Exposure-response relationships for bioaerosol emissions from waste treatment processes

Final report

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Exposure-response relationships for bioaerosol emissions from waste treatment processes

Executive Summary

INTRODUCTION

This study was undertaken to provide Defra with an updated understanding of the exposure-response relationship between bioaerosol emissions from waste treatment processes and the potential impact on human health.

The overall aims of the study were:

- To collect and critically review the literature on the sources of bioaerosols from waste treatment processes, identify the components of greatest relevance to human health and their potential effect, including consideration of sensitisation, allergy, infection and toxicity and sensitive receptors, such as asthma sufferers, the old, the very young, and immuno-suppressed individuals, who will be more at risk from opportunist pathogens (e.g. Aspergillus fumigatus) than the general public or workers close to the source;

- To establish exposure-response relationships for key bioaerosol components and health endpoints

- To place this information into a regulatory context including determining which bioaerosol components should be monitored to adequately assess the potential impact of waste treatment activities; and

- To identify the knowledge gaps and make recommendations for further research.

The bioaerosol components considered were:

- Dust
- Bacteria including actinobacteria more commonly termed Actinomycetes, a group of Gram-positive bacteria that play an important role in decomposition of organic materials and produce external spores, similar to fungi.
- Endotoxin - a structural component in some bacteria that is released when the cell wall is damaged. Endotoxin is not a single uniform substance and includes lipopolysaccharides (LPS) or lipo-oligo-saccharides (LOS). The symptoms of many infections with pathogenic Gram-negative bacteria are due to endotoxin.
- Fungi
- Beta(1→3) glucans ((1→3)β-D-glucan), polysaccarides that form part of the cell wall of certain fungi, particularly Aspergillus species.

MEASUREMENT OF BIOAEROSOL EXPOSURE

Despite considerable research effort, a wide range of parameters are used to characterise bioaerosol exposure and there is considerable variability in the way that these are determined. There is also interlaboratory variability in the determination of individual parameters using single assays. A wide range of instruments have been used to capture bioaerosols on filter, in fluid or onto gel. One of the issues with the collection of viable microorganisms is the preservation of their viability during and subsequent to sample collection. This has led to very short sample times ranging from a few minutes to an hour and half whereas bioaerosol concentrations can vary dramatically in the space of a few hours. This gives rise to uncertainty in the representativeness of measured concentrations versus actual exposure conditions. Conditions during the transport and storage of samples can have a profound effect on apparent bioaerosol concentrations. The analysis for viable organisms
involves culturing the sample in a suitable medium and at an appropriate temperature to support the growth of the organisms of interest and then performed by counting the number of colonies that develop. Counts of total viable and nonviable organisms are made microscopically using fluorescence staining to aid identification. Alternative approaches to the characterisation of bioaerosols have included the use of gas chromatography – mass spectroscopy (GC-MS) for the identification of characteristic marker compounds and quantitative polymerase chain reaction (PCR) to determine the presence and quantity of specific target DNA sequences linked to individual species. Endotoxin is most commonly measured using a chromogenic Limulus amebocyte lysate (LAL) assay and several assays have been developed for the measurement of beta (1→3) glucan based on LAL or immunological assays.

Initiatives aimed at method standardisation include a recent study by the EA (2007), guidelines produced by the Composting Association (1999), the recent formation of a CEN (European Committee for Standardisation) working group on the “Measurement of bioaerosols in ambient air and emissions” (CEN/TC 264/WG 28) and the publication of the American Society for Testing and Materials (ASTM) method E884-82 (ASTM, 2006). In addition to standardisation of measurement methods there is a need for a harmonised approach to sampling strategy.

There are no widely used biomarkers of exposure to bioaerosols.

BACKGROUND LEVELS OF BIOAEROSOL EXPOSURE

Most of the limited information available describes fungal concentrations which vary by location and season. Both UK data and the results of studies elsewhere suggest that concentrations in urban air are typically less than 1000 cfum$^{-3}$ but may be considerably higher, particularly during the autumn. Most studies of bioaerosol exposure in rural areas have focussed on occupational exposure associated with intensive livestock rearing and there has also been some interest in the impacts of sewage sludge spreading. There is very little information about typical concentrations of bacteria or fungi in rural areas. Both external air quality and building dampness play an important role in governing exposure to airborne moulds in indoor environments with reported concentrations in indoor air ranging from <100 to 20000 cfum$^{-3}$. Concentrations of bacteria in indoor environments are typically about 100 cfum$^{-3}$ but may be considerably higher in some environments.

Typical endotoxin levels in outdoor urban air are less than 1 EUm$^{-3}$, but slightly higher concentrations may arise in some rural locations where agricultural activities emit endotoxin. Indoor concentrations of endotoxin are heavily influenced by environmental tobacco smoke.

BIOAEROSOL EXPOSURES ASSOCIATED WITH WASTE HANDLING PROCESSES

Elevated levels of workplace exposure to bioaerosols are found widely throughout the waste industry including waste collection, materials recovery, composting and the storage of waste material prior to incineration. Exposure levels in most sectors tend to be higher during the summer but are not clearly linked with waste composition. There is limited evidence that waste storage may lead to increased exposure concentrations and more substantive evidence that increased activity levels are associated with increased bioaerosol exposures. Exposure levels vary within individual sectors suggesting that there is potential to reduce exposures through good practice. A number of measures have been suggested to reduce emissions from commercial composting operations including appropriate control of moisture content and airflow during composting, using water mist to dampen dust over screen conveyers, providing adequate ventilation in buildings and using in-vessel systems. Biofiltration may be an effective method for reducing odour nuisance and may also reduce microbial counts.

Studies of community exposure to bioaerosols emitted from composting have reported concentrations of $>10^2$ cfum$^{-3}$ of thermophilic actinomycetes, moulds, and total bacteria 200 m from a large composting site, dropping to near background concentrations within 300 m although raised microbial concentrations may under some conditions occasionally arise at distances of about up to about 0.5 km from composting operations. Studies in the UK have
found minimal concentrations within the site or within 5 or 10 m of the site boundary on some days whereas on other days concentrations of viable bacteria exceeded $7 \times 10^5$ cfum$^{-3}$.

The limited data available suggest the nature of bioaerosol emissions from stored waste in the home and its subsequent collection varies depending on the waste composition and its storage conditions. The potential for exposure to bioaerosols and biologically-active liquid leachate is anticipated to increase given the emphasis being placed on alternative and more sustainable waste management practices (e.g. composting, recycling) in homes across the UK and elsewhere. Exposure can be minimised, however, following good practice.

**HEALTH EFFECTS AND EXPOSURE RESPONSE RELATIONSHIPS**

The findings of a large number of individual studies and reviews of the published literature indicate that workplace exposure to bioaerosols in the waste and other industries is associated with increased risks of developing upper and lower respiratory symptoms and chronic respiratory illness. There is more limited evidence of increased risks of gastrointestinal illness or fatigue. Specific studies of residential exposure to bioaerosols arising from the domestic storage of organic waste have found no excess of respiratory symptoms (possibly because exposure levels are low) but evidence of an association with skin symptoms. There has been relatively little investigation of the effects of community exposure to bioaerosols arising from waste processes. Some extremely limited data suggest that living in close proximity to a compost facility may be associated with an increased risk of respiratory symptoms and the development of long term respiratory illness as well as symptoms such as excessive tiredness.

Table S1 summarises the key exposure-response information for bioaerosols that was identified in this study.

**Table S1: Summary exposure-response information for bioaerosols**

<table>
<thead>
<tr>
<th>Bioaerosol component</th>
<th>Health endpoint</th>
<th>Exposure-response information</th>
<th>Study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic dust</td>
<td>irritation of eyes and nose</td>
<td>Symptoms reported at 200 ug m$^{-3}$ Reported at 1 -2 mg m$^{-3}$, prevalence increases with concentration May arise at concentrations &gt;0.3 mg m$^{-3}$ but normally associated with concentrations &gt;1.2 mg m$^{-3}$.</td>
<td>Waste workers, Various industries, Cotton workers</td>
</tr>
<tr>
<td></td>
<td>chest tightness and wheeze</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>chronic respiratory illness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fungi</td>
<td>respiratory symptoms, nausea, headache etc</td>
<td>Symptoms reported at &gt;$10^4$ cfu m$^{-3}$ and between $10^3$-$10^6$ spores m$^{-3}$ Increased symptoms associated with concentrations of 2000 cfum$^{-3}$ in indoor air or 1000 spores m$^{-3}$ in outdoor air Mild adverse respiratory effects may arise at concentrations ≥ 350 cfum$^{-3}$ in household air.</td>
<td>Waste workers, General community, Children</td>
</tr>
<tr>
<td>Total microbes</td>
<td>respiratory symptoms, nausea, headache etc</td>
<td>Symptoms reported at $10^6$ cfum$^{-3}$, very limited evidence of increase in symptom prevalence with increasing exposure</td>
<td>General community near compost operations</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>respiratory symptoms, fatigue</td>
<td>Greater prevalence of symptoms at concentrations &gt;50 EUm$^{-3}$, but indications of nasal irritation reported in one study of waste workers at 4.5 EUm$^{-3}$, clear evidence that risks increase with increasing exposure</td>
<td>Workers in various industries</td>
</tr>
<tr>
<td>Beta(1→3) Glucan</td>
<td>respiratory symptoms, nausea, headache etc</td>
<td>Limited evidence of adverse effects at concentrations 1-0 ngm$^{-3}$, no adverse effects at 1 ngm$^{-3}$</td>
<td>Studies of indoor air quality</td>
</tr>
</tbody>
</table>

On consideration of all the available studies, it is apparent that there are no clear thresholds of effect for different bioaerosol components and some individuals may experience adverse
effects at background levels of exposure, in the absence of any waste derived bioaerosols. This may partly reflect the susceptibility of some individuals in the population to adverse effects (below), the importance of sensitisation in governing some health effects and the potential for infection. It is also worth noting that the concept of a no effects level comes from chemical toxicology and arises from the conduction of animal experiments with a limited gene pool and a limited number of animals. Virtually all the health information for bioaerosols arises from studies in human populations with a range of susceptibility to effects.

**SUSCEPTIBLE SUBGROUPS OF THE POPULATION**

There is wide variability in individual sensitivity to bioaerosol exposure. A relatively substantial proportion of individuals (perhaps >10% of the population) may be susceptible to developing respiratory symptoms at levels of bioaerosol exposure that are encountered in the general community, in the absence of any specific point sources of bioaerosols. A large proportion of these individuals are likely to be atopic (i.e., have a tendency towards the development of allergic disease). A small proportion of individuals with asthma and also some cystic fibrosis patients have a greatly increased risk of developing hypersensitivity to specific mould species leading to serious respiratory illness at ambient levels of exposure. A small proportion of individuals have highly compromised immune systems and are at particular risk of invasive fungal infection leading to a risk of developing serious illness at concentrations of airborne fungi of less than $10^3$ cfu m$^{-3}$. These individuals include transplant and cancer patients.

There is no clear evidence that children or the elderly are particularly susceptible to the effects of bioaerosol exposure. There is very limited evidence that women may have an increased susceptibility to effects during pregnancy and lactation and that high levels of exposure to endotoxin may be damaging to the unborn child.

**USE OF EXPOSURE-RESPONSE INFORMATION FOR BIOAEROSOLS IN REGULATION**

In principle the exposure-response information collated in this project could inform the setting of guideline levels of exposure, emissions limits or stand-off distances intended to control community exposure to bioaerosols to concentrations below those associated with adverse effects. It could also be used to quantify benefits arising from improved regulation of bioaerosol emissions and in the comparison of different regulatory options (including the “do nothing” option).

There are insufficient data to set exposure guidelines for most components of bioaerosols other than endotoxin. There is, however, almost no information about levels of endotoxin in ambient air. There would be major difficulties in demonstrating compliance with guidelines or emissions limits through either measurement or modelling. Alternative approaches to developing air quality guidelines include identifying marker species that could be used to monitor the specific impact of waste as opposed to other sources on community bioaerosol exposure. Another approach would be to develop modified PM$_{10}$ objectives for use in the vicinity of waste operations. Any air quality guidelines that are developed are unlikely to provide complete protection for the most vulnerable members of the population, including the immunocompromised, who may become ill at background levels of bioaerosol exposure.

There is little information to support the development of appropriate stand-off distances for different types and sizes of process. The use of stand-off zones may make it difficult to open new sites and may stifle other developments around existing sites regardless of the real level of risk to health.

A code of best practice is an attractive regulatory option because best practice would anyway be required of operators as part of permitting and licensing conditions and would have to be implemented to achieve any air quality guidelines.
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Abbreviations

ABPA  allergic bronchopulmonary aspergillosis
AM    arithmetic mean
BAL   bronchioalveolar lavage
CI    confidence interval
cfu   colony forming units
EU    endotoxin unit (about 9.5 ng)
FEV$_1$ forced expiratory volume in 1 second (measure of lung function)
FVC   forced vital capacity (measure of lung function)
GC-MS gas chromatograph-mass spectrometer
GM    geometric mean
GSD   geometric standard deviation
IL    interleukin (inflammatory mediator)
LAL   Limulus Amoebocyte Lysate Chromogenic Assay using the kinetic rather than endpoint protocol (analysis of endotoxin)
LPS   lipopolysaccharide (endotoxin)
LOEL  lowest observed effects level
NAL   nasal lavage fluid
NOEL  no observed effects level
ODTS  organic dust toxic syndrome
OR    odds ratio - the ratio of the odds of an event occurring in one group (exposed) to the odds of it occurring in another group (control), or to a sample-based estimate of that ratio
RR    relative risk - ratio of the probability of the event occurring in the exposed group versus the control (non-exposed) group
SD    standard deviation
TNF   tumour necrosis factor (inflammatory mediator)
1. Introduction

1.1 BACKGROUND TO REPORT

There is an increasing public awareness and interest in the changing profile of waste management in the UK and a need to fully understand the real and perceived hazards and risks associated with different waste management options. This project has specifically examined the health risks arising from exposure to bioaerosols emitted from waste management activities. Bioaerosols are complex mixtures and different components of the mix have variable potentials to cause illness in different individuals. For example, fungi and bacteria may cause illness directly through infection or indirectly as a result of fungal or bacterial toxins such as endotoxin or glucans. Alternatively complaints of ill-health may arise as a result of allergic reaction to specific bioaerosol components or as a result of exposure to malodours or chemical components of the aerosol mix. This study was undertaken to provide Defra with an up to date understanding of exposure-response relationships linking bioaerosol emissions from biowaste treatment processes to potential impact on human health, in order that actions to restrict bioaerosol releases are proportionate to the risk.

1.2 AIMS

The overall aims of the study were:

- To collect and critically review the literature on the sources of bioaerosols from waste treatment processes, identify the components of greatest relevance to human health and their potential effect, including consideration to sensitisation, allergy, infection and toxicity and sensitive receptors, such as asthma sufferers, the old, the very young, and immunosuppressed individuals, that will be more at risk from opportunistic pathogens (e.g. *Aspergillus fumigatus*) than the general public or workers close to the source;

- To establish exposure-response relationships for key bioaerosol components and health endpoints;

- To place this information into a regulatory context including determining which bioaerosol components should be monitored to adequately assess the potential impact of waste treatment activities; and

- To identify the knowledge gaps and make recommendations for further research.

