration (US)
OTDS	Organic toxic dust syndrome
REL	Recommended exposure limit
TWA	Time-weighted average
UEIL	Independent Union of the European Lubricants Industry
VOC	Volatile organic compound

EXECUTIVE SUMMARY

Water-miscible metalworking fluids (MWFs) become colonised by bacteria living in the environment if not maintained properly. The growth of bacteria is often accompanied by a decline in their nutrients and subsequent death leading to the release of toxins (e.g., endotoxin) from some types of bacteria. These toxins are potent hazards causing inflammation within the airways at concentrations as low as nanograms per cubic metre of air.

The risks to respiratory health from exposure to bacterial endotoxins are well established and have been subject to numerous reviews of the evidence. Inhalation of endotoxin has been linked with acute and chronic health effects caused by an inflammatory response within the airways.

Current HSE advice to duty holders is that they should control bacterial levels in water-miscible MWFs below 10^3 colony forming units / ml (cfu / ml) and take remedial action against the accumulation of viable bacterial when numbers rise up to 10^6 cfu / ml of fluid. This guidance also emphasises the importance of controlling respiratory exposure to mists of MWFs that derive from the machining processes. These concerns reflect evidence that poor management of water-miscible MWF can lead to the accumulation of large numbers of bacteria, fungi, and other reactive chemical residues and that these hazards may be subsequently aerosolised and inhaled. This link has been reinforced by many investigations of outbreaks of respiratory disease associated with use of water-miscible MWFs and where MWF mist was considered a contributory factor. These concerns apply to poorly managed conventional water-miscible MWFs as well as to new types of fluid designed to encourage the growth of specific bacteria to prevent the growth of more harmful species.

How good is the evidence linking the levels of biohazards in MWF sumps to the concentrations in mist that may be inhaled? Many studies of exposure to MWFs have reported on sump levels of micro-organisms or chemicals; other studies have reported levels in mist (based on personal or static sampling). It was less clear how many studies had examined machine sump levels and compared them to airborne exposures surrounding the machine. Another knowledge gap related to the levels of endotoxin in air measured in the mist samples and whether these were likely to provoke airway inflammation. The objectives of this study were therefore to:

- Review the evidence used to develop the health based recommended occupational exposure limit (HBROEL) for endotoxin of 90 endotoxin units per cubic metre (EU / m^3) over an 8-hour period proposed by the Health Council for the Netherlands (DECOS); and to assess its relevance as a ‘benchmark’ to assess risks to respiratory health caused by endotoxin in metal working fluid mists.

- Assess whether the published evidence on endotoxin concentration in metal working fluids provides sufficient evidence that concentrations in mist are sufficient to cause harm to human health.

Findings:

A critical review of the DECOS study was undertaken to assess the validity of the recommended HBROEL of 90 EU / m^3. The following conclusions were made:

- The DECOS HBROEL 90 EU / m^3 was mainly based on the results of a single volunteer human exposure study.
- For ethical reasons subjects with pre-existing disease that could have been exacerbated by exposure to endotoxin were excluded and therefore this health based limit may not protect all workers. There is evidence that levels of endotoxin lower than 90 EU / m^3 can cause inflammation in the airways of some workers.

Following a search of peer-reviewed publications and National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluation reports, only 19 papers were found to report endotoxin concentrations measured in air and corresponding bulk fluid. Some studies included measurements for airborne bacteria and mist mass. The quality of these studies was critically reviewed and data on endotoxin concentrations and mist mass were summarised to establish what range of endotoxin concentrations had been measured compared to those found in the sump MWF. The following conclusions were made:

- There was a large discrepancy between concentrations of endotoxin and viable bacteria in mist compared to the concentrations in bulk fluid with airborne endotoxin levels generally falling close to or beneath the DECOS recommended level of 90 EU / m^3, whilst sump levels generally exceeded these by 100 to 1000 fold.
- Levels of viable bacteria captured in air were low compared to the levels in the sumps.
- Despite these studies using different methods to determine the mass of airborne mist, most measurements were below 1.0 mg / m^3, which was the previous guidance limit that applied in the UK based on measurement of boron content.

Further research is required to determine whether the discrepancy between bulk endotoxin and airborne levels is real or whether this is due to the impact of sampling or analytical methodology. However, if levels of mist, airborne bacteria and airborne endotoxin are low it may be necessary to consider what combination of other factors may be contributing to the respiratory problems that occur with exposure to water-miscible MWF mist.

1.0 INTRODUCTION

1.1 Background

Water-miscible metalworking fluids (MWFs) by their nature become colonised by bacteria living in the environment if not maintained properly. The majority of MWF adapted bacteria belong to the *Pseudomonas* family and are Gram negative meaning their surface consists of a double surface membrane. HSE recommend that bacterial levels are kept below 10^3 colony forming units / ml (cfu / ml) but levels often increase in excess of 10^6 cfu / ml. Bacteria die off naturally due to limited availability of nutrients in the MWF and are killed by the addition of biocide and other additives in an endeavour to manage the MWF quality. Once cell death occurs, the outer surface membrane breaks down releasing immunologically active proteins and toxins into the MWF. The main one of concern, due to its known link to respiratory ill health, is endotoxin.

1.2 Endotoxin

Endotoxins are composed of proteins, lipids and lipopolysaccharides (LPSs) that are found in the outer membranes of Gram-negative bacteria. Inhalation of endotoxin has been linked with acute and chronic health effects, caused by the lungs inflammatory response to this agent. Evidence to support this has come from studies of human volunteers inhaling either endotoxin or nebulised LPS, and studies of workers exposed to endotoxin predominantly from animal faeces (e.g. poultry and swine workers) or contaminated plant material (e.g. cotton workers). Certain of these industries have traditionally been linked with very high exposure levels to endotoxin, with mean levels of exposure commonly measured in the 10,000-100,000 endotoxin units per cubic metre (EU / m^3) range (Liebers *et al*, 2006).

1.2.1 Acute Effects

The acute effects of inhaling endotoxin are a combination of respiratory and flu-like symptoms, typified by the organic dust toxic syndrome (ODTS). This usually occurs several hours after a high exposure to a dust contaminated with endotoxin, resulting in cough, breathlessness, chest tightness, shivering, and joint aches. These symptoms are self-limiting, and typically improve through the working week as tolerance develops. Following a break from exposure, such as a weekend, this tolerance is lost, resulting in recurrence of symptoms on return to work. This pattern of disease has long been recognised in brown lung disease, a chronic asthma like restriction of the airways associated with cotton workers. In addition to symptoms, occupational endotoxin exposure may result in acute falls in lung function, usually demonstrated by cross-shift measurements of Forced expiratory volume in 1 second (FEV1) or peak flow (Rylander *et al*, 1985; Castellan *et al*, 1987). Human volunteer studies have demonstrated a marked variability in this type of acute response, with some individuals experiencing marked airway narrowing at low doses of inhaled LPS, and others not showing any response at the maximum dose delivered (Kline *et al*, 1999). There is some evidence to support a genetic basis for an individual's endotoxin-responsiveness (Michel *et al*, 2003).

1.2.2 Chronic Effects

Chronic occupational exposure to high levels of endotoxin may also result in respiratory symptoms, particularly a chronic productive cough due to chronic bronchitis (Kennedy *et al,* 1987; Kirychuk *et al*, 2006). Longitudinal workplace studies have also investigated the risk of accelerated lung function decline in groups of exposed workers, with the subsequent risk of developing chronic obstructive pulmonary disease (COPD). Such longitudinal studies may be prone to survivor bias, under-estimating the size of any effect if those with the highest decline in lung function leave the industry due to ill health. Although findings have been variable, a number of these have used regression models in an attempt to derive equations to predict average FEV1 decline based on the level of endotoxin and years of exposure (Kennedy *et al*, 1987; Post *et al*, 1998). These studies have produced relatively modest estimates of excess FEV1 decline of 0.0052 and 0.0340 ml per year, respectively. In these models, the expected 25-30 ml a year expected loss due to aging in non-smokers would equate to working for a year in an environment of 1000-6000 EU / m^3.

1.2.3 Occupational exposure limit

In July 2010 the Dutch Expert Committee on Occupational Safety (DECOS) recommended a health based occupational exposure limit (HBROEL) for endotoxin of 90 EU / m^3 over an 8-hour period, after an extensive review of the evidence-base (Health Council of the Netherlands, 2010). The DECOS review summarised the evidence from human volunteer, and workplace studies that have measured immunological responses due to endotoxin in biological samples such as blood, sputum, or broncho-alveolar or nasal lavage fluid. The LPS volunteer studies have demonstrated responses, but have used relatively high exposure levels between 1000 (Peden *et al*, 1999) to 1000,000 EU / m^3 (Thorn, 2001). Workplace studies have however documented immune responses in nasal lavage fluid at much lower average exposures, between 13-39 EU / m^3, in waste handlers chronically exposed to endotoxin (Wouters *et al*, 2002; Heldal *et al*, 2003).