Specific objectives were to:

- Review the methods used to measure bioaerosol exposure arising from waste management activities, particularly in the context of available epidemiological information;
- Determine the nature of bioaerosols emitted from different waste handling processes;
- Review background levels of exposure to bioaerosols and levels of workplace and community exposure arising from waste management activities;
- Determine the importance of waste management activities as an influence on population exposure to bioaerosols;
- Review the health effects associated with bioaerosol exposure;
- Describe the exposure-response information available from studies of bioaerosol exposure in the waste industry;
- Describe exposure-response information for bioaerosols from studies of bioaerosol exposure in other industries or in the general community;
- Determine a no effects level and/or lowest effects level, if possible, and determine which bioaerosol components have important potential impacts on health;
Comment on the likely exposure-response relationships in potentially susceptible subgroups of the population; and

Consider how the available exposure-response information could be used to inform the development of best practice guidelines and quality standards for waste management activities and in the evaluation of alternative strategies of biowaste management.

1.3 INFORMATION SOURCES AND METHODS

Information on exposures and health effects was sought from earlier reviews of the health effects of bioaerosols (e.g., the HSE-sponsored study by Swan et al., 2003; Wouters et al., 2006) and a series of searches of the peer-reviewed literature were undertaken using PubMed (a free database of abstracts of the medical literature available from the internet). Searches were undertaken for studies about bioaerosols, exposures and health effects in workers in the waste industry, the effects of endotoxin exposure and more general information about community exposure to bioaerosols. Further information was sought from the websites of a range of organisations based in Europe and North America with interests in biowaste management as well as through existing IOM contacts and contacts provided by the project steering group. Given the small number of studies in the waste industry that provide information linking bioaerosol exposure to effects, a much wider literature search was undertaken to find relevant additional information from other industries including studies of agricultural, cotton, sawmill and paper mill workers. In addition information was sought about the effects of bioaerosol exposure in the general community in relation to ambient and indoor air quality.

The identified studies were critically assessed to identify the exposures and likely health effects associated with emissions from waste management activities. In considering dose-response relationships, consideration was given to the reliability of the source data, relevance to the waste industry and potential confounding factors in the source study, such as socio-economic status of the population at risk. The implications of acute versus long term or episodic exposure were considered, including the potential long term effects that may arise from a relatively short period of exposure. The review specifically sought:

1. Quantitative information linking exposure concentrations of specific bioaerosol components or other measures of dose (for example, biomarkers or residential proximity to specific types of operation of differing size) to health effects including evidence of threshold levels, below which no effects have been reported;

2. The characteristics of populations for which dose-response information is available that should be taken into account when considering potential impacts in the general population;

3. Information about the pattern or duration of exposure leading to effects; and

4. Potential to integrate across studies to derive dose-response.

Insufficient studies linking exposure to a specific measure of bioaerosol exposure to a given health endpoint were available to permit a formal meta-analysis. Data quality issues considered in the review of epidemiological studies are discussed in Appendix 1.

1.4 REPORT STRUCTURE

The early chapters of this report review bioaerosol composition and measurement, ambient concentrations and exposure to bioaerosols emitted from waste operations. The main part of the report reviews health effects and exposure-response information. The final chapters review how this information could be used to support regulation, identify key information gaps and make recommendations for future research.
2. Bioaerosol composition, measurement methods and methods used to assess effects

2.1 INTRODUCTION

Bioaerosols are defined as aerosols, aeroallergens, or particulate matter of microbiological, plant or animal origin. Bioaerosols can interact with living systems through infective, allergenic and/or toxic mechanisms. The primary exposure route of concern is inhalation. The biological agents that have been examined in relation to bioaerosol exposures associated with waste handling and treatment processes include pathogenic or non-pathogenic, live (viable) or dead (non-viable) bacteria, fungi, viruses, bacterial endotoxins, mycotoxins, and peptidoglycans. Although other types of biological component may also be present as airborne particles such as algal fragments, protozoa and nematodes, these have not been considered in studies of bioaerosols emitted by the waste industry. Bioaerosol emissions may be accompanied by releases of microbial volatile organic compounds (MVOCs) and gaseous emissions including acetone, ammonia, 2-butane, carbon dioxide and hydrogen sulphide, dependent upon the source material and conditions. An overview of the metrics used to describe airborne concentrations of bioaerosols that are of principal relevance in waste handling and disposal activities is given below. More detail about bioaerosol emissions from waste processes is given in Chapter 4 of this report and detailed descriptions of airborne micro-organisms and their components are provided by Swan et al (2003). The second part of this chapter describes the measurement methods available for bioaerosols and the final part of this chapter describes the methods that have been used to investigate the impacts of bioaerosol exposure on human health.

2.2 DUST

Concentrations of airborne particulate matter or dust are widely reported in studies of bioaerosols in the waste industry. Although not technically a bioaerosol, dust may carry microbial constituents from bacteria, fungi, and/or the raw waste material. A range of metrics are used:

- **Inhalable dust fraction**: the size fraction of airborne particles that are able to penetrate the airways and may be deposited within the respiratory system;
- **Respirable dust fraction**: the size fraction of airborne particles that may be deposited in the gas-exchange region of the lung;
- **PM$_{10}$** (thoracic function): the size fraction of airborne particles that may be deposited in the lung; and
- **PM$_{2.5}$** (high risk respirable): the size fraction of airborne particles that may be deposited in the gas-exchange region of the lung in those with compromised respiratory health.

In some studies, dust is specifically referred to as “organic dust” but this is likely to be an indication that no substantial sources of inorganic dust were present at the time of sampling rather than a specific measure of organic dust as a component of the total dust present.

2.3 BIOAEROSOL COMPONENTS

2.3.1 Bacteria

Bacteria are vital in recycling nutrients. The range of bacteria found in waste and in bioaerosols generated from waste depends on the nature of the waste and conditions during storage and handling. Most bacteria can be described as being - Gram-positive cocci, Gram-positive bacilli, Gram-negative cocci or Gram-negative bacilli. Gram positive/negative refers to whether bacteria do (+) or do not (-) retain crystal violet dye in the Gram staining protocol. Cocci are close to spherical in shape whereas bacilli are rod-shaped.
Concentrations of bacteria in air are described in terms of counts of viable (culturable) or total (viable and non-viable bacterial particles) per unit volume air.

Actinobacteria or Actinomycetes are a group of Gram-positive bacteria that play an important role in decomposition of organic materials, such as cellulose and chitin and are therefore abundant in compost. Most members of the species are aerobic, but a few, such as *Actinomyces israelii*, can grow under anaerobic conditions. Some Actinomycetes species produce external spores, similar to fungi.

The term thermophilic is applied to bacteria (or fungi) that thrive at high temperatures (above 45 °C). Mesophilic bacteria (or fungi) thrive at moderate temperatures (25-40 °C). Xerophilic is used to describe organisms that can survive under extremely dry conditions.

### 2.3.2 Fungi

Fungi play a major role in causing decomposition of organic material and are important during waste decomposition and composting. The term mould specifically refers to species of microscopic fungi that grow in the form of multicellular filaments, called hyphae. Microscopic fungi that grow as single cells are termed yeasts. Fungi proliferate through sporulation leading to the production of spores or conidia. Fungi are generally present in ambient air in the form of spores. Spores degrade rapidly in air and both viable spores and the remains of spores that are no longer viable may be present in air.

The common fungal mould *Aspergillus fumigatus* presents a potential risk of opportunistic infection in immunocompromised individuals. The species most commonly associated with allergic disease are *Aspergillus fumigatus* and *Aspergillus clavatus*. *Aspergillus* species are found in almost all oxygen-rich environments and are common contaminants of starchy foods (such as bread and potatoes), and grow in or on many plants and trees. In addition, many species of *Aspergillus* are capable of growing in nutrient-deplete environments, or environments in which there is a complete lack of key nutrients. More information about the species of fungi involved in composting and found in waste-derived bioaerosols is given in Chapter 4.

### 2.3.4 Microbes

Some studies of the waste industry have described exposure in terms of microbial colony forming units (cfu). This is essentially a combined measure of the total viable fungal and bacterial population under certain defined conditions (culture medium and temperature – see section 2.4.5 below).

### 2.3.5 Mycotoxins

Certain types of moulds produce toxic secondary metabolites that are termed mycotoxins such as aflatoxin, produced by the fungi *Penicillium*, *Aspergillus flavus* and *Aspergillus parasiticus*. Many of these toxins are pathogenic to animals and humans and cause immune system responses that vary considerably, depending on the individual. The role of mycotoxins in the causation of adverse health effects has not been widely evaluated in the waste industry.

### 2.3.6 Endotoxins and peptidoglycans

An endotoxin is a toxic structural component of a bacterium that is released if the bacterium is damaged. Endotoxins are lipopolysaccharide (LPS) or lipo-oligo-saccharide (LOS) compounds found in the outer membrane of various Gram-negative bacteria. LPS consist of a polysaccharide (sugar) chain and a lipid moiety, known as lipid A, which is responsible for the toxic effects. The polysaccharide chain is highly variable amongst different bacteria. The symptoms of many infections with pathogenic Gram-negative bacteria are due to endotoxin. Systemic effects include fever, a lowering of the blood pressure, and activation of inflammation and coagulation. Endotoxin is not a single uniform substance and this gives rise
Peptidoglycan, also known as murein, is a polymer consisting of sugars and amino acids that forms a homogeneous layer outside the plasma membrane of bacteria. It has not been widely used as a marker of bioaerosol exposure in the waste industry and has not been included in this study, although it has been used as a marker of bacterial contamination in indoor air (Fox et al, 2005). Peptidoglycan and partial structures of petidoglycan are potent stimulators of the human immune system and are associated with symptoms typical of bacterial infections such as fever, inflammation, leukocytosis and lymphocyte stimulation (Hauswirth & Sundy, 2004).

Endotoxins and peptidoglycans are frequently present in organic dusts arising from waste handling activities and can persist long after the death of the source bacteria.

2.3.7 Glucans

Beta (1→3) glucans (more correctly (1→3) beta D glucans) are polysaccharides that form part of the cell wall of certain fungi, particularly Aspergillus species and are commonly present in dusts generated from waste. Beta glucans consist of linear unbranched polysaccharides (sugars) of linked β-(13)- and β-(14)-D-lucopyranose units in a non-repeating but non-random order. In addition to forming a component of fungal cells, beta-glucans occur in some cereals such as barley, oats, rye and wheat. Rylander et al (1999) reported that the quantities in pollen ranged from 79 to 1800 ng/10⁶ pollen grains and levels of airborne beta (1→3) glucan present during periods with a high pollen content in the air exceed those associated with airways inflammation.

There is relatively little information about the health effects of beta (1→3) glucan. Rylander (2006) suggests that inhaled beta (1→3) glucan suppresses the immune system causing an increased susceptibility to inflammation or sensitisation. Beta glucan is, however, marketed on the internet as nutritional supplement to boost and modulate immune response and to enhance various therapeutic healing effects generated by the immune cells (www.betaglucan.org).

The biological activity of beta (1→3) glucans is thought to be related to their molecular conformation, degree of branching and their molecular weight with high molecular weight and high levels of branching both giving rise to a higher level of activity (Bohn and BeMiller, 1995). These authors suggested that immunological effects associated binding of a beta (1→3) glucan molecule or particle probably include activation of cytotoxic macrophages, helper T cells, and NK (natural killer) cells, promotion of T cell differentiation, and activation of the alternative complement pathway. The beta (1→3) glucans backbone is essential and the most active polymers have degrees of branching (DB) between 0.20 and 0.33. Triple helical structures formed from high molecular weight polymers may be important for potentiating immune activity and effects do not appear to be linked to any specific ordered structure. Other data indicate that the distribution of the branch units along the backbone chain governs activity, β-D-glucopyranosyl units are required for potentiation of the immune response and the specific nature of the substituent is unimportant. Some data suggest that the more watersoluble polymers are more active (up to a certain degree of substitution (DS) or DB) whereas other data indicate that some insoluble aggregates are more stimulatory than the soluble polymers.

2.4 MEASUREMENT

2.4.1 Overview

Many methods have been developed for the monitoring of bioaerosols and a fully referenced longer description of measurement methods is given in Appendix 1. There is a wide range in the parameters used to characterise bioaerosol exposure and considerable variability in the way that individual parameters are determined. The most commonly reported parameters are dust, viable fungi, viable bacteria and endotoxin. Some studies have also reported beta (1→3) glucan levels. Less commonly total fungi and/or bacteria counts including both viable and
nonviable particles have been reported. The few studies that have investigated interlaboratory comparability in the determination of individual parameters have found that considerable variability exists.

2.4.2 Sampling strategy

Bioaerosol concentrations typically show dramatic fluctuations over short periods (hours) and given that most sampling techniques involve sampling for only a few minutes, this leads to uncertainty as to the representativeness of individual measures of bioaerosol concentrations at a particular locality on a given day. There is no consensus on the number of samples and total time period of sampling required to characterise bioaerosol concentrations at a particular location on an individual day or over a longer time period. There is only limited consensus on the geographical distribution of sample points required to characterise the influence of a given bioaerosol source on local air quality. Most investigators have placed samplers both upwind and downwind of the source with an expectation that the upwind sample will be representative of ambient conditions in the absence of the waste management process. There is not, however, always a clear divide between upwind and downwind concentrations. This can be due to the presence of other sources of bioaerosols in the local environment but may also reflect the local complexity of airflow around emissions sources.

2.4.3 Sampling

The preservation of organism viability during the sampling (eg minimising collision with the sampler) and subsequent to collection is an important consideration for bioaerosol sampling that has led to the development of a wide range of collection devices with very variable collection efficiencies. The principle sampling methods include impaction, impingement, and filtration. The most widely used devices are slit impactors, rotating arm impactors, and sieve impactors (Levetin, 2004). The concentrations of micro-organisms in different studies are not necessarily comparable as the exact fraction of bioaerosol considered may not be equivalent. Generally studies of viable organisms have used gel or fluid media to reduce the loss of viability of organisms within the sampler although studies of personal exposure have collected samples on filter using IOM heads or other personal sampling devices. Samples collected on filter must be quickly transferred into a fluid once sampling is complete. The most commonly used samplers for static sampling are the Andersen impactor which collects onto a gel and liquid filled impingers (particularly the All Glass Impinger – AGI30). The Andersen impactor may be used with one, two or six separate sampling plates each representing a different size fraction. Samples for endotoxin analysis can be collected as dust on filter or using a liquid impinger. Samplers for beta (1→3) glucan are typically collected onto filter. Side by side comparisons of sampling devices have demonstrated considerable variability in performance, particularly in respect to viable microorganisms which are destroyed to greater or lesser degrees by different sampling devices and protocols.

2.4.4 Sample transport and storage

Conditions during the transport and storage of samples can have a profound effect on apparent bioaerosol concentrations. Samples for microbial analysis should be kept cool and analysed within a defined time period to ensure viability is preserved. The Composting Association (1999) recommend storage of samples at a temperature of less than 4°C and the initiation of laboratory processing within 12 hours.

Microbial growth under damp conditions or desiccation and disintegration under dry conditions could also give rise to an erroneously high measurement of endotoxin, although poor sample storage can also lead to a loss of apparent activity. Samples for endotoxin analysis can be frozen for periods of months before analysis, but as endotoxin is also destroyed by repeated freeze-thaw cycles, it is important to ensure that samples are only frozen and defrosted once.
2.4.5 Analysis

Microscopy and culturing are the most important approaches to sample analysis; but immunoassays, molecular methods such as polymerase chain reaction (PCR), and other new techniques are becoming more widely used to analyze samples (Levetin, 2004). In a review, Douwes et al (2003) concluded that although the traditional culture methods to quantify microbial exposures have proven to be of limited use, experience with non-culture methods and assessment methods for microbial constituents (e.g. allergens, endotoxin, beta (1→3) glucans, fungal extracellular polysaccharides) is generally limited. The analysis for viable organisms involves culturing the sample in a suitable medium and at an appropriate temperature to support the growth of the organisms of interest – either bacteria or fungi and at temperatures appropriate to mesophilic and thermophilic species. The analysis is then performed by counting the number of colonies that develop. Counts of total viable and nonviable organisms are made microscopically using fluorescence staining to aid identification. There are uncertainties in the counting of both viable and nonviable organisms that arise from the random distribution of organisms within samples and the random selection of fields of view for enumeration.

Recently developed alternative approaches to the characterisation of airborne microbial samples have included the use of gas chromatography – mass spectroscopy (GC-MS) for the identification of characteristic compounds associated with specific groups or specific species of microbe eg 3-hydroxy fatty acids (markers of endotoxin), ergosterol (marker of fungal biomass), and muramic acid (marker of peptidoglycan/bacterial biomass). GC-MS can also be used to identify characteristic compounds emitted by specific microbial species. A number of investigators have investigated the use of quantitative polymerase chain reaction (PCR) to determine the presence and quantity of specific target DNA sequences that can be linked to individual fungal or bacterial species. A few studies have used flow cytometry to analyse bioaerosol samples. None of these alternative assessment methods have been used in epidemiological investigations of the health effects of bioaerosol exposure in the waste industry and they are of limited relevance to the consideration of exposure-response relationships based on existing studies.

Endotoxin is most commonly measured using a chromogenic Limulus amebocyte lysate (LAL) assay based on blood derived from the horseshoe crab. There are several commercial suppliers of kits for the analysis and our experience in using these kits at IOM suggests that there is considerable variation in the reliability and sensitivity of kits originating from different suppliers. Two different approaches have been used with the LAL assay. The reaction can either be allowed to progress for a fixed time period and the concentration of endotoxin assessed from the colour intensity at the end of that period (endpoint assay) or the concentration can be assessed from the rate of change of colour during the reaction (kinetic assay). There is not a consistent correlation in the results obtained using the two methods. A smaller number of studies have used GC-MS to determine the LPS content of samples. LPS levels reported using GC-MS are not directly comparable with endotoxin levels determined in the LAL assay.

Several assays have been developed for the measurement of beta (1→3) glucan, in medicine because of its clinical importance in the detection and quantification of fungal infections:

- Glucan-specific limulus amebocyte lysate (LAL) assay;
- Inhibition Enzyme-Linked ImmunoSorbent Assay (ELISA); and
- Sandwich ELISA.

The intercomparability of these methods in the assessment of exposures in the waste industry is uncertain.

There are no widely used markers of exposure to bioaerosols. Specific IgG antibodies to moulds and actinomycetes do not appear to be an effective measure of exposure in waste workers in comparison to unexposed controls. Various studies have assessed inflammatory markers in nasal lavage and sputum, but these markers are not specific to bioaerosol exposure.
There are no standard methods of measurement in the UK. The Composting Association (1999) published a “Standardised protocol for the sampling and enumeration of airborne micro-organisms at composting facilities”. The protocol was based on the use of the Andersen sampler to collect bioaerosols. This sampler relies on the impaction of airborne particles onto the surface of agar plates, which are then incubated to grow microbial colonies for enumeration and identification. The protocol requires sampling at a minimum of three locations: upwind of the site, downwind of the site, and adjacent to the nearest sensitive receptor (occupied building), the collection of relevant weather data and stipulates that sampling should not be performed at temperatures less the 5°C or during precipitation.

Currently the EA’s preferred method of monitoring bioaerosols around waste facilities is active impaction onto agar using Andersen or split samplers or liquid impingers. They indicate that analysis can be for viable micro-organisms based on cultivation of the sample and colony counting or counting of total micro-organisms using optical microscopy and staining (EA, 2004). A draft EA report (EA, 2007) indicates that in the future samples may be collected onto filter and analysis is likely to focus on thermophilic actinomycetes and Aspergillus fumigatus, as these agents are judged to be the most representative of composting material and the most likely to present a respiratory hazard. A simple methodology for sample collection and analysis may be most appropriate for basic monitoring, even if it may reduce collection efficiency to some extent. This draft report also recommends that sampling at different locations round a site should be simultaneous in order to allow direct comparison of results. A CEN working group on the “Measurement of bioaerosols in ambient air and emissions” (CEN/TC 264/WG 28) has recently been formed but this group is still at the stage of investigating current practice in different EU Member States and no draft protocols have been proposed for wider adoption (see section A1.8 of Appendix 1).

The American Society for Testing and Materials (ASTM) method E884-82 (ASTM, 2006) is a standard developed in the US for the sampling of bacterial and fungal aerosols that is intended to be applicable to waste facilities and similar workplaces. The method recommends use of a multistage impactor (eg Andersen sampler) and all-glass liquid impingers with sampling times of 30 minutes and up to 1.5 hours, respectively. It is recommended that sampling is undertaken as a minimum at one site 300 m upwind and one site 100 m downwind of the site (three replicates upwind and five downwind).

There has been very little investigation of endotoxin or beta (1→3) glucan concentrations in ambient air and methods have primarily been developed for the sampling and analysis of workplace air.

In addition to standardisation of measurement methods there is a need for a harmonised approach to sampling strategy.

The uncertainty in bioaerosol measurement is a major issue affecting the interpretation of exposure-response information. The comparability of measurements of individual measures of bioaerosol exposure made by different groups is likely to be limited. The use of slightly different measures by different groups further limits interstudy comparison. In terms of any future regulatory approach based on monitoring, there is a need to develop standardised measurement and monitoring protocols for bioaerosols. Although the EA (2004) have provided limited guidance on sampling and analysis for microbial contamination, a more harmonised approach would be desirable. Because of the difficulties in preserving organism viability during sampling, sample times for bioaerosol measurement tend to be very short from a few minutes to an hour and half whereas bioaerosol concentrations can vary dramatically in the space of a few hours (see section 3.2.2). The EA (2004) highlight the importance of acquiring large number of samples over an extended period, in order to gain a representative picture of bioaerosol concentrations. It is unclear how well published data in epidemiological studies represent actual levels of exposure.
2.5 ASSESSMENT OF THE HEALTH EFFECTS OF BIOAEROSOLS

2.5.1 Workplace studies

Most of the available information about the health effects of bioaerosol exposure comes from workplace studies. These studies have assessed symptom prevalence through questionnaires, measured markers of inflammation in the upper and lower respiratory symptoms and/or measured lung function.

Studies have evaluated the presence/absence of symptoms, markers of inflammation or markers of lung function change in exposed workers. Symptoms have most commonly been assessed using questionnaire surveys. These have identified symptoms such as cough, shortness of breath, itchy eyes, sickness, nausea, diarrhoea and fatigue. Inflammatory responses in the upper airways have been assessed through analysis of inflammatory cytokines (messenger molecules) and cell counts in nasal lavage (a procedure in which the nose is rinsed with a small volume of fluid and the fluid analysed for relevant biochemical and cellular markers). Inflammatory responses in the lower airways have been assessed by similar analysis of induced sputum and systemic inflammation has been assessed from measurement of body temperature, blood cell counts (certain cell types are specifically associated with an inflammatory response) and analysis for inflammatory cytokines. Lung function tests have been used to examine cross shift changes in lung function, changes in lung function through a working week and changes in lung function over a longer period of time. The most commonly reported lung function parameters are Forced Expiratory Volume in one second (FEV₁) – the volume of air that can be exhaled in one second; Forced Vital Capacity (FVC) - the volume change of the lung between a full inspiration to total lung capacity and a maximal expiration to residual volume; and Peak Flow Rate. Some studies have investigated immunological markers of exposure to specific bioaerosol components such as immunoglobin (Ig) antibodies to common fungi, with IgE being perceived as being of particular importance in the development of an allergic response. Skin prick tests have also been used to determine whether specific allergen challenges elicit a response.

The significance of any symptoms reporting inflammatory response or lung function changes may be assessed by comparison with unexposed workers in similar manual occupations but without bioaerosol exposure (a control group). Alternatively, workers may act as their own controls and studies may compare findings in workers following exposure to the findings in the same individuals prior to exposure (pre-employment or at the beginning of the shift or working week), after a break from exposure (eg return from holiday) or at an earlier stage in their employment history (eg tracking of lung function changes over a 5 year period). For some parameters, results for exposed workers can be compared with established norms for the wider population (eg comparison of 5 year decline in lung function versus standardised predicted decline in lung function by age and body mass). For some health endpoints such as mortality and cancer, it is possible to compare incidence rates in a study population with national rates but these endpoints have not been studied in relation to bioaerosol exposure.

Exposure-response relationships are usually investigated by assigning workers to high, medium, low and no exposure groups and ideally an indication of exposure concentrations is provided for each group, although this is frequently not done. Reported exposure concentrations are usually based on measurement data but may be entirely based on expert judgement. More sophisticated studies model exposure histories for individual workers and investigate the risk of adverse effects against a continuous scale of cumulative exposure (the product of average exposure concentration times duration of exposure). Typically exposure is only considered in terms of exposure concentration, although the effects of long term exposure are likely to be related to both the intensity and duration of exposure.

Some major limitations on the quality and reliability of information available from studies in waste workers include:

- The typically small size of the workforce investigated;
- Most studies have focussed on short term effects;
- For industries such as composting, MRF, there is a lack of long term experience; and
• Uncertainties and inconsistencies in exposure assessment.

Where only a few workers are involved in a study, it is unclear whether their susceptibility to effects is representative of the wider population e.g. less common health outcomes may not occur or may be relatively over-expressed. The confidence intervals on any risk estimates will be large and most findings are likely to lack statistical significance.

Long term studies of worker health are costly to undertake and there are likely to be substantial practical difficulties in tracing workers’ employment histories in an industry that has a relatively high staff turnover. In addition, composting and recycling are only now becoming widespread in the UK, so there is no UK experience of the health effects that may arise as a result of long term employment in these industries. It is likely that the development of short term and long term effects is linked with repeated respiratory irritation being likely to predispose to chronic respiratory disease.

Uncertainties in exposure measurement are a major difficulty in the investigation of exposure-response relationships with chemical agents and the difficulty is potentially greater for bioaerosols because of the inconsistencies in measurement methods and the parameters selected for investigation.

2.5.2 Community health

There have been a small number of studies of community health that have used questionnaires to assess symptom prevalence. Inherent problems include poor response rate and the possibility that symptom reporting may be influenced by people’s beliefs about environmental exposures such as the presence of a nearby compost plant or the transition from a one week to two week waste collection regime.

2.5.3 Experimental studies

There have been experimental studies in which small numbers of volunteers have been exposed to inhalation of endotoxin, specific fungi or beta (1→3) glucan over periods of minutes to hours. Health endpoints assessed include systemic temperature, reported symptoms, respiratory function, markers of inflammation. The advantage of volunteer studies is that exposures are well characterised. The disadvantages are that volunteers must be in good health and thus not representative of the wider population and the pattern of exposure is very different from that encountered in the wider environment.

There have also been a small number of animal studies that have exposed rats, mice or guinea pigs to inhalation of endotoxin, specific fungi or beta (1→3) glucan and endotoxin/glucan mixtures. Such studies allow investigation of the mechanisms by which specific components of bioaerosols may cause disease. There are uncertainties in the extrapolation of the findings of animal studies to the prediction of effects in humans.
3. Ambient bioaerosols in the urban and rural environment

3.1 INTRODUCTION

In order to understand the potential significance of bioaerosol emissions from the waste industry, it is important to understand the background levels of exposure to bioaerosols that are experienced by the general population in the absence of specific sources of bioaerosols from the waste industry. Concentrations in air are governed by source emissions and by weather conditions that dictate how bioaerosols are dispersed in the atmosphere (Jones & Harrison, 2004). In addition to the waste industry, other potentially important sources of bioaerosol exposure include agriculture, waste water treatment, food processing and the textile industry. This chapter reviews published information describing bioaerosol concentrations in outdoor air in urban and rural environments in the absence of waste management activities and also concentrations in indoor air. It also reviews information about background levels of exposure available from specific studies of the waste industry (for example, from upwind versus downwind comparisons at individual sites). Appendix 2 provides more detail about individual studies.

3.2 AMBIENT BIOAEROSOLS: OUTDOOR AIR

3.2.1 Endotoxin

Reported concentrations in urban air in several German, Danish and US urban areas have ranged from 0.02 to 2 EU m\(^{-3}\) with mean concentrations typically being less than 1 EU m\(^{-3}\) (Schultze et al, 2006; Heinrich et al, 2003; Carty et al, 2003; Park et al, 2000; Hines et al, 2000; Madsen et al, 2006; Mueller-ANNELING et al, 2004). There is limited evidence that higher temperatures and humidity are associated with increased levels of endotoxin in PM\(_{2.5}\) (Carty et al, 2003). Although few data are available for urban air within the UK, the data from elsewhere in Europe and from North America suggest that concentrations are likely to be less than 1 EU m\(^{-3}\). This is consistent with the results of IOM analyses of endotoxin concentrations in workplace air that suggest that background levels in the UK are typically less than 0.5 EU m\(^{-3}\) (unpublished data from commercial contracts).

Higher endotoxin concentrations have been reported in rural air. Schultze et al (2006) reported that mean ambient concentrations of endotoxin in a rural area reached a maximum of 23.2 EU m\(^{-3}\) during the summer and in a recent review, Madson (2006) reports that the median endotoxin concentration on an agricultural field was 2.9 EU m\(^{-3}\). There are limited data that indicate that bioaerosol emissions from intensive animal rearing houses raise ambient bioaerosol levels some distance downwind (SCAIFFE et al, 2007). Hartung et al (1998), for example, reported endotoxin concentrations of about 600 and 150 EU m\(^{-3}\) at 50 and 115 m downwind from livestock buildings.