1.3 Project Justification

The risks to respiratory health from exposure to Gram-negative bacterial endotoxins are well established and have been subject to numerous reviews of the evidence (Donham *et al*, 1989; Donham *et al*, 2000; Heldal *et al*, 2003; Kirychuk *et al*, 2006, Liebers *et al*, 2006). Current HSE advice to duty holders is that they should ideally keep bacterial levels in water-miscible MWFs below 10^3 colony forming units / ml (cfu / ml) taking remedial action against the accumulation of viable bacterial numbers up to 10^6 cfu / ml of fluid. Above this level, the MWF should be replenished (COSHH Essentials "Managing sumps and bacterial contamination" MW5 http://www.hse.gov.uk/metalworking/ecoshh.htm). This guidance also emphasises the importance of controlling respiratory exposure to mists of MWFs that derive from the machining processes. These concerns reflect evidence that poor management of water-miscible MWF can lead to the accumulation of large numbers of bacteria, fungi, and other reactive chemical residues and that these hazards may be subsequently aerosolised and inhaled. This link has been reinforced by many investigations of outbreaks of respiratory disease associated with use of water-miscible MWFs (Burton *et al,* 2012) and where MWF mist was considered a contributory factor. These concerns apply to poorly managed conventional water-miscible MWFs, bio-stable fluids, and bioconcept fluids.

HSE recognised that there is a knowledge gap about potential airborne exposure to endotoxin in mists of MWF generated by machining operations. This information is needed to ensure that if stringent controls are required for controlling exposure to mists of MWF these can be justified in terms of evidence.

Aims: To determine whether levels of bacterial endotoxin in mists derived from water-miscible MWFs (conventional, long-life, and bioconcept) pose a risk to respiratory health.

Objectives:

- To review the findings of the DECOS recommendation for the HBROEL for endotoxin of 90 EU / m^3 over an 8-hour period; and to assess its relevance as a benchmark to assess risks to respiratory health caused by endotoxin in MWF mists.
- To assess whether the published evidence on endotoxin concentration in MWFs provides sufficient evidence that concentrations in mist are sufficient to cause harm to human health.

2.0 METHODOLOGY

2.1 Assessment of DECOS report

DECOS produced a substantial review of occupational endotoxin exposure including studies in which the hazardous effects of endotoxin had been studied using experimental models and human volunteer studies. The purpose behind assessing this document was to consider the findings and the recommended HBROEL of 90 EU / m^3 in terms of its relevance to endotoxin exposure in MWFs. This also included assessing whether the HBROEL could be used as a benchmark to assess whether reported concentrations of endotoxin in MWF mists exceeded this value. The assessment of the DECOS review was undertaken by Dr Chris Barber, Centre of Workplace Health HSL, who is a respiratory physician and member of the national Group of Occupational Respiratory Disease Specialists (GORDS) and the British Thoracic Society Occupational Lung Disease Specialist Advisory Group.

2.2 Peer-reviewed evidence of airborne endotoxin in water-miscible MWF mist

2.2.1 Literature search

The aim of this literature search was to find research studies where airborne (mist) and sump measurements of endotoxin had been taken along with information about estimated mass of exposure to MWF and other measures of bacteria numbers. The key requirements were for studies to have measured endotoxin in both air and bulk fluids.

HSL staff identified appropriate search words and associated synonyms (see Annex 7.1) and in consultation with the HSE information service team constructed an appropriate search matrix (see Table 1). The searches were carried out by combining each term in list one with each term in list two or three. The searches were based upon proximity of the terms irrespective of their order within the document but they had to be no more than five words apart.

The searches were completed using OSHROM (HSELINE, NIOSHTIC, CISDOC, RILOSH and OSHLINE) databases, and using online PubMed, GoPubMed, ToxNet and Web of Science databases between 1990 and 2011.

A total of 125 references were added to an Endnote reference database and the titles and abstracts reviewed by three HSL staff. Relevant abstracts were identified and sifted on the basis of the specific links between these topics. The published studies were selected on the basis of the priority topics (see Table 2), specifically the measurement of endotoxin in MWF (both airborne and sumps). Studies reporting only sump concentrations were not included. Once this final criterion had been applied there were only 19 studies published during this period that contained data on exposure to endotoxin both in mist (either personal or static samples or both) and in the bulk fluids.

2.2.2 Quality sift of relevant studies

Principles applied in systematic reviews were used to rank the quality of the published studies to assess the robustness of the findings. The first stage of this involved data extraction forms (Annex 7.2). This form contains a series of requirements used by each reviewer to identify information relevant to the research questions.

- Have endotoxin concentrations been measured in airborne MWF mist as well as in the bulk MWFs?
- Are the levels of endotoxin in airborne mist higher than levels defined by DECOS as the threshold for adverse health outcomes?
- Are high airborne endotoxin measurements supported by other measures of microbial exposure such as numbers of bacteria or mass of MWF mist?

Table 1: Search terms employed in the sift and number of associated papers

Combined search terms			**Number of references**
Endotoxin	+ Mist	+Metal Working Fluid (MWF)	1
Endotoxin	+ Mist	+Sump	1
Endotoxin	+ Mist	+ Water-miscible	1
Endotoxin	+ Mist	+ Water Mix	1
Endotoxin	+ Mist	+ Semi-synthetic	1
Endotoxin	+ Mist	+ Machining	3
Endotoxin	+ Mist	+ Metal Removal Fluid	0
Endotoxin	+ Mist	+ MWF	8
Endotoxin	+ Mist	+ Lubricant	1
Endotoxin	+ Mist	+ Machining Fluid	3
Endotoxin	+ Mist	+ Cutting Fluid	0
Endotoxin	+ Mist	+ Tank	0
Endotoxin	+ Mist	+ Reservoir	0
Endotoxin	+ Mist	+ Exhaust	0
Endotoxin	+ Mist	+ Ventilation	1
Endotoxin	+ Mist	+ Local Exhaust Ventilation	0
Endotoxin	+ Mist		17
Endotoxin	+ Machining		9
Endotoxin	+ Aerosol	+ Machining	8
Endotoxin	+ Aerosol	+ MWF	16
Endotoxin	+ Aerosol	+ Metal Working Fluid	6
Endotoxin	+ Aerosol	+ Metal Removal Fluid	0
Endotoxin	+ Aerosol	+ Lubricant	2
Endotoxin	+ Aerosol	+ Coolant	0
Endotoxin	+ Aerosol	+ Machining Fluid	6
Endotoxin	+ Aerosol	+ Cutting Fluid	0
Endotoxin	+ Aerosol	+ Sump	4

Combined search terms			Number of references
Endotoxin	+ Aerosol	+ Tank	1
Endotoxin	+ Aerosol	+ Reservoir	0
Endotoxin	+ Aerosol	+ Exhaust	9
Endotoxin	+ Aerosol	+ Local Exhaust Ventilation (LEV)	1
Endotoxin	+ Aerosol	+ Ventilation	35
Endotoxin	+ Aerosol	+ LEV	0
Endotoxin	+ Aerosol	+ Water-miscible	0
Endotoxin	+ Aerosol	+ Water Mix	1
Endotoxin	+ Aerosol	+ Semi-synthetic	1
Endotoxin	+ Aerosol	+ Milling	1
Endotoxin	+ Aerosol	+ Grinding	3
Endotoxin	+ Aerosol	+ Turning	2
Endotoxin	+ Aerosol	+ Inhalable	21
Endotoxin	+ Aerosol	+ Respirable	14

The reviews of the papers selected as relevant were assessed by two reviewers who conducted the data extraction and applied a quality score to each study independently (see Annex 7.3). Three different scores were applied based on the type of research on worker exposure to endotoxin and bacteria in mists of MWFs. At the last stage of this assessment, both reviewers met to discuss their scores and overall view of each study. Where there was a discrepancy in the scores allocated to the same paper, the data extraction tables were considered in full and a consensus score agreed.

2.2.3 Summary of data

Due to the small number of studies containing evidence about the concentrations of endotoxin in bulk fluids and corresponding air (and their variable quality) it was decided not to pool the data for further analysis. The results therefore were summarised as provided by the source papers without further modification. For some studies, this involved single measurements or for multiple pooled measurements central estimates (e.g., arithmetic and geometric means and medians), with some studies providing estimates of uncertainty (e.g., confidence intervals or estimated error values).

Table 2: Criteria used to assess the quality of the published studies

Questions that were applied to the published studies
Primary purpose of study
Is the main focus on endotoxins or another parameter (e.g., bacteria or dust)?
Was the study an investigation of ill health or hygiene research?
Type of exposure sampling
Were bulk fluid and air samples collected at the same point?
If air samples were collected, were they personal or fixed static samples?
Was airborne exposure to mist assessed by total mass, a marker (e.g., boron) or particle counting?
Was the method of determining airborne exposure based on inhalable, thoracic or respirable fractions?
Were bacterial concentrations determined?
Was the duration of sampling time short (minutes) or long (hours)?
Methodology and data analysis
Is enough detail provided to understand the study design, sampling methods, and data analysis?
How is the data summarised (are individual data points provided or only summary statistics)?
Was the study of sufficient size to provide a robust assessment of exposure (e.g., numbers of samples and variables)?
Were samples taken in replicate?
Other supporting questions
Was the study longitudinal?
Was it focused on a particular type of machining?
Were multiples types of MWF in use where the samples were collected?

3.0 RESULTS

3.1 Assessment of DECOS review of Endotoxin Exposure

3.1.1 Basis for exposure limit

A number of studies of endotoxin-exposed workers have attempted to determine a threshold level of exposure by logistic regression, below which no significant cross-shift fall in lung function would be expected. This was around 330 EU / m^3 for cotton workers for 4-hour exposures (Rylander *et al,* 1985), 1800 EU / m^3 for swine workers with 2-8 hour exposures (Donham *et al*., 1989), and 614 EU/m^3 for poultry workers with 2-4 hour exposures (Donham *et al*, 2000). DECOS selected the level of 90 EU / m^3 for an 8-hour work period as a health based exposure limit, based on evidence that this would prevent acute symptoms at work and cross-shift fall in FEV1 responses. The DECOS review stated that they had not chosen a lower exposure limit that would prevent demonstrable immune responses, as they felt that some of these would not be associated with health effects.

Evidence to support the 90 EU / m^3 level came predominantly from a study of healthy volunteers exposed experimentally to cotton dust (Castellan *et al*, 1987). They selected individuals who had falls in FEV1 between 5-30% after exposures of 1000 EU / m^3, and exposed them to endotoxin levels ranging from 60-7790 EU / m^3 for six-hour periods. Using a regression model, they then calculated that there would be a zero fall in FEV1 for 6-hour exposures at 90 EU / m^3. It should be noted however that the volunteers in this study had been carefully pre-selected, excluding those with asthma, chronic bronchitis, exertional breathlessness, baseline FEV1 < 80%, or any fall in FEV1 > 30% during the screening cotton exposure. The DECOS state that an exposure limit of 90 EU / m^3 over an 8-hour period (as opposed to 6 hours in Castellan *et al*, 1987) should therefore protect all workers, as the study volunteers were pre-selected as being sensitive to endotoxin. This assumption is however likely to be limited, as Castellan *et al*, (1987) screened out the most sensitive individuals from participating in the study. Any volunteer who had an acute fall in FEV1 of over 30% during the pre-screening exposure to 1000 EU / m^3 was felt to be ineligible due to safety concerns. Given the additional exclusion of volunteers with pre-existing respiratory conditions, the evidence-base for the exposure limit is also not applicable to those with asthma or COPD. The final limitation to consider is that Castellan *et al,* (1987) noted that the level of FEV1 response might have been higher if individuals had been asked to exercise during the exposures, as would expected to be the case in the cotton industry. The basis for the DECOS no effect limit is therefore based on experimental inhalation challenge tests on volunteers rather than data from workplace studies.

Once DECOS had selected a level of exposure to endotoxin that would prevent acute workplace effects, they considered how the chronic effects in terms of how this would translate into loss of lung function over a 40-year working life. They calculated that 40 years of exposure at 90 EU / m^3 would equate on average to an extra 120 ml loss of FEV1, a level they felt would not equate to ill health in an otherwise healthy worker. This is in keeping with estimates from another study, where a predicted excess fall of 200 ml of FEV1 was calculated for a 40 year working exposure at 150 EU / m^3 (Smid *et al*, 1994). To put this into context, the DECOS estimate would represent an excess decline of around a tenth of the normal age-related FEV1 decline over that period of approximately 1000-1200 ml.

3.2 Summary of published endotoxin exposure data in MWFs

3.2.1 Overall quality of the evidence

Of the 125 papers that contained key words relevant to the search criteria, only 19 contained measurements of endotoxin in the air and MWF sumps. Within this group one unpublished paper was included as this contained data on the concentrations of endotoxin in bioconcept fluid. The studies were published between 1996 and 2010 (for summary, see Annex 7.4). Based on an assessment of the study quality, three were ranked as high, seven of medium and ten of low quality. For the medium and low quality studies, the implication was that due to the design or execution of the study, confounding and biases were likely to add uncertainty to the results. The categorisation of these studies did not suggest that some studies were under or over reporting exposure and overall the estimates obtained were broadly comparable across the studies.

Five of the studies were based on health incident investigations mostly in the United States and undertaken by the National Institute for Occupational Safety and Health (NIOSH). These health investigations were not specifically designed to address endotoxin exposure but undertaken to investigate causes of ill health. Others studies included hygiene surveys as well as research about control interventions for mist. There were no narrative literature reviews, systematic reviews or meta-analysis of studies that had measured exposure to endotoxin in MWF mists.

The types of plants where measurements were undertaken varied from car manufacture, machining of titanium and nickel alloys, grinding, milling and machine casting. The majority of studies were based on a single site visit, but two studies involved multiple site visits over more than a year. The numbers of samples varied from a few to over 250 in larger studies. The types of MWF considered were all water-miscible MWF emulsions but in some studies comparisons were made between mineral oil and synthetic MWFs.

Wide differences in study design and methodology used to sample and assess exposure to MWF mist, microbial numbers and microbial constituents such as endotoxin was immediately apparent. These were such that direct comparison or pooling of the data was not possible and so the exposure measurement data (mass, bacterial numbers, and endotoxin) were reported without any further adjustment or analysis. The type of air sampling undertaken included personal and static sampling, with most of the personal samples based on a sampling rate of 2.0 litres per minute. The choice of sample collection devices included staged impaction devices, closed faced cassettes, and IOM samplers. The choice of filters used in the air samplers varied from polycarbonate, Teflon, and glass fibre. The details of any measurement of MWF mass and extraction and analysis of endotoxin concentrations were only provided in detail by some studies.

3.2.2 Mist Exposure

Only eight studies made an assessment of personal exposure to MWF mist and six also included static air measurements in accordance with national guidance or enforcement levels. The means of expressing the exposure to MWF mist varied, with some studies providing total inhalable mass fractions and others thoracic particulate mass. At the time these studies were undertaken, the Occupational Safety and Health Administration (OSHA) total inhalable Occupational Exposure Limit (OEL) of 5.0 mg / m^3 and the NIOSH recommended thoracic particulate mass exposure limit (REL) of 0.4 mg / m^3 applied. For studies conducted in the UK, the guidance value of 1.0 mg / m^3, based on the boron elemental marker, applied until 2005. For the studies from Finland, the OEL value of 5.0 mg / m^3 applied.

Mist measurements are summarised in Figure 1. These were collated from studies (see Annex 7.4) investigating exposure to MWF mists in engineering plants using water-miscible MWFs. The individual points represent either single or central estimates of exposure. However, it needs to be borne in mind that these estimates were made using different sampling and analytical measurements. Despite this, more than half of the values suggest exposure levels of less than 1.0 mg / m^3, with a smaller number suggesting mist exposure as high as 10.0 mg / m^3. This general trend applied to both personal and static area samples.