3.2.2 Fungi

There is little published information about ambient levels of fungi in outdoor air in the UK. In studies reviewed by Swan et al (2003) for HSE (Health and Safety Executive), Jones and Cookson (1983) reported mean concentrations of mesophilic fungi of 273 cfum\(^{-3}\) (range 0-7200) and mean concentrations of thermophilic fungi of 2.1 cfum\(^{-3}\) (range 0-193) and Lacey and Crook (1988) reported concentrations of 1200 and 300 cfum\(^{-3}\) for mesophilic and thermophilic species respectively. In an investigation of the relationship between levels of fungal spores in ambient air and asthma, Newsona et al (2000) reported that mean concentrations of total spores in Derby air were 10101 with a 90th percentile of 23790. In a similar study of the relationship between fungal spores and asthma exacerbations, Atkinson et al (2005) reported total spore counts of 4425 spores m\(^{-3}\) (daily mean concentration; range 39-16119) for samples collected in central London. Anderson et al (2001) reported that concentrations of total (viable and nonviable) fungal spores in central Cardiff range from 100 spores m\(^{-3}\) in the winter to 3500 to 4000 spores m\(^{-3}\) during the summer.

Extensive measurements made by DSTL (Defence, Science & Technology Laboratory) during the 1990s demonstrated that concentrations of individual microbial species varied from below
detection limit levels to thousands of organisms/m$^3$ on different days (Table 3.1). Concentrations also varied during the course of individual days. For example, measurements of Aspergillus/penicillium made in Birmingham Botanic Gardens during the course of one day ranged from 439 to 16176 spores/m$^3$ rising to 21231 spores/m$^3$ early the next day and then falling to 472 spores/m$^3$ by that evening. There was also a strong seasonal variation in microbial concentrations with many species being most prevalent in the autumn.

Table 3.1: Summary of selected fungal counts (spores/m$^3$) from DSTL study of background bioaerosol concentrations in the UK (see Table A2.3; Appendix 2 for the full species range investigated)

<table>
<thead>
<tr>
<th>Location</th>
<th>Birmingham</th>
<th>Lichfield</th>
<th>Lizard</th>
<th>Pershore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Botanic gardens, 3 miles from city centre</td>
<td>Army training camp in area of arable farming</td>
<td>Little used airfield, 0.5 miles from coast</td>
<td>Agricultural area, landfill 0.5 miles from site</td>
</tr>
<tr>
<td>Ascospores</td>
<td>mean: 102 max: 3067</td>
<td>mean: 55 max: 1428</td>
<td>mean: 469 max: 10177</td>
<td>mean: 221 max: 5610</td>
</tr>
<tr>
<td>Aspergillus/penicillium</td>
<td>mean: 1274 max: 21231</td>
<td>mean: 1247 max: 16682</td>
<td>mean: 324 max: 3539</td>
<td>mean: 16682 max: 10880</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>mean: 1239 max: 16811</td>
<td>mean: 1454 max: 14826</td>
<td>mean: 501 max: 6679</td>
<td>mean: 2548 max: 27200</td>
</tr>
<tr>
<td>Myxomycete</td>
<td>mean: 67 max: 3943</td>
<td>mean: 4 max: 67</td>
<td>mean: 5 max: 135</td>
<td>mean: 10 max: 641</td>
</tr>
</tbody>
</table>

Studies in north America and elsewhere in Europe have reported mean outdoor concentrations ranging from <500 to >3500 cfu m$^{-3}$ (Shelton et al., 2002; Lugauskas et al., 2003; Horner et al., 2004) with concentrations being higher in summer than in the winter (Lugauskas et al., 2003; Horner et al., 2004). In a Polish publication, Krajewski et al. (2001b) reported that the number of fungal spores, 10$^4$ m$^{-3}$ of air on city streets was similar to that observed in the waste reloading and composting plants, but lower than that associated with waste collection.

In conclusion both UK data and the results of studies elsewhere suggest that concentrations are typically less than 1000 cfu m$^{-3}$ but may be considerably higher, particularly during the autumn.

3.2.3 Bacteria

There appears to be little information about ambient airborne concentrations of bacteria in urban areas. In studies reviewed by Swan et al. (2003), mean bacterial concentrations in suburban air of 79 cfu$^3$ (range 42-1600; Jones and Cookson, 1983), 500 cfu$^3$ (Lacey and Crook, 1988) and 850 cfu$^3$ (range 100-4000; Bovallius et al., 1978) were reported. The DSTL study reported bacterial concentrations ranging from 100 to 65000 organisms/m$^3$ (Table 3.2).

Table 3.2: Summary of selected microbial counts from DSTL study of background bioaerosol concentrations in the UK

<table>
<thead>
<tr>
<th>Location</th>
<th>Birmingham</th>
<th>Lichfield</th>
<th>Lizard</th>
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<td>Little used airfield, 0.5 miles from coast</td>
<td>Agricultural area, landfill 0.5 miles from site</td>
</tr>
<tr>
<td>Bacteria</td>
<td>mean: 100 max: 33000</td>
<td>mean: 100 max: 53000</td>
<td>mean: 100 max: 65000</td>
<td>mean: 100 max: 33000</td>
</tr>
</tbody>
</table>

In an investigation of bacterial populations in two US cities over 17 weeks, Brodie et al. (2007) determined that at least 1,800 types of bacteria were present in urban air including the consistent presence of bacterial families with pathogenic members. Seasonal and meteorological influences were more important than location determining bacterial compositions. In a Polish publication, Krajewski et al. (2001b) reported that on city streets
concentrations of Actinomycetes were below detection limit (<10^2 cfum^{-3}). Tsai and Macher (2005) reported that mean outdoor concentration of culturable bacteria in the vicinity of 100 office buildings in the US were 194 cfum^{-3} in the winter and 165 cfum^{-3} in the summer.

A number of studies have been conducted in rural areas to investigate the impacts of sewage sludge spreading on health (NRC, 2000; Tanner et al, 2005; Forcier, 2002) but there is no evidence to date to indicate any excess risk to community health as a result of increased offsite exposure to bioaerosols arising from sludge spreading. Intensive animal rearing is another important rural source of bioaerosols and effects on airborne bacterial concentrations have been reported at distances of up to 450-480m from pig and poultry units (Scaife et al, 2007). Plaztz et al (1995) reported concentrations of airborne bacteria at a distance of about 100 m from pig houses of 17800 cfum^{-3} in winter and 930 cfum^{-3} in summer.

Allowing for uncertainties in the measurement data and the small quantity of data available, it seems probable that typical bacterial concentrations in urban air are less than 1000 cfu m^{-3}, whereas much higher concentrations may arise locally in rural areas in association with particular agricultural activities.

3.3 AMBIENT BIOAEROSOLS IN THE INDOOR ENVIRONMENT

3.3.1 Introduction

There has been substantial interest in the role of ambient bioaerosols in contributing to the symptoms of sick building syndrome, particularly in the US. A large number of measurement surveys have been carried out in US offices and studies have also been conducted in Scandinavia, elsewhere in northern Europe and also in the Far East. Given the substantial differences in building ventilation and climate in the UK compared to elsewhere in Europe and in the US, the data described below may be of limited relevance to the UK situation.

3.3.2 Endotoxin

Limited published data from studies conducted in North American and Polish homes suggest that airborne endotoxin levels are typically much less than 1 EUm^{-3} although some individual measurements have been as high as 20 EU m^{-3} (Park et al, 2000; Gorny & Dutkiewcz, 2002; Miller et al, 2007). Cigarette smoke can be a major source of endotoxin in indoor environments. Sebastian et al (2006) reported that concentrations of endotoxin were 4-63 times higher in the rooms of smoking students than in identical rooms of non-smoking students.

3.3.3 Fungi

Most studies have focussed on exposure in moisture-affected buildings. In a UK study of 24 homes reviewed by Swan et al (1993), Hunter and Lea (1994) reported mean fungal concentrations of 1096 cfum^{-3} (range 28 - >35000) with maximum levels arising during the autumn. There was little difference between concentrations found in urban or rural locations.

Studies undertaken in homes and offices elsewhere in Europe and in the US have typically reported mean fungal concentrations of less than about 500 cfum^{-3} (Horner et al, 2004; Lee et al, 2006; Chao et al, 2002; Macintosh et al, 2006; Shelton et al, 2002). Concentrations of up to 2000 cfum^{-3} have been reported in houses without mould problems with concentrations exceeding 10000 cfum^{-3} in some homes with moisture problems (Gorny & Dutkiewicz, 2002). Some authors such Chao et al (2002) and Shelton et al (2002) have found significant seasonal variation in office concentrations with concentrations being highest in summer and autumn and lowest in winter whereas others such as Macintosh et al (2006) failed to identify any seasonal variability in indoor air, although outdoor concentrations were markedly lower in winter. Chao et al also reported that concentrations positively correlated with relative humidity and negatively related to carbon dioxide concentrations. In a Finnish study of personal exposures to viable fungi and bacteria, associations were found between personal exposure and concentrations in the home and work environment (Toivola et al, 2004). Geometric mean personal exposure concentrations were 3-12 cfum^{-3} for total viable fungi, 0.6-3.7 cfum^{-3} for
Penicillium and mainly under 1 cfum\(^{-3}\) for other fungi with a total of 39 genera being identified in personal samples.

In summary, levels of exposure to airborne moulds in indoor environments are hugely variable (from <100 to 20000 cfum\(^{-3}\)). Only some of this variability could be attributable to measurement variability. Both building dampness and concentrations in outdoor air are important influences on concentrations.

### 3.3.4 Bacteria

Limited data from studies conducted in the US and elsewhere in Europe suggest that typical concentrations of bacteria in home and office environments are about 100 cfum\(^{-3}\) but may be considerably higher in some buildings (Tsai and Macher, 2005; Bouillard et al, 2005 Gorny & Dutkiewicz, 2002; Krajewski et al., 2001b; Lee et al., 2006).

### 3.3.5 Beta (1\(\rightarrow\)3) glucan

Few studies have investigated ambient levels of exposure to beta (1\(\rightarrow\)3) glucan. Miller et al (2007) reported that median levels of glucan in Canadian homes were less than 2 ngm\(^{-3}\) (range 0.3–8.5 ngm\(^{-3}\)). Beijer et al (2002) reported that (1\(\rightarrow\)3) beta glucan concentrations in 35 Swedish homes ranged from 0.2-51.7 ngm\(^{-3}\).

### 3.4 BACKGROUND LEVELS REPORTED IN STUDIES OF WASTE MANAGEMENT ACTIVITIES

Background bioaerosol concentrations reported in studies of the waste industry are summarised in Appendix 2 (Table A2.6). The reported concentrations are extremely variable, ranging from < 100 to about 6000 cfum\(^{-3}\) for total bacteria, <5 to >8000 cfum\(^{-3}\) for viable fungi and about 2 EU m\(^{-3}\) for endotoxin. The background levels of bacteria and fungi in several studies are higher than would be typical of urban air or indoor air but are not dissimilar to concentrations of fungi and bacteria found in some buildings with damp problems. Measured levels of bacteria and fungi tend to be lower in the winter than in the summer. The background levels of endotoxin reported in waste studies appear to be fairly typical of those reported in other studies of ambient air quality or indoor air quality.

### 3.5 CONCLUSIONS

Background levels of exposure to bioaerosols are hugely variable. Typical endotoxin levels in outdoor urban air are less than 1 EU m\(^{-3}\), but slightly higher concentrations may arise in some rural locations where agricultural activities emit endotoxin. Indoor concentrations of endotoxin are heavily influenced by environmental tobacco smoke. Fungal concentrations in outdoor air vary by location and season. Although concentrations are typically less than 1000 cfum\(^{-3}\), they may be considerably higher, particularly during the autumn. Reported fungal concentrations in indoor air range from <100 to 20000 cfum\(^{-3}\) and are affected by both external factors and by building dampness and ventilation. There are few data describing background exposures to bacteria. Concentrations of bacteria in indoor environments are typically about 100 cfum\(^{-3}\) but may be considerably higher in some environments.

Background concentrations of bacteria and fungi measured in studies of the impacts of waste management process tend to be higher than those typically found in indoor or outdoor air in urban areas but are comparable to levels reported in some buildings with damp problems.
4. Emissions and exposure to bioaerosols from waste management activities

4.1 INTRODUCTION

This chapter reviews information about bioaerosol emissions from the waste industry and information about workplace and community exposure to bioaerosols arising from the waste industry. It is based on the detailed review of emissions and exposure data provided in Appendices 3 and 4.

Bioaerosols become airborne by processes that include forcible release, mechanical disruption and nebulisation. Bioaerosol emissions from waste are dependent on a wide range of factors that consequently affect the potential for exposure. Principally, these are i) waste composition, ii) conditions of storage, and iii) treatment processes. Associated with these factors are more specific attributes including: how the waste was generated and its physical form; the storage container type; the profile of microbiological species present; and physical conditions including temperature and pH.

The likelihood and magnitude of exposure to bioaerosols in the waste processing industry have been shown to depend upon a number of factors including an individual’s job role, their proximity to various mechanical processes handling the waste, and the season. Exposure measurements, using techniques described earlier in Chapter 3 are generally either conducted at the individual level using personal monitoring, or at the area level using environmental monitoring. Most workplace studies have focused on total organic dust, endotoxin and some measure of microbial exposure (e.g. total or viable micro-organisms or fungal colony forming units). A few studies have also measured exposure to beta (1→3) glucan – a marker of fungal contamination in air, and volatile organic compounds (VOCs). A small number of studies have investigated community exposure to bioaerosols in the vicinity of waste processes. Exposures to other components of bioaerosols: protozoa, algae, cyanobacteria, nematodes and insect larvae have not been investigated in the waste industry.

4.2 BIOAEROSOL EMISSIONS FROM WASTE HANDLING PROCESSES

4.2.1 Waste Collection

The limited data available suggest the nature of bioaerosol emissions from stored waste in the home and its subsequent collection varies depending on the waste composition and its storage conditions (Appendix 3). The potential for exposure of waste collection to bioaerosols and biologically-active liquid leachate is anticipated to increase given the emphasis being placed on alternative and more sustainable waste management practices (e.g. composting, recycling) in homes across the UK and elsewhere. Source separated biowastes, unsorted waste and residuals are associated with higher bioaerosol emissions than dry recyclate such as paper. The indoor storage of biowastes may also give rise to increased residential exposure to mould and endotoxin, although emissions can be minimised by ensuring that waste is stored in closed containers that are kept in cool conditions.

4.2.2 Composting

Composting is likely to be the major source of bioaerosol emissions from the waste industry. The change in microbial population in waste during the composting process, the methods and extent of materials handling during composting, the quantities of waste handled and whether the process is undertaken in the open air or a closed building are likely to be a major influence on emissions.

The composting process can be divided into three key stages (Gilbert et al, 2001):

- High rate composting when micro-organisms consume forms of carbon that can be readily broken down and a high rate of biological activity is associated with high oxygen demand and heat generation;
- Stabilisation when micro-organisms break down the less available forms of carbon such as cellulose, biological activity starts to decline and oxygen demand and temperature reduce; and
- Maturation when the compost is recolonised by soil microbes and lower rates of biological activity are associated with a low to medium oxygen demand and temperatures of less than 50°C.