3.2.3 Exposure to endotoxin

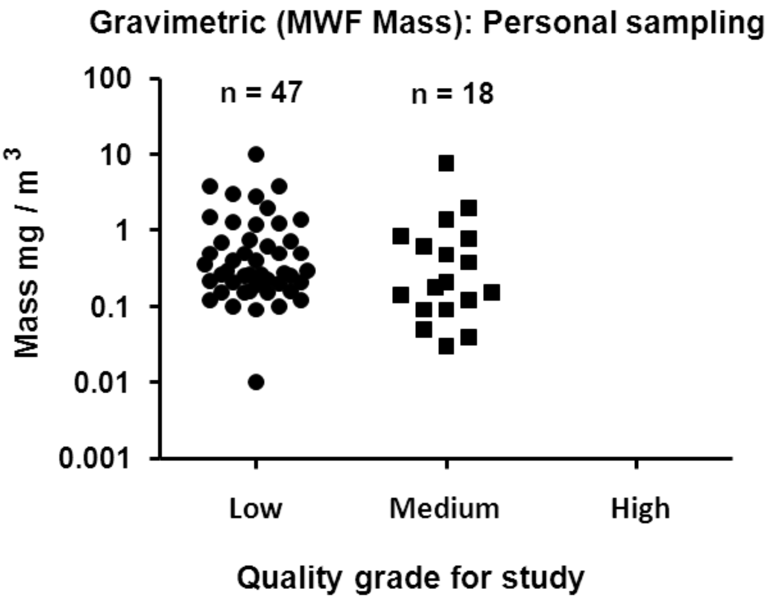
In most cases, the analysis of endotoxin was undertaken using the standard international method the Limulus amoebocyte lysate (LAL) assay, but some studies used the more advanced Kinetic Limulus Assay with Resistant-parallel-line Estimation (KLARE) procedure. One study measured total endotoxin using 3-hydroxy fatty acid measurements by gas chromatography mass spectroscopy (GC-MS) and another study quantified total pyrogens using the monocyte activation test (MAT).

Concentrations of endotoxin in the personal breathing zone and within bulk fluids are summarised in Figure 2. The data was collated from studies (see Annex 7.4) investigating exposure to MWF mists in engineering plants using water-miscible MWFs. These estimates of endotoxin were based on different sampling measurements; despite this most of the exposures were less than the HBROEL of 90 EU / m^3 set by DECOS with only a minority of the samples containing endotoxin at higher levels. This also applied to the static samples (summarised in Figure 4 upper panel) where the levels of endotoxin were generally well below the HBROEL. These results were in contrast to the concentrations of endotoxin in the bulk MWF samples which were mostly in excess of 1000 EU/ml and ranged from as little as 10.0 to >100,000 EU / ml.

3.2.4 Exposure to viable bacteria

Data was collated from studies (see Annex 7.4) investigating exposure to MWF mists in engineering plants using water-miscible MWFs. Only one study made an assessment of personal airborne exposure to viable bacteria and only three studies made area measurements of viable bacteria in mist. No national bodies have set guidance or exposure limits for bacterial numbers in mist although HSE guidance recommends that viable bacterial numbers should be kept below 10^3 cfu / ml in MWF sumps. Figure 3 summaries the results for personal airborne exposure and concentrations in bulk fluids. Most personal air samples contained about a thousand to a few thousand viable bacteria for each cubic metre of air sampled. In the static samples (Figure 4 lower panel) the numbers were lower, typically less than 1000 cfu / m^3. This is in contrast to the estimated number of viable bacteria in the bulk MWF samples which were generally in excess of 100,000 cfu / ml (ranging from ~10 to >10^8 cfu / ml).

Figure 1: Summary of mist measurements. The upper panel shows the results for personal sampling and the lower panel results for static sampling. The data sets are organised on the basis of quality of the study (low, medium or high). Individual points either represent single estimates or central estimates (means or medians).



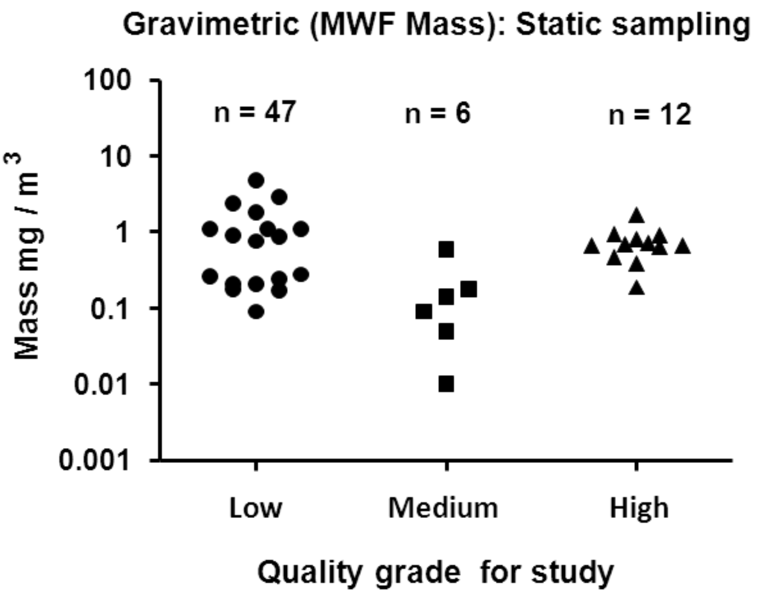
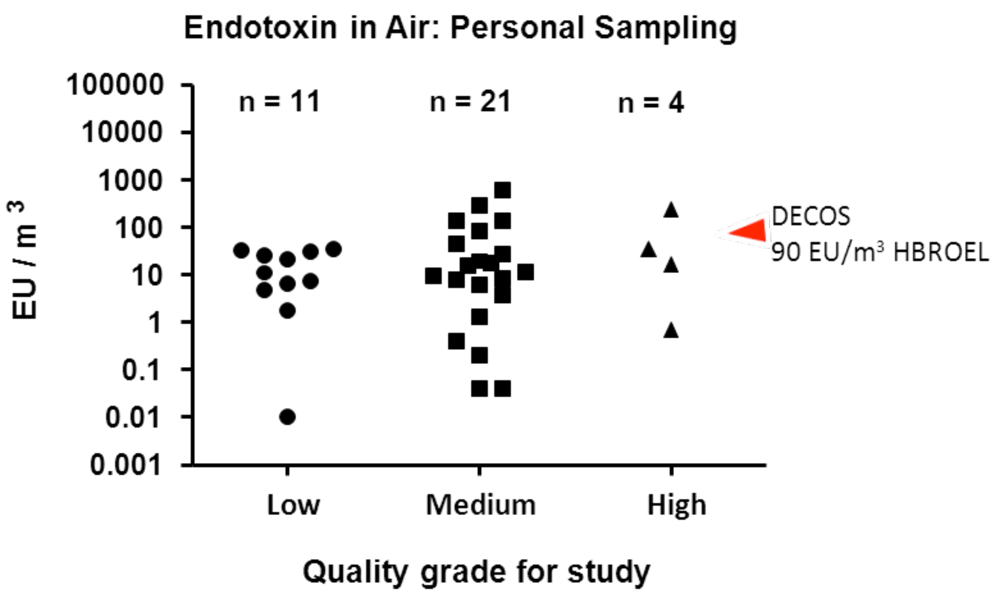


Figure 2: Summary of endotoxin concentrations. The upper panel shows the results for personal sampling and the lower panel results for sump sampling. The data sets are organised on the basis of quality of the study (low, medium or high). The individual points either represent single estimates or central estimates (means or medians). The red arrow marks the value of the proposed DECOS HBROEL of 90 EU / m^3.



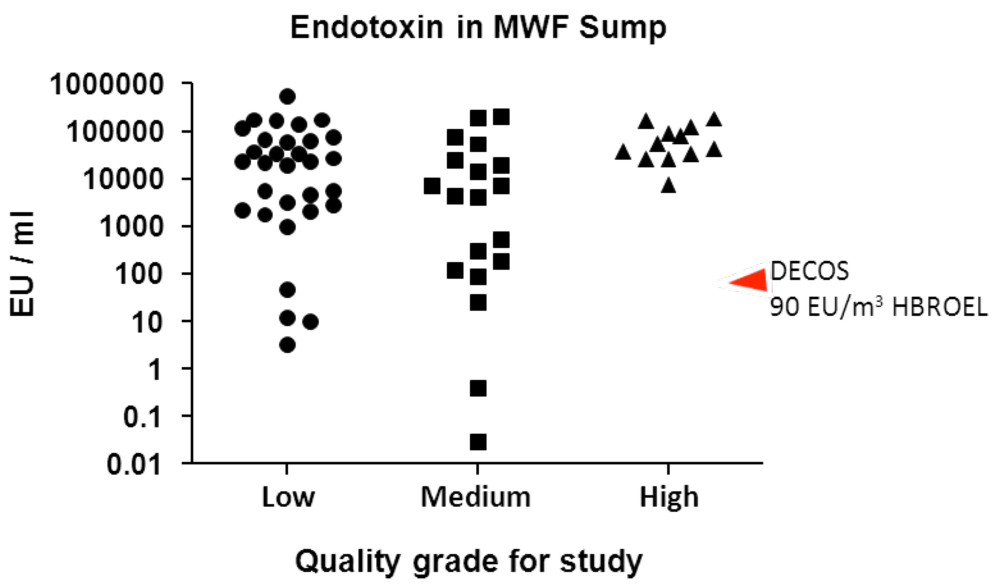


Figure 3: Summary of bacterial concentrations. The upper panel shows the results for personal sampling and the lower panel results for bulk fluids. The data sets are organised on the basis of quality of the study (low, medium or high). The individual points either represent single estimates or central estimates (means or medians).

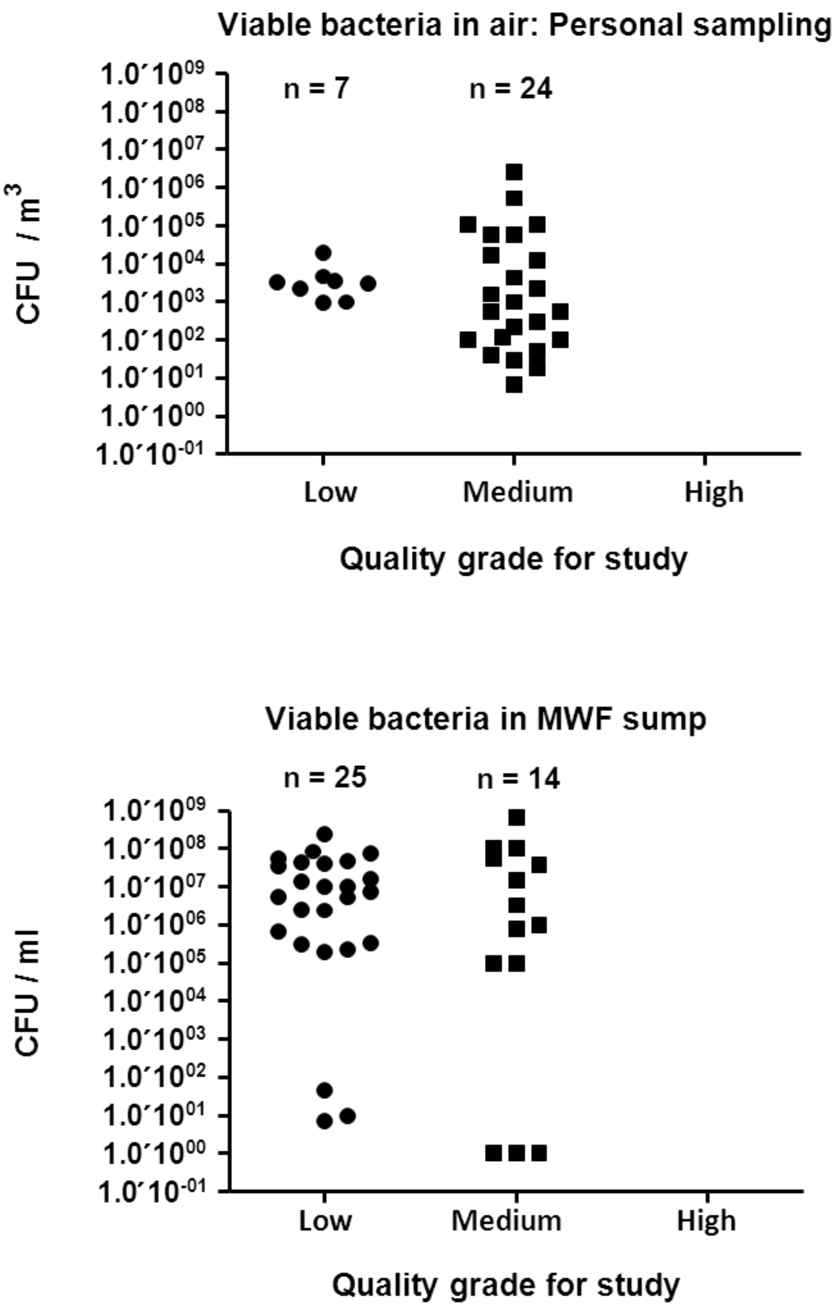
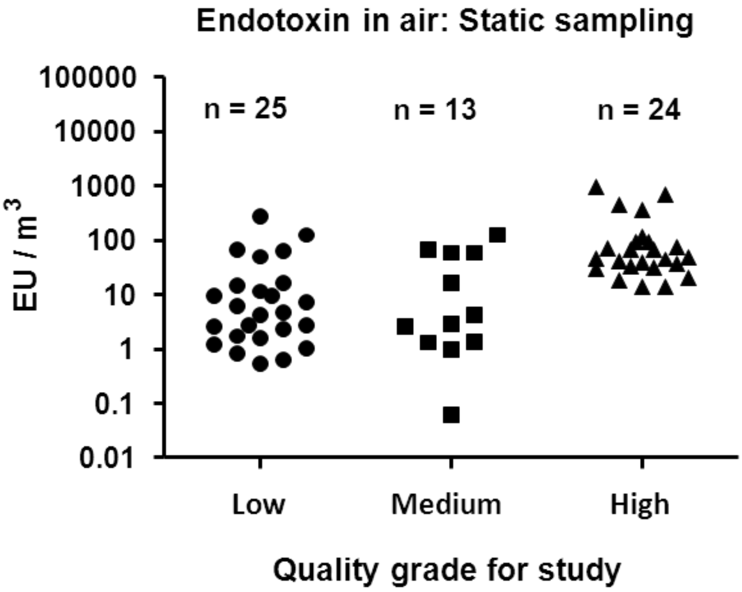
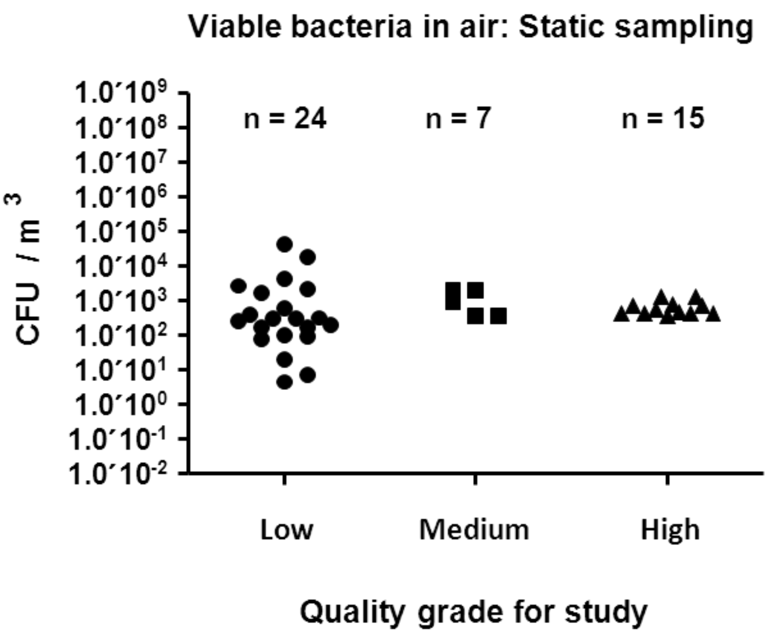


Figure 4: Summary of static sampling data. The upper panel shows the results for airborne endotoxin and the lower panel results for airborne viable bacteria. The data sets are organised on the basis of quality of the study (low, medium or high). The individual points either represent single estimates or central estimates (means or medians).





4.0 DISCUSSION

4.1 The relevance of the DECOS HBROEL for endotoxin

The DECOS review represents a comprehensive summary of the evidence relating to occupational and experimental endotoxin exposure. It highlights the difficulties of selecting a health based exposure limit based on the results of historical studies, but DECOS nevertheless opted for a limit of 90 EU / m^3. The review considered evidence from a large number of occupational exposure scenarios relevant to the UK. Most of these addressed exposure scenarios where endotoxin concentrations are typically high. For example, agriculture, storage of dusty organic material, cotton dust and recycling and disposal of organic waste. The DECOS review included a small number of studies on exposure to endotoxin in MWFs.

The critical studies used to recommend a HBROEL for endotoxin were not based on workplace studies but human volunteer exposure challenge studies. These were designed to establish a dose relationship between inhaled endotoxin and the onset, prevalence, and severity of symptoms. The specific study used to derive the HBROEL came from a challenge study reported by Castellan *et al*, (1987). This investigated exposure to cotton dust containing a known concentration of endotoxin. The authors considered their selection of 90 EU / m^3 for the HBROEL should protect all workers. However, in their selection of recruits they screened out (for ethical reasons) the most sensitive individuals to ensure no adverse reactions. Therefore, the DECOS limit may underrepresent the lowest adverse effect concentrations of endotoxin.