During the first stage, the microbial population is dominated by bacteria, particularly actinomycetes, and bacterial counts initially increase as temperature increases with a concurrent replacement of mesophilic species by thermophilic species. Provided sufficient heat is generated during the composting process, the number and diversity of bacteria is reduced as temperature increases and any human pathogens should be destroyed (Swan et al, 2003). During cooling and stabilization, fungal species, including Aspergillus fumigatus, dominate.

Emissions of volatile organic compounds (VOCs; and odour arising from VOCs) are influenced by the microbial population present in composting waste.

4.2.3 Other waste handling activities

There is very little information about bioaerosol emissions from landfill or other waste handling operations. However, data are available about workplace exposures in other waste sectors that provide some indication of the variability in emissions rates and exposure control between sites.

4.2.4 Control measures

Several measures have been suggested to reduce the build up of bioaerosols, VOCs and any biologically-active liquid during the storage of household waste prior to collection including timely waste preparation, the wrapping of wastes in newspaper, storage in a closed container, cleaning of the container prior to refilling, and storage in a cool place (Appendix 3).

A number of measures have been suggested to reduce emissions from commercial composting operations including appropriate control of moisture content and airflow during composting, using water mist to dampen dust over screen conveyers, providing adequate ventilation in buildings and using in-vessel systems. Biofiltration may be an effective method for reducing odour nuisance and may also reduce microbial counts. The Composting Association has published a good practice guide (Appendix 3). Several studies have demonstrated the importance of heat in the reduction of the bacterial content of wastes, particularly in faecal waste in animals. These studies have also demonstrated, however, that incomplete sterilisation or subsequent recontamination by leachate may lead to bacteria recolonising the waste during maturation and cooling of the compost. Current practices may be insufficient to completely remove pathogenic species from the end product and therefore, potentially from emissions (Appendix 3).

4.3 BIOAEROSOL EXPOSURES ASSOCIATED WITH WASTE HANDLING PROCESSES

4.3.1 Waste collection

Bioaerosol exposure during waste collection has been investigated in a number of studies in various European countries that are generally climatically similar to the UK (Table A4.1, Appendix 4). There are clear differences in the pattern of exposure in different studies with the highest levels of exposure to dust, fungi, endotoxin or beta (1→3) glucan arising in separate studies. Reported mean or median concentrations of viable fungi vary from 30 to 10000 cfum⁻³, mean/median concentrations of bacteria range from 1700 to 80000 cfum⁻³, mean/median dust concentrations range from 0.4 to 8 ngm⁻³, mean/median endotoxin concentrations from 13 to about 370 EUm⁻³ and mean/median betan (1→3) glucan concentrations range from about 10 to more than 1000 ngm⁻³ (Heldal et al 2003a; Krajewski
et al., 2002; Thorn 2001; Wouters et al., 2002; Bunger et al., 2000). The highest reported mean level of dust exposure for waste collection workers is 7.7 mg m\(^{-3}\) for the loader with only a marginally lower exposure for the driver (mean 6.3 mg m\(^{-3}\); Krajewski et al., 2002). Levels of endotoxin exposure were also particularly high in this study (36 ng m\(^{-3}\), about 350 EU m\(^{-3}\)) and were associated with gram-negative rod bacteria (primarily intestinal). Viable microorganisms in the air samples collected from the breathing zone of waste collection workers in Poland include various species of Gram-positive bacteria (Micrococcus, Staphylococcus, Enterococcus, Bacillus, Listeria, ory nebacterium, Rhodococcus) and Gram-negative rods (Enterobacteriaceae, Pseudomonas and other aerobic bacteria) as well as faecal bacteria (Escherichia coli, Enterococcus faecalis and Enterococcus faecium; Krajewski et al., 2001a, b). Several studies have reported higher levels of bioaerosol exposure during the summer than in the winter (Thorn et al., 2001; Neumann et al., 2002).

Neumann et al. (2005) specifically investigated factors of workplace design that affect the bioaerosol concentration to which refuse collectors are exposed: compaction method, lifting device control, rave rail height, hopper depth, design of intake area and dust interception. Notably higher total fungal counts were recorded with rotating drum compaction than with packer plate compaction. Bioaerosol exposures were reduced by the use of a hinged lid closure at the lifting device in conjunction with a suction unit. In addition, the automatic lifting system reduced levels of bioaerosol exposure associated with rear-end loading vehicles, as did loading operation control from the closed driver’s cab on side loaders. Exposures could be reduced through regular internal and external high-pressure cleaning of the lifting device at intervals of not more than 14 days. Previously, Neumann et al. (2002) reported that workplace hygiene, the prevailing 1-week collection interval, and the low in-process exposure time resulting from the effective deployment of automatic lifting devices contributed to low levels of bioaerosol exposure for waste collection workers and that concentrations in the cab of the collection vehicle were at least a factor of ten lower (for all components) than outside. In a Canadian study of waste collection workers, Lavoie et al. (2006) reported that the highest personal exposures to bacteria were observed for urban compostable waste collectors (median = 50,300 cfum\(^{-3}\)), whereas the highest fungal counts were found among a group of rural compostable waste collectors on a fortnightly collection cycle (median = 101,700 cfum\(^{-3}\)).

### 4.3.2 Waste transfer stations

There appears to be very little published information about bioaerosol exposure at waste transfer stations. Van Tongeren et al. (1997) recorded particularly high concentrations of total fungi and bacteria (10\(^5\) cfum\(^{-3}\)) in the dumping pit at one of the waste-transfer stations in their study (Appendix 4, Table A4.2).

### 4.3.3 Composting

**On-site concentrations**

In a review for The Composting Association of Ireland, Prasad et al. (2004) concluded that reported dust concentrations at composting sites ranged from 0.1 to 12 mg m\(^{-3}\), but were generally below 2 mg m\(^{-3}\), and dependent upon the type of activity taking place. Reported bacterial concentrations range from about 10\(^4\) to 10\(^7\) cfum\(^{-3}\) with Acetinomycetes ranging up to about 10\(^5\) cfum\(^{-3}\). The balance of mesophilic to thermophilic species varies between studies, probably as a result of changes in the microbial content of compost as the composting process progresses. Fungal concentrations at compost sites range from about 10\(^2\) to 10\(^7\) cfum\(^{-3}\) (Table A4.3; Appendix 4). Fungal concentrations tend to be higher during the summer than winter probably as a result of lower temperatures curbing microbial growth (Prasad et al., 2004). Reported endotoxin concentrations range from 1 to about 1000 EU m\(^{-3}\) and beta (1→3) glucan concentrations range from <1 to about 5000 ng m\(^{-3}\). In one study the seasonal variation in endotoxin concentrations was from 1 to 640 ng m\(^{-3}\) (Prasad et al., 2004).

In a study of the composting of green waste in windrows, Sanchez-Monedero et al. (2005) reported that the shredding of fresh green wastes and pile turning gave rise to bioaerosol concentrations that were 100 times higher than background levels at 40 m downwind of the composting pad. Screening of mature compost gave rise to concentrations of A. fumigatus...
that were about 10 times higher than background levels.

Tolvanen et al (2005) reported relatively high concentrations of bioaerosols and odour in the working air of a drum composting plant treating source-separated catering waste. Odour concentrations measured using an olfactometer were 23,000 OU m\(^{-3}\) at the output end of the composting drum, 6300 OU m\(^{-3}\) in the exhaust pipe and 500 and 560 OU m\(^{-3}\) inside the composting hall.

**Worker exposure**

Reported personal exposures to bioaerosols are highly variable with some individual measurements indicating dust concentrations that exceeded 20 mg m\(^{-3}\) and endotoxin concentrations that exceed 20,000 EU m\(^{-3}\), although most reported exposure concentrations are less than 10% of these levels (Table A4.3; Appendix 4). Van Tongeren et al (1997) reported particularly high levels of exposure of inhalable organic dust (up to 9.7 mg m\(^{-3}\)) and exposure to micro-organisms associated with the screening of compost. Krajewski et al (2002) reported a slight predominance of mesophilic species in personal samples from workers operating the bulldozer and composting plant machine at a compost site.

**Community Exposure**

Several groups have investigated community exposure to bioaerosols emitted from composting (Table A4.4). Herr et al (2003a) reported concentrations of >10\(^5\) cfum\(^{-3}\) of thermophilic actinomycetes, moulds and total bacteria in air 200 m from a large composting site in Germany, dropping to near background concentrations within 300 m. In an investigation of bioaerosols emitted from a suburban yard waste composting facility in northern Illinois, Hryhorczuk et al (2001) reported that on-site microbial concentrations showed a statistically significant pattern of decreasing concentration with distance from pile and were higher downwind compared with upwind. Mean on-site and offsite (at 150 m distance downwind) concentrations of viable bacteria, viable fungi, and endotoxins were significantly higher during periods of activity compared to periods of no activity. The highest concentrations of total particulates, endotoxin, and beta-1,3-glucans were observed in personal samples for workers at the facility. However, in a study published in Germany, Knop et al (1996) reported that measured mould concentrations of up to 8.4 \times 10^3 cfum\(^{-3}\) within a compost facility, were associated with levels of microbial contamination in the filtered air from the compost facility that did not exceed the concentration measured in air outside the plant. The data were obtained during the winter months and probably represented the lower end of the average exposure range over the entire year. In a review of studies that had investigated the dispersal of bioaerosols from composting sites, Swan et al (2003) reported that bioaerosol concentrations generally reduce to background levels at distances of about 200 m from source (The Composting Association, 1999; Gilbert et al, 2002; Passman, 1983; Reinthaler et al, 1998/99; Sanchez-Monedero et al, 2007). Syzdek and Haynes (1995), Kohary et al (1984) and Schilling et al (1999), however, all reported raised concentrations of *Aspergillus fumigatus* at sampling sites at much greater distances from composting facilities (540 m, 250 m and 500 m respectively).

Muller et al (2004a and b) collected air samples at distances ranging from 50 to 800 m in a downwind direction from three different waste composting facilities. Compost-derived and microbial volatile organic compounds (MVOC) were found at distances of up to 800 m from the composting facilities. Concentrations of single compounds (alcohols, ketones, furanes, sulphur-containing compounds and especially terpenes) ranged from 10\(^2\) up to nearly 10\(^3\) ng m\(^{-3}\). Terpenes like alpha-pinene, camphene and camphor were the dominant compounds and coincided with typical compost odour, whereas several typical MVOC were not found at greater distances.

The Environment Agency has funded several studies of bioaerosol emissions from composting and municipal solid waste activities (Tables A4.4, 5). In a study of three selected composting sites, Wheeler et al (2001) found that all operations released bioaerosols but emissions were very variable due to the batchwise nature of the composting process whereas Crook et al (2006) found that elevated bioaerosol concentrations were not consistently
associated with specific operations on site. Wheeler et al (2001) reported that on-site concentrations of viable bacteria ranged from less than detection limit to \(7 \times 10^6\) cfu m\(^{-3}\). Concentrations of bioaerosol were shown to decline with distance from the site and concentrations measured at distances of 100 – 150 m were mostly close to zero. Concentrations of viable bacteria measured 80 m from the site were generally less than 100 cfu m\(^{-3}\) and the levels at 150 m were less than 5 cfu m\(^{-3}\). At one site concentrations of 300 cfu m\(^{-3}\) and 50 cfu m\(^{-3}\) were measured at distances of 50 m and 100m respectively. Crook et al (2006) found that bioaerosol concentrations were generally but not always higher downwind than upwind of compost operations and generally reduced with distance from source.

4.3.4 Materials recovery facilities

Mean workplace exposure concentrations for bioaerosols and VOCs at materials recovery facilities (MRFs) tend to be towards the upper end of the range of those reported for waste collection workers and landfill which possibly reflects work within an enclosed space rather than outside (Van Tongeren et al, 1997; Kiviranta et al 1999). Reported median/mean dust concentrations for MRFs are between \(<1\) and 8 mgm\(^{-3}\) and are associated with endotoxin levels of about 50 – 4000 EU ngm\(^{-3}\) (Gladding et al, 2003; Van Tongeran et al, 1997; Krejewski et al, 2002 and Kiviranta et al, 1999; Table A4.6). Krejewski et al (2002) reported that exposures to endotoxin of 61 ngm\(^{-3}\) at a Polish waste sorting facility were combined with the presence of gram-negative rods (primarily intestinal). In other studies that have examined microbial exposures, mean total viable bacterial counts have ranged from less than \(10^3\) to more than \(10^6\) cfu m\(^{-3}\) and mean fungal counts have ranged from about \(10^3\) to about \(10^6\) cfu m\(^{-3}\) (Lavoie and Guertin, 2001; Van Tongeran et al, 1997). Gruner et al (1999) reported that some refuse sorting workers had exposures to moulds that exceeded \(10^6\) cfu m\(^{-3}\) and exposures to bacteria exceeded \(10^5\) cfu m\(^{-3}\) (study published in German). Only one study, Gladding et al (2003), has reported exposures to beta (1\(\rightarrow\)3) glucan which were about 15 ngm\(^{-3}\). In a study that investigated the potential for community exposure to bioaerosols arising from MRFs (Table 6.4), a small elevation in microbial concentrations was observed downwind of a MRF relative to samples collected upwind.

Increased levels of workplace exposure may arise in the waste delivery area of MRFs with dust levels being correlated with the frequency of deliveries (Knop et al, 1996) and also during the sorting of waste with Lavoie & Guertin (2001) reporting the highest exposures in sorting workers. In addition elevated exposure to bacteria and fungal spores associated with receiving, sorting and shipping recyclate were considerably greater in the summer than in the winter (Lavoie and Guertin, 2001). Gladding et al (2003) determined that similar levels of bioaerosol exposure were associated with the sorting of box-collected, bag-collected, twin bag collected or mixed waste.

4.3.5 Other waste treatment processes

Reported mean levels of workplace exposure to dust and total organisms (viable and unviable) arising during the production of refuse-derived fuel (RDF) are less than 1 mgm\(^{-3}\) and \(10^7\) m\(^{-3}\), respectively with endotoxin concentrations ranging up to 1000 ngm\(^{-3}\) in the summer but being generally less than 33 ngm\(^{-3}\) in winter (Mahar et al, 1999; Tolvanen, 2001; Table A4.7, Appendix 4). The personnel cleaning the plants had significantly higher exposures than other workers (Mahar et al, 1999). A Finnish study of the concentrations of dust, microbes and endotoxins at a plant treating catering waste found elevated concentrations of microbes and endotoxins during waste crushing and in the bioreactor hall (Table A4.8; Tolvanen and Hanninen, 2006). Overall bioaerosol concentrations were comparable with those measured at other closed waste treatment plants in Finland and lower than in some Finnish dry waste treatment plants.