This caveat suggests that for workers with a predisposition for endotoxin sensitivity, a level of 90 EU / m^3 may not be protective. Whilst it is likely that the proposed HBROEL will protect the majority of workers from endotoxin-related symptoms, there is evidence that this level of endotoxin is capable of inducing inflammatory responses. Given the basis for the limit, concerns remain as to whether it will adequately protect all exposed workers, particularly those with a genetic predisposition to endotoxin responses (Michel *et al*, 2003), and those with existing airway conditions such as asthma (Michel *et al*, 1989). The DECOS review also considered a number of other human challenge studies in addition to that of Castellan *et al,* (1987). These included estimates of the threshold levels of adverse effect of 330 EU / m^3 for cotton workers (Rylander *et al,* 1985), 1800 EU / m^3 for swine workers (Donham *et al*, 1989), and 614 EU / m^3 for poultry workers (Donham *et al*, 2000). Taken together, these suggest that for airborne exposures to endotoxin lasting more than a few hours and in excess of ~100 EU / m^3, there is sufficient evidence for concern about respiratory function and health. These effects include respiratory inflammation and progressive declines in lung function exceeding those expected from normal aging.

The question is whether the DECOS HBROEL is a suitable benchmark to assess risks to respiratory health in MWF mists? Whilst the HBROEL is based on a challenge study to cotton dust, most of the other exposure scenarios that DECOS considered (including MWFs) are complex. The impact of endotoxin has to be considered in relation to other hazards present in used MWF mists and vapours. This includes chemical, volatile organic compounds, other microbial toxins, and metal ultra-fines (White & Lucke, 2003; Cohen & White, 2006). Zucker & Fluri (unpublished[1]) used the human MAT to assess levels of bacterial pyrogens, not just endotoxin levels, in bioconcept and conventional MWFs. Pyrogens are constituents of bacteria that cause fever and include endotoxin as well as exotoxins. It was shown that the pro-inflammatory effects of one type of 'used' MWF could be mostly abrogated by Polymixin B an inhibitor of endotoxin. This suggests that most of the pro-inflammatory effects of the 'used' MWF on isolated human cells were in this case related to endotoxin.

4.2 Published Exposure Data

A literature search has been undertaken to evaluate studies where airborne (mist) and sump measurements of endotoxin have been taken along with supporting data for MWF mist exposure and numbers of viable bacteria. The key requirements were for studies to have measured endotoxin in both air and sump. A number of important caveats had to be applied to the summary of relevant literature. The first was the small number of qualifying studies. The sufficiency of this evidence is further undermined by the variable quality of these studies and inconsistencies in their design and methodology to quantify exposure. Another limitation was the small number of studies that examined bacterial numbers in bulk fluids and air samples.

The present summary excluded studies that considered only the endotoxin content of MWF sumps. These studies are more substantial in number and many report large numbers of bacteria and endotoxin in poorly managed water mix MWFs. This has led many experts to conclude that levels of bacteria and endotoxin in MWF mist are likely to be high (and therefore a risk to health) since the corresponding sump levels are high. This lies behind concerns expressed in government and industry guidance about the need to control exposure to mists based on the assumption that they contain high concentrations of micro-organisms and endotoxin. The evidence supporting this assumption has not been questioned previously and therefore has been considered here.

The main finding arising from this evaluation of the evidence is that there is a large discrepancy between concentrations of endotoxin and viable bacteria in mist compared to the concentrations in bulk fluid. Taken at face value, this suggests that endotoxin concentrations in mist generally fall close or beneath the HBROEL of 90 EU / m^3 raising uncertainty about the proposed risks to health. The relatively low numbers of viable bacteria (100-1000 cfu / m^3) in MWF mist samples are also at odds with the large numbers reported in the corresponding sumps. The data also suggests that the levels of mist collected were correspondingly lower than international enforcement or guidance values (i.e., those applied in the US and Europe or historically in the UK).

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[1] Zucker B & Fluri A. Characterising the pro-inflammatory potential of bactericide-free and preserved coolant-lubricants and their aerosols at workplaces in the metalworking industry (unpublished).

However, all of these comparisons need to be treated with caution since the measurement of each parameter was obtained using different endpoints and measurement techniques. For example, most studies only report numbers of viable bacteria but this may underrepresent the total number of bacteria (i.e., alive and dead).

Areas of uncertainty, with respect to methodology, relate to the varying means of capturing the mist such as the type of sampling head and filter as well as the analytical assay employed. The extent of recovery from a filter can vary upon many factors and different researchers have employed the addition of a variety of extraction methods. For example, chemical extractions have been employed to improve the accuracy of mist measurements, as has the addition of detergents to release bacteria and endotoxins from filters. The use of different analytical assays to determine concentrations of endotoxin can also impact any comparisons made. The traditional enzymatic based endotoxin assays to which test samples are added directly may be affected by interfering substances present in used MWFs.

There have been very few studies that have examined the particle size distribution of MWF mist and it may be possible that the majority of fluid droplets containing bacteria and endotoxin fall out of the air rapidly as the droplets produced are too large to remain airborne essentially meaning the majority are splatter. This was certainly the observation when the impact of compressed air use on mist formation was previously examined at HSL (Scaife *et al,* HSE Research Report RR904). In contrast, Wang *et al,* (2007) investigated mist particle size distribution and endotoxin levels in the laboratory workplaces and found the highest concentrations of airborne droplets were in the fine particle size ranges in the areas affected by MWFs. Relatively high concentrations of endotoxin were detected at particle sizes below 0.4 μm, which is smaller than the size of intact bacterial cells and suggests sheering of bacteria during the machining process.

In addition, a variety of other factors have been shown to affect mist formation such as fluid viscosity and type of machining. A further paper by Reponen *et al,* (2005) confirmed past observations that increasing tool rotation speed in grinding operations increases the mist concentrations. An original finding of this study was that microbial contamination of MWF increases the aerosolization of particles. This effect was seen more clearly with semi-synthetic MWF than with soluble oil and in the fine particle size range (<1.0 μm).

5.0 SUMMARY

5.1 Conclusions

A critical review of the DECOS study has been undertaken to assess the validity of the HBROEL of 90 EU / m^3 which has been recommended. The following considerations were made:

- The DECOS HBROEL of 90 EU / m^3 was mainly based on the results of a single volunteer human exposure study.
- For ethical reasons, subjects with pre-existing disease that could have been exacerbated by exposure to endotoxin were excluded and therefore this health based limit may not protect all workers. There is evidence that levels of endotoxin lower than 90 EU / m^3 can cause inflammation in the airways of some workers.

Published evidence of endotoxin measured in air and corresponding bulk fluid was summarised to examine the extent to which endotoxin became airborne during machining processes. The following conclusions were made:

- There is a large discrepancy between concentrations of endotoxin and viable bacteria in mist compared to the concentrations in bulk fluid with airborne endotoxin levels generally falling close to or beneath the HBROEL.
- Similarly, levels of mist mass and bacteria captured in air were low.

5.2 Knowledge gaps

- What is the relevance of the DECOS HBROEL in setting a guidance value for airborne endotoxin exposure associated with MWFs?
- Regarding the observed discrepancy between bulk endotoxin and airborne levels: is this a sampling or analytical measurement problem? Further details about monitoring water-miscible MWF mist were provided by Senior & Evans (2014).
- If bacteria and endotoxin are present at very small concentrations in the MWF mist it may be necessary to consider what combination of other factors (e.g., volatile organic compounds) may be contributing to the respiratory problems that occur with exposure to water-miscible MWF mist?

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7.0 Annex

Annex 7.1 Key search terms for endotoxin in MWF mist

Endotoxin(s)	Mist	Metalworking fluid	Sump	Water-miscible	Machining	Inhalable
Pyrogen(s)	Aerosol(s)	Metal removal fluid	Tank	Water-mix	Milling	Respirable
Lipopolysaccharide	Bioaerosol(s)	MWF	Reservoir	Semi-synthetic	Grinding	Inhalation
LPS	Airborne	MRF	Exhaust		Turning	
	Dust	Lubricant	Ventilation			
	Particle(s)	Coolant	Local exhaust ventilation			
	Spray	Machining fluid	LEV			
	Cloud	Cutting fluid				
	Droplets					
	Fog					
	Haze					
	Fine particles					

Annex 7.2 Data extraction form used to summarise relevant date for the published exposure studies

<table>
<tr><td colspan="4">Data Extraction Form</td></tr>
<tr><td colspan="4">Study identification (Include author, title, year of publication, journal title, pages)</td></tr>
<tr><td colspan="2">Guideline topic:</td><td>Key Question No:</td><td>Completed by:</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4">Key Questions
1) Do mists of water-miscible MWF contain endotoxin?
2) Do the levels of endotoxin constitute an inhalation risk according to the HBROEL proposed by DECOS?</td></tr>
<tr><td colspan="4">The following information is required to complete evidence tables facilitating cross-study comparisons. Please complete all sections for which information is available. PLEASE PRINT CLEARLY</td></tr>
<tr><td>1</td><td colspan="2">1. Is the main focus of the paper endotoxins or is the focus on other airborne contaminants?
Give level of focus that is given to endotoxin,
What other samples were measured in parallel e.g. dust / bacteria?
Name other parameters measured</td><td></td></tr>
<tr><td>2</td><td colspan="2">2. Is the paper describing an investigation following ill health or is it research driven?
State investigation or research
State lab or workplace</td><td></td></tr>
<tr><td>3</td><td colspan="2">Why was the site (s) chosen?
What type of plant was it?
Size of plant
State whether focus on specific type of machining / MWF / wide range of fluids or machining
Give info on above</td><td></td></tr>
<tr><td>4</td><td colspan="2">What was the scale of the study?
Was the study longitudinal?
State timeline, no. of visits
State number of sites</td><td></td></tr>
<tr><td>5</td><td colspan="2">What endotoxin sampling was undertaken?
State number of bulk and air samples taken at each site
If both taken, were they done at same point?</td><td></td></tr>
<tr><td>6</td><td colspan="2">Were air samples personal or fixed position?
If both, were samples taken close to each other?</td><td></td></tr>
<tr><td>7</td><td colspan="2">Give information on sampling devices
Glass quartz, polycarbonate, other filters
Open or closed cassettes
Positioning</td><td></td></tr>
<tr><td>8</td><td colspan="2">What was the duration of air sampling or volume of air sampled?
Give information e.g. flow rate and time</td><td></td></tr>
<tr><td>9</td><td colspan="2">How were samples analysed for endotoxin?
LAL / KLARE/ other</td><td></td></tr>
</table>

Data Extraction Form		
	Were replicate samples analysed? Was the method modified? Was more than one method used?	
10	**Was raw data reported?** State raw or averages What ranges were given for bulk fluids? What ranges were given for air?	
11	**What statistics were performed on the data?**	
12	**Do any values exceed the 90 EU / m^3 limit set by DECOS?** Is the proportion high or low?	

Annex 7.3 Scoring scheme for reviewed papers

Score 1	Score 2	Score 3
Low quality	**Medium quality**	**High Quality**
Large uncertainties apply to the study	**Moderate uncertainties apply to the study**	**Small uncertainties apply to the study**
The number of samples* is small (<10)	The number of samples* is medium (>10 <30)	The number of samples* is large (>30)
Only aggregate (or relative) data is provided with no summary of variation in the data	Summary data (e.g., means) are provided with estimates of the range only	Appropriate summary data (e.g. means, medians, etc) are provided with appropriate estimates of the distribution of the data; or all of the individual data values are provided
No statistical analysis (e.g., p values) of the data has been undertaken or the methods used are inappropriate	Some statistical analysis of the data has been undertaken but has not considered the data appropriately	Statistical analysis of the data has been undertaken and is appropriate
The core§ analytical methods* used are inappropriate, flawed or inadequately described	The core§ analytical methods* used are not described in sufficient detail to enable comparison with other studies	The core§ analytical methods* are appropriate and described sufficiently to compare with other studies
There is either no, or inadequate, additional data to support the primary* measurements and conclusions; there is an absence of controls groups	There is some data to support the primary* measurements and conclusions; control groups are included but may be deficient	There are other data which support interpretation of the primary* measurements; control groups are included
Other factors	**Other factors**	**Other factors**
The monitoring of exposure to endotoxin in air is not a primary purpose of the study	The monitoring of exposure to endotoxin in air is an objective of the study	The monitoring of exposure to endotoxin in air is the primary purpose of the study
The study has not been peer-reviewed	The study has been peer-reviewed	The study has been peer-reviewed and published in an international journal
The outcomes of the study are not well summarised	The outcomes are summarised	The outcomes are well summarised
The study is theoretical and not related directly to worker exposure	The study is related to worker exposure	The study is directly relevant to worker exposure

* - refers to endotoxin measurements or any other measurements that support the interpretation of the endotoxin data such as gravimetric data including total inhalable and thoracic fractions, bulk MWF endotoxin measurements, total and biologically-active endotoxin, and total and viable Gram-negative bacterial numbers.

§ - refers to the main methods of primary interest, e.g. sampling, collection and analysis of endotoxin, Gram-negative bacteria or of gravimetric mass of the sample.

Annex 7.4 Summary scores for papers included in the review

Study paper and number	Comments	Score (1, 2, 3)
1) Abrams *et al,* (2000) Appl Occup Environ Hyg **15** (6) 492-502	This is a well-designed ill health investigation at a car plant with repeated measures of the same individuals at different points of time. Controls of no MWF use (final assembly) and MWF but no ill health (valve production) were included. The numbers of different samples is reasonably large and also each variable is supported by other measurements (e.g., particulates, bacterial counts and endotoxin, personal and area and sump samples). The data was corrected for effects of environmental tobacco smoke exposure**.**	**3** **High**
2) Bracker *et al,* (2003) Appl Occup Environ Hyg **18** (2) 96-108	An investigation of an outbreak of EAA at a plant machining titanium & high nickel alloys. Whilst the study has a longitudinal design with some repeated measures the number of bulk and air samples quantified for mist, bacteria, fungi and endotoxin were generally small (<10). Some of the single sump measurements have been reported only as single mean values or as a percentage changes. Inconsistent study design and the way that the endotoxin samples (bulk vs air) were quantified reduced the quality of the paper.	**2** **Medium**
3) Cervelli *et al,* (2010) Italian J Occup Environ Hyg **1** (3-4): 139-145	Whilst the paper focused on airborne endotoxin exposure in several industries including metal machining, waste handling and waste water treatment, the study is marked by lack of information about the sampling regime and detail about the factories visited and processed sampled. Another problem is the unorthodox way in which the data is summarised. An attempt is made to compare the measured endotoxin concentrations to an earlier proposed Dutch exposure limit of 45 EU m^3 8hrTWA.	**1** **Low**
4) Cook & Mattorano (1996) HETA-96-0020-2610	A NIOSH ill health evaluation at a manufacturer of sprockets and gears. Some of the analysed data is not reported; the overall mean (and SD) values are not reported. The design of the study was not clear. It did not specifically look at quantifying exposure to MWF mists and its constituents. No assessment was made of bacterial or fungal – concentrations in the sumps or air. The collected ill-health information was scant due a very poor response rate.	**1** **Low**
5) Gilbert *et al,* (2010) J Occup Environ Hyg **7** (5) 280-289	An exposure assessment across a large number of metal machining sites that covered 25 different industries and a wide variety of MWFs. The study is well designed with a good number of sample replicates, consistency in the method used at all sites, inclusion of appropriate controls for sampling and analysis. The only minor deficiencies are the lack of data on endotoxin levels in the bulk samples and the reliance on static sampling and not personal sampling	**3** **High**
6) Hodgson *et al,* (2001) Am J Ind Med **39**: 616-628	This is a further paper reporting the ill health investigation at a car plant described in Abrams *et al,* (2000). From the perspective of assessing exposure to MWF mist this	**2**

Study paper and number	Comments	Score (1, 2, 3)
	study is of limited quality due to the small number of samples collected, the absence of personal air samples, and the inconsistent application of sampling methods to collect air samples.	**Medium**
7) Kiefer & Trout (1998) HETA 98-0030-2697	A NIOSH ill health investigation at an aircraft parts manufacturer. The numbers of personal or static samples taken for endotoxin measurement was small and other accompanying measurements (e.g., sump levels of endotoxin) were also limited in number. The majority of these samples (n=32) were assessed only for MWF and particulate mass and suggested that the majority of exposures were below the NIOSH REL for particulates. No viable bacteria were found consistent with biocide treatment; nevertheless endotoxin levels were very high suggesting that previous accumulation of bacteria had occurred. The methodology was described in sufficient detail to provide confidence about the analytical results but not sufficient detail to compare with the results of other studies.	**1** **Low**
8) Laitinen *et al,* (1999) Int Arch Occup Environ Health **72**: 443-450	This study is of medium quality which has grouped 18 machining sites according to fluid type and machining task. The study specifically addresses the issue of airborne exposure to bacteria and endotoxin and supported the airborne data with measurements in the bulk fluid. The number of measurements is relatively large >100 but does not distinguish between area and personal samples. However this is also a potential deficiency in the study in that there is limited data from each site for any particular type of MWF. The study does provide a lot of supporting hygiene data including information about the MWF and the history of management. No data on airborne exposure to MWF mist mass (i.e., gravimetric data) is provided. However, the study does provide good detail about the methods used to sample and analyse the endotoxin and bacterial numbers. The results demonstrate that even with very high levels of viable bacteria in MWF sumps the airborne levels of bacteria and endotoxin can be very much lower. The authors also suggest that the number of bacteria and concentration of endotoxin in the air is not related in a straight forward way to the levels in the bulk MWF but those activities such as the type of machining processes and other variables affect airborne levels.	**2** **Medium**
9) Laitinen *et al,* (2001) Ann Agric Environ Med **8**: 213-219	This study was a general survey of endotoxin and other bacterial inflammatory markers over a range of different industries. The samples sizes for the metalworking industry were small and only fixed samples were taken. There is no data on the sump levels of endotoxin or bacteria. No details are provided about the plant visited, or the type of MWF or other supporting hygiene data.	**1** **Low**