There has been little investigation of bioaerosol exposure arising at waste incineration plants but the limited available data suggest that exposure concentrations associated with waste storage may be extremely high in some plants (Table A4.9; Tolvanen and Hänninen 2005; Swan et al, 2003). One study reported mean concentrations of \(3.3\) mgm\(^{-3}\) for dust, \(24500\) and \(2670\) cfu m\(^{-3}\) for mesophilic and thermophilic bacteria, \(118225\) and \(5235\) cfu m\(^{-3}\) for mesophilic and thermophilic fungi and \(39500\) EUm\(^{-3}\) for endotoxin.
There is little information about bioaerosol exposure in relation to landfill operations (Appendix 4; Section A4.9). Reported mean exposure concentrations for landfill workers for dust range up to 1.5 mg m\(^{-3}\), concentrations of fungi range from <100 cfu m\(^{-3}\) to more than 2.6\(\times\)10\(^5\) spores m\(^{-3}\), concentrations of thermophilic bacteria range from <10\(^3\) to 21\(\times\)10\(^3\) organisms m\(^{-3}\) and concentrations of total bacteria of up to 8.2 \(\times\)10\(^5\) cfu m\(^{-3}\) have been reported. Reported workplace exposures to endotoxin are variable – about 4000 EU m\(^{-3}\) in one study and less than 200 EU m\(^{-3}\) in another. Concentrations of bioaerosols reported on landfill sites, downwind of active operations are generally lower than the personal exposure concentrations reported for workers. Bioaerosol concentrations measured downwind of active operations are not consistently higher than those measured upwind.

**4.3.6 Factors influencing bioaerosol exposure**

Elevated levels of workplace exposure to bioaerosols are found widely throughout the waste industry including waste collection, materials recovery, composting and the storage of waste material prior to incineration. Exposure levels in most sectors tend to be higher during the summer but are not clearly linked with waste composition. There is limited evidence that waste storage may lead to increased exposure concentrations and more substantive evidence that increased activity levels are associated with increased bioaerosol exposures. Exposure levels vary within individual sectors suggesting that there is potential to reduce exposures through good practice.

**4.4 CONCLUSIONS**

Elevated levels of workplace exposure to bioaerosols are found widely throughout the waste industry including waste collection, materials recovery, composting and the storage of waste material prior to incineration. Reported exposure concentrations range from less than 100 to up to about 10\(^7\) cfu m\(^{-3}\) for viable bacteria and similar levels have also been reported for viable fungi. Reported concentrations of endotoxin range up to about 40000 EU m\(^{-3}\) but are more commonly between about 50 and 500 EU m\(^{-3}\). Exposure concentrations for beta (1\(\rightarrow\)3) glucan are less widely reported but vary from about 10 to 10000 ng m\(^{-3}\). Exposure levels in most sectors tend to be higher during the summer but are not clearly linked with waste composition. There is limited evidence that waste storage may lead to increased exposure concentrations and more substantive evidence that increased activity levels are associated with increased bioaerosol exposures.

There are clear differences in the exposures reported in different studies of waste collection workers with the highest levels of exposure to dust, fungi, endotoxin or beta(1\(\rightarrow\)3) glucan arising in separate studies. The results of the few studies of bioaerosol exposure at MRFs suggest that waste sorting is associated with particularly high exposures in some plants with reported concentrations of moulds that exceed 10\(^5\) cfu m\(^{-3}\) and concentrations of bacteria that exceeded 10\(^4\) cfu m\(^{-3}\). Reported mean/median workplace concentrations for composting are hugely variable. For dust, they range from about 0.5-5 mg m\(^{-3}\), for bacteria from about 5 \(\times\)10\(^3\) to 10\(^7\) cfu m\(^{-3}\) and for fungi from about 20 to 10\(^7\) cfu m\(^{-3}\). Reported mean/median endotoxin concentrations range from less than 100 to over 700 EU m\(^{-3}\) and mean/median beta (1\(\rightarrow\)3) glucan concentrations range from about 0.5-5 ug m\(^{-3}\). A few studies of community exposure to bioaerosols emitted from composting have found evidence of raised levels of bacteria and fungi in air at distances of up to 0.5 km from compost sites whereas most have found no evidence of increased bioaerosol concentrations at distances greater than about 200-250m. This variability suggests that good practice could reduce exposure to bioaerosols.

Elevated workplace exposure to bioaerosols has been reported in the waste storage area at an incineration plant and associated with the production of refuse-derived fuel (RDF) with reported endotoxin concentrations exceeding 10000 EU m\(^{-3}\) in one plant in the summer. The limited data available for landfill workers suggest that levels of bioaerosol exposure are highly variable, even at a single site.
5. Health effects of bioaerosol exposure and exposure-response information

5.1 INTRODUCTION

This chapter reviews published information relevant to understanding exposure-response relationships for bioaerosols from studies conducted in the waste industry, other industries and experimental data. This chapter is organised by bioaerosols component: organic dust, bacteria, endotoxin, fungi and beta (1→3) glucan. In practice, workplace or environmental exposure to bioaerosols involves combined exposure to all of these agents although individual study authors may have only included some components in their analysis. Most studies in the waste industry have focussed on the short term rather than long term effects of bioaerosol exposure.

5.2 NON-SPECIFIC EXPOSURES ASSOCIATED WITH WASTE HANDLING

A number of investigators have investigated the health of waste workers exposed to bioaerosols in the absence of detailed exposure information. Studies of workers at transfer stations, landfills and incineration plants have reported an increased risk of pulmonary disorders and gastrointestinal problems that may be associated with elevated exposures to total airborne dust, bacteria, faecal coliform bacteria and fungal spores (Poulsen et al, 1995). Workers involved in manual sorting of unseparated domestic waste, as well as workers at compost plants experience symptoms of organic dust toxic syndrome (see section 5.3 below), gastrointestinal problems such as nausea and diarrhoea, irritation of the skin, eye, nose and upper airways (Poulsen et al, 1995a and b). In addition cases of severe asthma, alveolitis (inflammation within the gas-exchange region of the lung) and bronchitis have been reported. (Poulsen et al, 1995a and b). Workers in plants sorting source-separated domestic waste, have generally lower exposures but may have an increased risk of gastrointestinal symptoms and irritation of the eyes and skin (Poulsen et al, 1995a and b).

A number of Danish investigations of recycling workers undertaken during the early 1990s, found an increased prevalence of respiratory, gastrointestinal and skin symptoms in comparison to other blue-collar workers (Sigsgaard, 1999). Most cases of occupational asthma were associated with poor control of exposure to organic dust arising from lack of knowledge about its potential harmfulness. However, even with good industrial hygiene and use of the proper protective equipment, waste handling was associated with a small but significant risk of occupational asthma.

In a study of household waste collection workers in Taiwan, Yang et al (2001) reported that household waste collection is associated with excess risks for the development of chronic respiratory symptoms (cough, phlegm, wheezing, and chronic bronchitis).

Heldal et al (2003) used nasal lavage to demonstrate a progressive development of upper airways inflammation in waste handlers during the working week, as evidenced by increases in cellular markers of inflammation and the development of nasal swelling. In a study in organic waste loaders, De Meer et al (2007) reported a slight decline in lung function over the working week. Airway responsiveness increased in workers who reported regular symptoms whereas it declined slightly over the week in other workers.

In a study of community exposure to bioaerosols arising from stored organic waste in the home, Herr et al (2004a) reported that longer indoor storage of organic waste was associated with "skin rash", "itching skin rash", lifetime diagnoses of skin disease by a doctor and allergies other than hay fever. Atopic subjects (i.e. those with a predisposition to develop allergies) were at increased risk for these skin complaints. In a related study, Herr et al (2004b) reported an increased reporting of irritative airway complaints in residents living in an area with elevated bioaerosol exposure. In an earlier study of communities around 3 composting plants, Herr et al (2003b) demonstrated that self-reported symptoms were influenced by respondents’ perception of their exposure to emissions from the composting operations.
Overall the findings of both individual studies and reviews of the published literature indicate that workplace exposure to bioaerosols in the waste industry is associated with increased risks of developing upper and lower respiratory symptoms and chronic respiratory illness. Studies of residential exposure to bioaerosols arising from the domestic storage of organic waste found no excess of respiratory symptoms (possibly because exposure levels are low) but evidence of an association with skin symptoms.

5.3 ORGANIC DUST

5.3.1 Overview

The results of a large number of studies suggest that the health effects associated with workplace exposure to organic dusts differ from those typically associated with inorganic dust (Poulsen et al; 1995a and b). There are no specific studies of the effects of environmental exposure to organic dusts in the general community. Although there is a well established association between exposure to ambient particulate in urban air and premature mortality from cardiovascular and respiratory causes (WHO, 2000), it is not known whether exposure to bioaerosols gives rise to similar effects. The underlying difficulty in relating exposure to organic dust to health effects is the variability in dust composition and associated pollutants in different workplace environments. Two conditions are specifically linked to exposure to organic dusts in a range of different settings:

- Organic Dust Toxic Syndrome (ODTS) is a flu-like syndrome that can occur after inhalation of cotton, grain, wood chip dusts, or other organic dusts or aerosols. ODTS is a non allergic response that occurs 4-8 hours after exposure and is characterised by chest tightness, shortness of breath, dry cough, fever, chills, aching muscles and fatigue. The condition resolves within a few days.
- Hypersensitivity pneumonitis (also known as extrinsic allergic alveolitis) is an immunologically mediated (i.e. allergic) inflammatory disease of the lung involving the terminal airways.

The dust concentrations associated with ODTS are about ten times higher than those typically associated with hypersensitivity pneumonitis. Several fungal and bacterial components of organic dusts have been specifically linked with hypersensitivity pneumonitis. Neither ODTS nor hypersensitivity pneumonitis are commonly reported in waste workers and are not known to arise following environmental exposure to organic dust. The following sections summarise the exposure-response information outlined in Tables A5.1-A5.3 in Appendix 5.

5.3.2 Waste Industry

Most of the few studies of waste workers that have considered organic dust have reported adverse effects on respiratory health, but not a significant association between dust concentrations and respiratory effects (Appendix 5; Table A5.1). Exposures to dust in these studies were generally lower than the UK Workplace Exposure Limit (WEL) for inhalable dust (10 mg/m³) and the WEL for respirable dust (4 mg/m³). There are few data relating exposure concentrations to effects. Eye and nasal irritation may arise at dust levels of 0.2 mg/m³ (Heldal & Eduard, 2004; Kennedy et al, 2004) and respiratory symptoms such as chest tightness and wheeze may arise at exposure levels of about 1-2 mg/m³ (Douwes et al, 2000). There are limited data that suggest an increased prevalence of respiratory symptoms at dust levels exceeding 5 mg/m³ (Gladding et al, 2003) and other studies have failed to find an excess of respiratory symptoms in workers exposed to concentrations of up to 2.1 mg/m³ (Heldal et al, 2003 a and b). The variability in the apparent response to organic dusts in different workplace settings within the waste industry suggests that the composition of organic dusts plays an important role in determining potency.
5.3.3 Exposure-response information for organic dust from other industries

Cross industry study

In a cross sectional study of 1032 UK workers exposed to organic dusts in 9 industries, Simpson et al. (1998) reported a dose-response relationship between organic dust concentrations and symptoms (upper and lower respiratory symptoms) and an association with ODTS was also reported (Fig. 5.1). Symptoms were strongly correlated with endotoxin exposure (section 5.5).

Figure 5.1: Exposure-response information for symptom prevalence in 9 industries in relation to organic dust concentrations (Simpson et al., 1998)

Cotton industry

Exposure to organic dust in the cotton industry is associated with the development of byssinosis, an obstructive respiratory disease specific to the cotton industry characterised by coughing, wheezing and chest tightness. Workers typically habituate to cotton dust exposure during the working week and their respiratory symptoms are worst on Monday morning following a weekend away from work. This effect has not been widely reported in waste workers. No increased risks of byssinosis were reported in studies of cotton workers exposed to dust concentrations of 0.4-1.2 mgm$^{-3}$ (Sigsgaard et al., 1992) or 0.3-15 mgm$^{-3}$ (Cinkotai et al., 1977). Similarly no impacts on lung function were reported at 0.2-2.5 mgm$^{-3}$ (Kennedy et al., 1987) or 0.12-0.55 mgm$^{-3}$ (Castellan et al., 1987), or on respiratory symptoms at 0.2-2.5 mgm$^{-3}$ (Kennedy et al., 1987). In contrast, Haglind et al. (1981) reported an association between dust concentrations of 0.3-2.0 mgm$^{-3}$ and byssinosis where dust and bacterial concentrations were strongly correlated. Haglind and Rylander (1984) reported an association between dust and lung function at concentrations of 0.5-6.9 mgm$^{-3}$ where dust was strongly correlated with endotoxin. In a study of lung function decline over 20 years in cotton workers, Wang et al. (2005) found a stronger relationship between endotoxin and lung function decline than with dust. On cessation of exposure, the rate of lung function decline in non-smokers, but not smokers, reduced to that seen in a control population. Some of the variability in the findings of different studies is likely to reflect differences in the dust, particularly with respect to endotoxin content (section 5.5).

Agriculture

A significant percentage of agricultural workers have clinical symptoms associated with exposure to organic dusts including hypersensitivity pneumonitis, ODTS, chronic bronchitis, mucus membrane inflammation syndrome, rhinitis and asthma-like syndrome. The reported prevalence of rhinitis in farm workers ranges from 20 to 50% and the prevalence of acute symptoms consistent with asthma-like syndrome (cough, chest tightness, wheeze and breathlessness in the absence of persistent airways inflammation or hyper responsiveness) has been as high as 50% in some studies of pig workers and grain elevator operators (Kirkhorn and Garry, 2000). In a cross sectional study of 297 people attending an agricultural trade show, ODTS symptoms were described by 36% of the respondents (von Essen et al,
As in the cotton industry, the acute inflammatory effects of exposure to pig house air are reported to be most pronounced in subjects with no prior exposure suggesting that adaptation or tolerance to endotoxin or other substances in this environment is induced by repeated exposures (von Essen & Romberger, 2003).

Exposure response data are variable. No associations between organic dust and respiratory symptoms or lung function was found at dust concentrations of 0.5-23 mgm\(^{-3}\) on pig farms (Heederik et al., 1991) or 0.4-15.3 mgm\(^{-3}\) in poultry farming (Hagmar et al., 1990). In contrast, work-related symptoms consistent with ODTS were reported by 31 of 53 pig farm employees exposed to concentrations of 3.03-14.05 mgm\(^{-3}\) (Mackiewicz, 1998) and 23 of 29 pig farmers exposed to mean concentrations ranging from 1.66 to 21.04 mgm\(^{-3}\), reported work-related chest tightness/wheeze and nasal and eye irritation (Crook et al., 1991). Donham et al. (1995) reported significant short term decrements in lung function in pig workers exposed to a mean organic dust concentration of 2.8 mgm\(^{-3}\). The threshold concentration associated with a 10% decline in FEV\(_1\) increased with increasing duration of exposure suggesting either the development of tolerance or a healthy worker effect with those susceptible to respiratory illness leaving the industry. Reynolds et al. (1996) reported that short term decrements in lung function in pig workers with more than 6 years of exposure were significantly associated with dust exposure whereas endotoxin was associated with lung function effects in workers with shorter exposures. The authors suggested that dust exposure may be of most relevance to chronic respiratory disease whereas endotoxin may have more significance for shorter term respiratory effects. Reynolds et al concluded that levels of 2.5 mgm\(^{-3}\) (total dust) and 7.5 ppm (ammonia) were reasonable guidelines for occupational exposure limits in pig farming.