Study paper and number	Comments	Score (1, 2, 3)
10) Lewis *et al,* (2001) Int Biodeter Biodegrad **47:** 89-94	This study was a follow up of three ill health investigations at machining plants and focussed on the use of non-culture based techniques for the assessment of microbial contamination of MWFs. Although endotoxin was examined at three sites, both airborne and sump levels were only tested at 1 site. Methodology for the analysis of bulk fluids was detailed but lacked information for the sampling of air. No information regarding the type of workplace was given or whether airborne data was for personal or area samples.	
11) Linnainmaa *et al,* (2003) AIHA Journal **64**: 496-500	This was a further paper covering the investigations of Laitinen *et al,* (1999) that undertook exposure assessments at 18 machining sites. The study is poorly designed, includes different study designs, none of which have been carried out thoroughly. There is a lack of data for endotoxin measurements and no comparison between airborne and bulk measurement of endotoxin or bacteria can be made. There is a lack of experimental detail and statistical analysis.	**1** **Low**
12) Marchand *et al,* (2010) J Occup Environ Hyg **7** (6) 358-366	This study is an assessment of sump cleaning of four lathes at a machining plant and contains very limited data regarding endotoxin levels. The study was limited to just one type of machine and no other parameters relevant to monitoring mist emissions were measured. The number of endotoxin air samples was small. No comparison between endotoxin levels and bacterial numbers in the bulk fluid or airborne samples was made.	**1** **Low**
13) Park *et al,* (2008) J Occup Health. **50:** 212-220	The study is of limited value. The main focus of this study is the clinical endpoints of workers at a piston manufacturing plant. The study only reports airborne levels of endotoxin and not data that would allow these to be related to levels of bacteria or endotoxin in the MWF sump. Levels of airborne culturable fungi are reported but not levels of airborne bacteria. Insufficient detail is provided about the methods used and sampling procedures. The interesting finding in this study is that a high prevalence of nasal symptoms was found amongst a workforce exposed to moderate levels of MWF mists but which contained relatively low levels of endotoxin. High odd rations were found for work in grinding and manufacture and nasal symptoms.	**2** **Medium**
14) Piacitelli & Washko (1999) HETA **96**-0232-2776	Taking into account this is a NIOSH health hazard investigation report at a machining plant manufacturing roof bolts there is insufficient rigor in the design of the study particularly those aspects related to the measurement of airborne endotoxin and bacteria. Whilst bulk samples were assessed for endotoxin and viable bacterial numbers endotoxin contact in the air was only assessed for static samples with no measurement of airborne bacterial load. There is a lack of detail provided about the sampling methods and analysis of the samples. The one aspect of this study, which is in line with other	**1** **Low**

Study paper and number	Comments	Score (1, 2, 3)
	studies, is that despite very heavy contamination of the bulk samples with bacteria and endotoxin, very low levels of endotoxin were encountered in the air samples. Whilst the workers reported symptoms, these were mostly consistent with irritant and nausea and no long-term decline in lung function or serious lung disease. The presence of a high thoracic level of MWF dust above the NIOSH REL is noted	
15) Suuronen *et al,* (2008) Ann Occup Hyg **52**(7) 607-614	This is an exposure assessment where samples were spread around a variety of workshops and MWFs. Whilst this provides some measure of the variability it does not provide much certainty about the variation in levels of endotoxin within a workplace. The main relevant conclusion was that levels of endotoxin were low and beneath the proposed DECOS HBROEL. The major caveats are: Whilst the study was designed to monitor exposure to MWF mist the focus was on chemical not biological constituents No measurements in MWF bulk samples of bacteria numbers and endotoxin were undertaken to compare to the mist levels. It is not therefore possible to say how well these MWFs were managed in terms of microbial growth.	**2** **Medium**
16) Thorne *et al,* (1996) AIHA Journal **57**: 1163-1167	The study involved an exposure assessment at a single car manufacturing plant and provides some data suggesting that levels of endotoxin in air can be found above the DECOS HBROEL and that these levels were influenced by numbers of bacteria in the bulk fluid. The results also suggest that where control on mist was less efficient (ie the old machining plants) a larger proportion of the airborne samples contained endotoxin concentrations above the DECOS HBROEL. The value of these results is limited by the inadequate quality of the data reported in the paper. Whilst a large number of samples were taken, in a repeated measures design, the summary data for these samples are not provided. The methodological detail and statistical analysis of the data are not clearly explained. The positioning and type of air sampling is not specified.	**1** **Low**
17) Wang *et al,* (2007) J Occup Environ. Hygiene 4(3) 157-165**.**	The main objective was to investigate the size distribution of airborne particles as well as endotoxin in MWF environments. This study consisted of both a laboratory study as well as workplace sampling. Workplaces sampled had cases of ill health and manufactured metal parts using lathes, milling and drilling. Good levels of detail regarding methodology were given. However, the number of samples was very limited.	**1** **Low**
18) Zucker & Fluri (NOT PUBLISHED)*	This study was undertaken at a single machining workshop and compared levels of airborne endotoxin produced when bioconcept fluid or conventional MWF was used with grinders and lathes. The study consisted of a few samples and not enough information about how and where the samples were taken. The low number of samples	**1** **Low**

Study paper and number	Comments	Score (1, 2, 3)
	is likely to mean that there are large uncertainties surrounding these estimates. No comparison was made between airborne and sump endotoxin and bacterial numbers. No other measures of mist formation were made. The one interesting aspect of the paper is the application of the MAT test, which suggests very little detectable endotoxin was present in the air samples. In contrast the bulk fluid samples gave much clearer MAT responses; significantly higher than might be predicted from an inspection of the LAL results. This may suggest that a lot more endotoxin (or pyrogen) is present in the MWF not detected by the LAL**.**	
19) Zucker *et al,* (2006) Gefahrstoffe-Reinhaltung der Luft **66**: 369-372	An exposure assessment was undertaken at six workplaces in various unspecified metalworking plants. The study was limited by sample sizes, lack of sampling methodology, novel extraction method for endotoxin. Poor study design, lack of supporting methodology and statistical analysis of the data led to a paper of poor quality. There is a lack of data supporting measurement of airborne MWF (gravimetric etc.), and a lack of data providing estimates of variation of exposure, as well as lack of information about the sites visits and hygiene assessment about working practices. No comparison between airborne and bulk fluid levels of endotoxin and bacteria was provided.	**1** **Low**

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Endotoxin in metal working fluid (MWF) mist

The risks to respiratory health from exposure to bacterial endotoxins are well established. The aims of this research were to:

- Review the evidence used to develop the health based recommended occupational exposure limit (HBROEL) for endotoxin of 90 endotoxin units per cubic metre (EU/m3) over an 8-hour period proposed by the Health Council for the Netherlands (DECOS); and to assess its relevance as a 'benchmark' to assess risks to respiratory health caused by endotoxin in metal working fluid mists.

- Assess whether the published evidence on endotoxin concentration in metal working fluids provides sufficient evidence that concentrations in mist are sufficient to cause harm to human health

The research concluded that there was a large discrepancy between concentrations of endotoxin and viable bacteria in mist compared to the concentrations in bulk fluid with airborne endotoxin levels generally falling close to or beneath the DECOS recommended level , whilst sump levels generally exceeded these by 100 to 1000 fold. Levels of viable bacteria captured in air were low compared to the levels in the sumps.

Further research is required to determine whether the discrepancy between bulk endotoxin and airborne levels is real or whether this is due to the impact of sampling or analytical methodology

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