The results of several experimental studies in which volunteers were exposed for short periods to farm dusts or grain dusts suggest that endotoxin is more strongly associated with the development of respiratory symptoms than total dust concentrations (Zhiping et al., 1996; Jagielo et al., 1996). Experimental exposure of volunteers to 4 mgm\(^{-3}\) endotoxin-rich grain dust gave rise to increased bronchial hyper reactivity and a significant transient decrease in lung function in subjects with mild asthma but not in normal subjects (Sigurdarson et al., 2004).

**Animal feed**

In a study of 240 workers at two Ukrainian fodder production plants, Kuchuk et al. (2000) reported that the prevalence of chronic bronchitis was 26% at one plant compared with 0% in workers at the same plant with no exposure to organic dust. The prevalence of chronic bronchitis at the second plant was 8.8%, not significantly greater than the prevalence in unexposed workers at the same plant (3.4%). Dust concentrations were 48.2 mgm\(^{-3}\) and 16.8 mgm\(^{-3}\) and endotoxin levels were 240 ngm\(^{-3}\) and 1.8 ngm\(^{-3}\) at the two plants respectively. The length of service at the first plant was more than twice that at the second. Respiratory symptoms were more strongly associated with dust exposure than with smoking. ODTS was diagnosed in 40% of exposed workers at the first plant and 15% at the second plant. Lung function decreased with increasing duration of employment. In a separate study, Smid et al. (1992) reported that dust levels of 0.2-150 mgm\(^{-3}\) were associated with reduced lung function in an animal feed mill, but a stronger relationship was found for endotoxin.

**Pooled exposure-response information from other industrial sectors**

There are clear inconsistencies in the exposure-response information linking organic dusts to respiratory endpoints in different studies, even where studies have been conducted in a single industrial sector. There are a number of causes leading to these inconsistencies:

- Differences in the exact health endpoints assessed;
- Differences in the severity of different health endpoints;
- Differences in dust composition and thus toxic potential;
- Differences in exposure patterns (hours exposed each day, intensity of exposure);
- Differences in the duration of exposure; and
- Interindividual differences in sensitivity to organic dust.

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1999).
Under some circumstances, workers prone to respiratory health problems may move to other jobs leading to the creation of a workforce with better than average baseline respiratory health and greater tolerance of low level dust exposure.

The available data suggest that there is a small increased risk of respiratory symptoms leading to the development of chronic respiratory disease associated with exposure concentrations of less than 1 mgm$^{-3}$ for some organic dusts, with effects potentially arising at concentrations as low as 0.3 mgm$^{-3}$. Other studies have not detected adverse effects at these levels of exposure (Tables 5.1, A5.1-3). The data also suggest that workplace exposure to organic dusts at a concentration of 10 mgm$^{-3}$ (the UK WEL for low toxicity inhalable dust) or greater, is likely to be associated with a substantial risk of developing chronic respiratory illness.

Table 5.1: Concentrations (mgm$^{-3}$) of inhalable or total dust associated with no effects or effects on respiratory symptoms, chronic respiratory illness and lung function in workers exposed to organic dusts in other industries; data from studies described above and in Appendix 5.1, each entry represents a separate study

<table>
<thead>
<tr>
<th></th>
<th>Respiratory symptoms</th>
<th>Chronic respiratory illness</th>
<th>Lung function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No association</td>
<td>Effects</td>
<td>No association</td>
</tr>
<tr>
<td>Cross sectional study in 9 industries</td>
<td>0.1-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotton industry</td>
<td>0.2-2.5</td>
<td>0.4-1.2</td>
<td>0.3-2.0</td>
</tr>
<tr>
<td>Pig workers</td>
<td>0.5-23</td>
<td>16-25</td>
<td>3.03-14.05</td>
</tr>
<tr>
<td>Poultry workers</td>
<td>0.4-15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animal feed workers</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.3.4 Conclusions

Short term workplace exposure to organic dusts in the waste industry is associated with irritation of the eyes, nose and throat and respiratory symptoms. The results of some studies suggest that eye and nasal irritation may arise at dust levels of 0.2 mgm$^{-3}$ (200 ugm$^{-3}$) in some workplaces, respiratory symptoms such as chest tightness and wheeze may arise at exposure levels of about 1-2 mgm$^{-3}$ (1000-2000 ugm$^{-3}$) and there are limited data that suggest an increased prevalence of respiratory symptoms at dust levels exceeding 5 mgm$^{-3}$. These effects are an immediate response to dust inhalation rather the result of prolonged exposure. The results of studies in the cotton industry and in some agricultural settings suggest that some workers may develop a degree of tolerance to organic dust on repeated exposure.

Prolonged high levels of workplace exposure to organic dust in other industries are associated with chronic respiratory illness including ODTS and byssinosis-like obstructive respiratory conditions. These conditions do not appear to be common in the waste industry. This may reflect differences in dust composition compared with other organic dust exposures, differences in the pattern of exposure or the relatively short periods typically spent in the waste industry by waste workers. The recent changes in the waste industry leading to greatly increased numbers of workers at composting and recycling facilities may lead to a greater prevalence of these conditions in the future, if adequate measures to control exposures are not in place. There is no evidence that these serious irreversible respiratory conditions arise at workplace exposure levels less than 0.3 mgm$^{-3}$ (300 ugm$^{-3}$) and most reports of adverse effects are associated with much higher levels of exposure (>1.2 mgm$^{-3}$).
The apparent potency of organic dusts varies in different workplace environments. Endotoxin has been widely identified as a key component of organic dusts giving rise to adverse effects in exposed workers but adverse effects have also been reported in dust-exposed waste workers with relatively low levels of endotoxin exposure (eg Kennedy et al, 2004).

There is no readily available information about the effects of community exposure to organic dusts. It seems highly unlikely that ODTS and hypersensitivity pneumonitis would arise at the levels of exposure likely to be experienced in the general community. In contrast, given that irritation of the eyes and upper respiratory tract has been observed at dust concentrations of 200 ugm⁻³ in the workplace, it is possible that these effects could arise in the general community at similar concentrations. Although the development of respiratory symptoms in the workplace occurs at concentrations that are vastly higher than would arise in the general environment (1000-2000 ugm⁻³), these workplace exposures would correspond to exposures of 200-400 ugm⁻³, if time-averaged for continuous exposure (200x8 hour working days versus 365x24 hour days per year). It is possible that respiratory symptoms could arise among more susceptible members of the general community at lower concentrations (see Chapter 6).

5.4 BACTERIA

5.4.1 Overview

Airborne bacteria have not been widely used as a metric of bioaerosol exposure in the waste industry or in other industry sectors where mixed exposures to bacteria and fungi occur. A variety of measurement metrics have been used which limits inter-study comparison.

The harmful effects of bacteria would be expected to be species dependent and to therefore vary considerably between different industry sectors. Several studies have identified actinomycetes species as being of particular concern as is discussed in more detail below.

5.4.2 Waste industry

Adverse effects on respiratory health and more general health (eg excessive tiredness) have been reported at concentrations exceeding 10⁶ as total bacterial count m⁻³ or 10⁵ cfum⁻³ (Appendix 5, Table A5.5). No specific relationship between bacterial exposure and health has been established. Data presented by Heldal & Eduard (2004) suggest relationships between airborne bacteria levels and irritation of the eyes and nose, weaker relationships with headache and fatigue and no relationship with lower respiratory symptoms (Fig. 5.2). The data are insufficient to be certain which bacterial components are of greatest relevance to health. In a community study, Herr et al (2003a, b; 2004) demonstrated a relationship between living near a composting site and a range of reported symptoms (Fig. 5.3). Concentrations of total micro-organisms within 150-500 m of the facility ranged up to levels exceeding 10⁵ cfum⁻³. Although the relative importance of bacteria and fungi in contributing to microbial concentrations is not clearly described, the discussion centres on bacteria rather than fungi.

Figure 5.2: Median exposure levels associated with the presence and absence of symptoms in organic waste collectors (Heldal & Eduard, 2004).
Figure 5.3: Percentage prevalence of respiratory symptoms (above) and other symptoms (below) in a community living at distances of 150-500 m from a composting facility (Herr et al., 2003a).
Itching eyes >10 times/year
Smarting eyes
Loss of appetite
Nausea/vomiting >5 times/year
Diarrhoea >5 times/year
Excessive tiredness >5 times/year
Shivering
Fever >5 times/year
Joint trouble >5 times/year
Muscular complaints >10 times/year
Current intake medication/vitamins
5.4.3 Other industries

The nature of exposure to airborne bacteria varies hugely between different workplaces. Few studies were found in PubMed that were of possible relevance to the prediction of effects associated with emissions from the waste industry. Crook et al. (1991) reported work-related respiratory symptoms, typically chest tightness/wheeze and nasal and eye irritation, in 23 of the 29 piggery workers exposed to concentrations of airborne microorganisms ranging from $10^5$ to more than $10^8$ cfum$^{-3}$, mostly as bacteria. No specific relationship was detected between microbiological exposure and effects.

Poulsen et al. (1995a and b) reported that the development of byssinosis in the cotton industry was associated with concentrations of gram-negative bacteria of $10^3$-$10^5$ cfum$^{-3}$ (Haglind et al., 1981) and concentrations of viable bacteria of $10^3$-$10^5$ cfum$^{-3}$ (Cinkotai et al., 1977). Exposures to gram negative bacteria in pig farms of $10^3$-$10^5$ cfum$^{-3}$ and $10^3$-$3 \times 10^6$ total bacteria were associated with acute respiratory symptoms but not with effects on lung function (Heederik et al., 1991). Hagmar et al. (1990) reported cross shift increases in respiratory symptoms and reductions in lung function in a small study of poultry workers but found no significant association with exposure conditions. Concentrations of airborne bacteria were $4 \times 10^5$-$4 \times 10^6$ cfum$^{-3}$ mainly coagulase-negative staphylococcal strains. Concurrent fungal concentrations were 500-4000 cfum$^{-3}$.

In a study of paper mill workers, Haug et al. (2002) reported an association between exposure to bacteria ($10^4$-$>10^5$ cfum$^{-3}$) and increased risks of cough, dyspnoea, gastrointestinal symptoms, skin infections and systemic infection. The increased infection risk was marked by an increased incidence of treatment with antibiotics against infection (Fig. 5.4).

**Figure 5.4:** Relationship of symptoms in paper mill workers to exposure to airborne bacteria reported by Haug et al. (2002); High exposure: >20% of time spent in areas with high concentrations; medium exposure:10-20% time spent in areas with usually moderate concentrations; low exposure 10-15% of time spent in areas with usually low concentrations.

5.4.4 Actinomycetes

A number of studies in different workplace settings have linked exposure to Actinomycetes species with the development of hypersensitivity pneumonitis. The condition is associated with intense or repeated exposure to inhaled organic dusts and the most common form is farmers’ lung. Farmers’ lung is believed to typically develop as a result of inhalation exposure to thermophilic Actinomycetes species that flourish in areas of high humidity (Wild & Chang, 2007).
The symptoms of farmers’ lung depend on the intensity, frequency, and duration of exposure and on the individual’s response to the causative antigen. Acute effects following exposure include airways inflammation and a reduction in lung function and efficiency. Prolonged exposure leads to collagen deposition and destruction of the lung structure leading to reduced lung volume. Farmers’ lung appears to involve both immune complex–induced tissue injury (type III hypersensitivity) consistent with the presence of antigen-specific immunoglobulin and also cell-mediated, delayed-type hypersensitivity (type IV hypersensitivity).

The estimated prevalence of farmers’ lung in the UK is 420-3000 cases per 100,000 at-risk persons, compared with 400-700/100,000 in the US, 250-15300/100,000 in France and Sweden and 700/100,000 in Finland. The main causal agent depends on the relative abundance of micro-organisms in workplace air and varies between different regions. In the UK Farmers’ lung appears to be associated with Micropolyspora faeni whereas in Finland it seems to be associated with Thermoactinomyces vulgaris (Husman et al, 1987) consistent with the much greater abundance of Thermoactinomyces vulgaris spores relative to Micropolyspora faeni on Finnish farms. Serum antibodies are not necessarily predictive of adverse effects. Amishima et al (1995), for example, reported that the presence of serum antibody to Micropolyspora faeni and/or Thermoactinomyces vulgaris was not associated with bronchial hyper-responsiveness among dairy farmers.

Hypersensitivity pneumonitis associated with antibodies to actinomycetes species has been reported in mushroom growers exposed to concentrations greater than $10^9$ cfu m$^{-3}$ (Van den Bogart et al, 1993) and in workers exposed to thermophilic actinomycetes contaminated indoor air (van Assendelf et al, 1979; Fink et al, 1976).

The absence of widespread hypersensitivity pneumonitis in the waste industry may be due to the small number of workers who were historically employed in composting. Given that hypersensitivity pneumonitis generally takes several years to develop, compost workers’ lung may emerge as a recognised syndrome in the next 5-10 years.

5.5 ENDOTOXIN

5.5.1 Overview

The toxic effects of endotoxin have been widely studied in humans and animals. Inhaled endotoxin is associated with lung inflammation that can produce symptoms of fever, malaise, cough, shortness of breath and nausea. Endotoxins can lead to bronchoconstriction in asthmatics potentially leading to an asthma attack. The variety of methods used to measure endotoxin exposure gives rise to uncertainty in cross study comparisons of exposure data (Liebers et al, 2006). In addition, there are likely to be differences in the molecular composition and, potentially the potency of, endotoxin from different sources. The following sections are based on exposure-response information tabulated in Tables A5.5 to A5.7 of Appendix 5 and describe exposure-response information from the waste industry, other industries, human volunteer experiments and animal studies.
5.5.2 Waste industry

Endotoxin has been the most widely used measure of bioaerosol exposure in workplace studies in the waste industry. The results of a number of studies suggest that concentrations exceeding about 50 EUm$^{-3}$ (proposed Dutch Occupational Exposure Limit; OEL) are typically associated with an increased risk of respiratory symptoms (Appendix 5; Table A5.5; Gladding et al. 2003; Douwes et al. 2000; Thorn et al., 1998; Bunger et al. 2007; Wouters et al. 2002). Other studies have failed to detect adverse effects at exposure levels of 200-300 EU m$^{-3}$ (Douwes et al., 2000). The results of a small number of studies suggest that minor effects on respiratory health may occur at concentrations below 50 EUm$^{-3}$ (Mahar et al., 1999; Heldel et al., 2003 a and b; Heldal & Eduard, 2004). These effects include small impacts on inflammatory markers in nasal lavage fluid that are not clearly related to symptoms and are of unknown health significance. There are also limited data that are suggestive of increased risks of nasal irritation, cough, unusual tiredness and diarrhoea at exposure levels <10 EUm$^{-3}$ (Fig. 5.5). The results of several individual studies demonstrate exposure-response relationships between endotoxin and markers of respiratory inflammation or gastrointestinal symptoms (Ivens et al., 1997, 1998; Fig. 5.6). Other studies failed to find exposure-response relationships (e.g. Heldal et al., 2003 a and b). There is a paucity of studies describing the long-term impacts of endotoxin exposure in the waste industry. Gladding et al. (2003) show data that suggest that the prevalence of symptoms may increase with increasing duration of exposure to endotoxin and/or beta (1→3) glucan in the waste industry but their data are inadequate for the prediction of risk arising from decades rather than months of exposure (Fig. 5.7). Mahar et al. (1999) found that workers employed seven years or more had significantly larger cross-shift decrements in lung function than those employed for a shorter period. In contrast Wouters et al. (2002) found no significant differences in symptom prevalence in waste workers with less than or more than 6 months exposure. In all studies, workers were exposed to a mixture of bioaerosol components and it is unclear to what extent observed effects were due to endotoxin, some other bioaerosol component(s) or the interaction between endotoxin and other bioaerosol component(s). In addition, the small study populations in most studies limited their power to detect small to moderate increases in risk.

**Figure 5.5:** Median concentrations of endotoxin associated with symptoms/absence of symptoms in organic waste collectors (Heldal & Eduard, 2004)
Figure 5.6: Exposure-response relationships for nausea (above) and diarrhoea (below) in waste workers (Ivens et al. 1999): For endotoxin exposures – High: >500; Medium: >100-500; Low: 0.1-100; Comparison <0.1 EUm⁻³; For viable fungal – High exposure: >10⁷; Medium: >10⁶-10⁷; Low: >10⁵-10⁶; comparison: <10⁵ cfu m⁻³; For total fungi – High: >2x10⁷; Medium: >2x10⁶-2x10⁷; Low: >2x10⁵-2x10⁶; comparison: <2x10⁵ cell m⁻³; For total microorganisms – High: >6x10⁷; Medium: >6x10⁶-6x10⁷; Low: >6x10⁵-6x10⁶; comparison: <2x10⁵ cell m⁻³.

Figure 5.7: Change in symptom prevalence with duration of exposure to bioaerosols in recycling workers (Gladding et al, 2003)
5.5.3 Studies in other industry sectors

Endotoxin has been recognised as a potential cause of ill-health across a number of industries (Table A5.6, Appendix 5). Studies of workers exposed to grain dust and to cotton dust have established that respiratory symptoms are more strongly associated with endotoxin than other measures of dust exposure (Poulsen et al, 1995a and b). Exposure to endotoxin in the cotton industry is linked to the development of byssinosis. Rylander (1987) proposed a series of OELs for different health effects in the cotton industry: fever – 500-1000 ng m$^{-3}$, chest tightness – 300-500 ng m$^{-3}$, cross shift reduction in lung function – 100-200 ng m$^{-3}$ and bronchial inflammation – 20 ng m$^{-3}$ (uncertain). Sigsgaard et al (1992) presented limited exposure response information for a range of respiratory health endpoints (Fig 5.8). In a cross sectional study across 9 industries, Simpson et al (1998) reported exposure response relationships linking increased levels of symptom reporting with exposure concentrations above a threshold of about 5 ng m$^{-3}$ (about 45 EU m$^{-3}$; Figure 5.9). The pooling of data across a number of industries may reduce the confounding effects of other dust components. Schwartz et al (1995) reported exposure response information for respiratory symptoms in grain workers (Fig, 5.10). In a recent review, Liebers et al (2006) reported no effects levels for endotoxin range from 90 to 1800 EU m$^{-3}$ and that the potency of endotoxin appeared to vary between different workplace environments. Both duration and intensity of exposure were perceived to be important influences on health outcome as were individual genetic susceptibility (see Chapter 6). In addition, there is substantial measurement uncertainty that contributes to difficulty in identifying threshold levels for effects, and the role of other bioaerosol components in giving rise to effects is also uncertain.

**Figure 5.8:** Exposure-response relationships based on an approximate measure of exposure to total endotoxin (the endotoxin fraction of total inhalable dust) based on mean of personal samples collected in cotton, wool and man made fibre (MMF) mills; odds ratio based on disease prevalence in cotton and wool mills versus the MMF mill (Sigsgaard et al, 1992).

![Figure 5.8](image_url)

**Figure 5.9:** Exposure-response information for symptom prevalence in relation to endotoxin exposure in workers exposed to organic dusts in nine industries (Simpson et al, 1998).

![Figure 5.9](image_url)
**Figure 5.10**: Exposure-response information for symptom prevalence in relation to endotoxin exposure in grain handlers (Schwartz et al, 1995; concentrations shown of endotoxin in total inhalable dust)

Table 5.2 represents an attempt to pool the available data (Table A5.6, Appendix 5). There is limited evidence for thresholds of about 50 EUm$^{-3}$ for respiratory effects and eye irritation, 900 EUm$^{-3}$ for fever, and between 130 and 450 EUm$^{-3}$ for effects on lung function, although the results of one study suggest possible impacts at less than 20 EUm$^{-3}$ (Fig 5.11). Significantly increased risks of long term respiratory illness have been reported in workers exposed to between 20 and 5500 EUm$^{-3}$.

**Table 5.2**: Summary of exposure-response information for endotoxin from studies outside of the waste industry

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>No effects levels</th>
<th>Exposure levels in studies where endotoxin was associated with effects</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory symptoms, eye irritation</td>
<td></td>
<td>130-1500 ngm$^{-3}$ (about 1100-13500 EUm$^{-3}$) 25-30 ngm$^{-3}$ 6.91 ngm$^{-3}$</td>
<td>5 ngm$^{-3}$ (about 45 EUm$^{-3}$) 25 ngm$^{-3}$ (about 225 EUm$^{-3}$)</td>
</tr>
<tr>
<td>Fever</td>
<td></td>
<td>130-390 ngm$^{-3}$ 1200 ngm LAL – 1200 (900-1400) ngm$^{-3}$ LPS(GC-MS) 3900 (2500 to 4900) ngm$^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Chronic respiratory</td>
<td></td>
<td>2-550 ngm$^{-3}$</td>
<td></td>
</tr>
<tr>
<td>Lung function</td>
<td>200 EU m$^{-3}$</td>
<td>2-550 ngm$^{-3}$ (20-5000 EUm$^{-3}$) IH 18.5 and R 5.6 EUm$^{-3}$ 31-340 ngm$^{-3}$ (280-3100 EUm$^{-3}$) 40-350 ngm$^{-3}$ (360-3100 EUm$^{-3}$) 130-1500 ngm$^{-3}$ (1150-13500 EUm$^{-3}$) 0.2-470 ngm$^{-3}$ (2 – 4200 EUm$^{-3}$) 6 to 779 ngm$^{-3}$ (55 – 7000 EUm$^{-3}$) 130 EUm$^{-3}$</td>
<td>450 EU m$^{-3}$.</td>
</tr>
</tbody>
</table>

*only in workers with less than 6 years exposure
5.5.4 Volunteer experiments

A large number of volunteer studies have investigated the effects of inhaled LPS (endotoxin; Table A5.7; Appendix 5). The results of these experiments demonstrate that inhalation of LPS gives rise to a reduction in lung function and an inflammatory response in the airways and at systemic level. Systemic effects include a rise in temperature and fatigue. LPS can increase the airway’s response to other airborne contaminants. Individuals vary widely in their response to LPS. This variation in responsiveness is not clearly linked to atopy. The symptoms and effects of LPS inhalation generally develop and persist during the 24 hours following exposure, but resolve within a week.

Sundy et al (2006) reported exposure response relationships from data pooled across 199 volunteers in a series of experiments (Fig. 5.12). The data provide strong evidence of a causal link between inhaled LPS and effects on lung function, respiratory symptoms and wider systemic effects including headache, muscular pain and chills. The data also provide some evidence of a link with nausea.
Thorn (2001) identified inhalation of 30-40 ug LPS as a threshold level for inducing clinical symptoms and lung function changes in healthy subjects whereas the threshold dose for inducing changes in blood neutrophils (white blood cells involved in the development of inflammation) appeared to be less than 0.5 ug LPS. Averaged over a 24 hour period, for a typical adult inhaling 20m$^3$ of air, these intakes equate to exposure to concentrations of 1.5-2 ugm$^{-3}$ and $<$0.025 ugm$^{-3}$ or about 13500-18000 EUm$^{-3}$ and $<$220 EUm$^{-3}$. In a pooling of data from 108 exposure sessions with 32 cotton dusts, Castellan et al (1987) reported that there was little evidence of adverse effects on lung function at exposure concentrations $<$ 10 ngm$^{-3}$, whereas a significant reduction in lung function was reported in all experiments at concentrations $>$ 50 ngm$^{-3}$. Linear relationships were found between the percentage mean loss in FEV$_1$ or proportion of volunteers with greater than 5% loss of FEV$_1$ and the log of endotoxin concentration. Less than 10% of volunteers showed a greater than 5% decrement in FEV$_1$ at about 10 ngm$^{-3}$, about 20% of volunteers at about 50 ngm$^{-3}$ and about 30% at about 100 ngm$^{-3}$. Students with no previous exposure were more sensitive to the effects of cotton dust than cotton workers. There is some evidence from experiments in which volunteers have been exposed to swine dust that repeated exposure to endotoxin leads to some degree of tolerance (Hoffmann et al, 2005).

A number of studies have used endotoxin injection in human volunteers as a means of studying systemic inflammation (Table A5.8). Measurable effects on plasma concentrations of pro-inflammatory cytokines have been reported at considerably lower levels of exposure than in inhalation experiments. The data are consistent with the initiation of inflammatory processes at levels of exposure equivalent to less than 24 hours exposure to a concentration of 25 EU m$^{-3}$. A small proportion of individuals may be particularly sensitive to endotoxin. Van Eijk et al (2004) describe four cases of extreme slowing or stopping of the heart in volunteers, approximately 1 hour after administration of endotoxin. The systemic dose arising from injection may be greater than that following administration by inhalation whereas the dose directly experienced by lung tissue would be smaller. Systemic effects would be expected to be similar following either route of administration, but inhalation might give rise to more serious adverse effects on the lung.

Experiments with volunteers normally involve small numbers of healthy individuals that are unlikely to be representative of the full range of susceptibility within the general population. The doses administered in experiments with endotoxin are greater than those likely to arise as a result of community exposure to bioaerosols emitted from waste processes but some waste workers are likely to experience exposure levels equivalent to those demonstrated to cause adverse effects in volunteer studies.

5.5.5 Animal studies

A large number of animal studies have investigated the specific effects of endotoxin on respiratory health and the mechanisms by which endotoxin influences respiratory health. A review is provided in Appendix 5, Section A5.3.3. The results of these studies confirm the potential harmfulness of inhaled toxin but do not provide much information on exposure-response relationships. Inhalation exposure of guinea pigs to concentrations of 0.03 to 50.5 ugm$^{-3}$ aerosolized endotoxin over 4 hours, for example, was associated with a significant increase in total cell count and lactate dehydrogenase levels in bronchoalveolar lavage fluid except at the lowest dose of 0.03 ugm$^{-3}$ (about 300 EUm$^{-3}$; Gordon, 1992). Significant airway constriction was observed following two hours exposure to 9.6 and 50.5 ugm$^{-3}$ endotoxin but not at lower levels of exposure. There is some evidence that repeated exposure to endotoxin can reduce the sensitivity of hamsters, rats and mice to endotoxin (Lantz et al, 1985). Several investigators have demonstrated that co-exposure of animals to ozone and endotoxin gives rise to a greater response than observed with either agent alone (Harkema et al, 2005; Wagner et al, 2001). The results of other studies indicate that endotoxin may enhance the response to inhaled allergens (eg Slater et al, 1998; Wan et al, 2000; Gerhold et al, 2002). Other studies have investigated the variation in toxicity of endotoxin from different sources (Baseler et al, 1983; Mizoguchi et al, 1986).

5.5.6 Conclusions
Endotoxin has been the most widely used measure of bioaerosol exposure in workplace studies. The presence of other components within bioaerosols are likely to have an important influence on apparent exposure-response relationships. The results of a number of studies in the waste industry suggest that concentrations of 50 EUm$^{-3}$ (proposed Dutch OEL) or greater are typically associated with adverse effects on health and there are limited data that suggest minor health effects may occur at levels of exposure below 50 EUm$^{-3}$. These effects include nasal symptoms, cough, unusual tiredness and diarrhoea at exposure levels <10 EUm$^{-3}$. In studies of workers from other industries there is limited evidence for thresholds of about 50 EUm$^{-3}$ for respiratory effects and eye irritation, 900 EUm$^{-3}$ for fever, and between 130 and 450 EUm$^{-3}$ for effects on lung function, although the results of one study suggest possible impacts at less than 20 EUm$^{-3}$. Significantly increased risks of long term respiratory illness have been reported in workers repeatedly exposed to concentrations between 20 and 5500 EUm$^{-3}$. In a cross sectional study across 9 industries, the prevalence of symptoms ranged from 3% at 1 ngm$^{-3}$, 10% at 10 ngm$^{-3}$, 18% at 100 ngm$^{-3}$ to 25% at 1000 ngm$^{-3}$ (Simpson et al., 1998). The pooling of data across a number of industries may reduce the confounding effects of other dust components.

5.6  FUNGAL SPORES

5.6.1  Overview

The most common mechanism by which airborne fungi cause adverse effects is through an allergic, IgE-induced, response (Nordess et al, 2003) and about 5% of individuals are predicted to have some allergic airway symptoms from moulds over their lifetime (Hardin et al, 2003). The non-IgE-mediated effects of fungi, including hypersensitivity pneumonitis, infectious disease, and mycotoxicoses are rare (Nordess et al, 2003). Most airborne fungi are not pathogenic to man but some are capable of invasive infection. The Health Protection Agency estimates that there are about 4200 cases of aspergillosis in immunocompromised individuals in the UK each year, of which about 60% may be fatal (HPA, 2006).

Most individuals with allergies to fungi develop rhinitis or asthma, and sinusitis may occur secondarily due to obstruction. Allergic bronchopulmonary aspergillosis (ABPA) is a rare complication of persistent asthma and cystic fibrosis (Moss, 2005). ABPA is characterized by recurring episodes of asthma, pulmonary infiltrates, and bronchiectasis (dilation, inflammation and weakening of the airways, resulting in airflow obstruction and impaired clearance of secretions), that may progress to fibrosis (the development of scar tissue). It is an adaptive immune response with elevated levels of A. fumigatus-specific IgG, IgA and IgE antibodies, and a profound non-specific IL-4-dependent elevation in total IgE. Inhaled conidia of A. fumigatus are able to persist and germinate, releasing fungal products that further compromise clearance, breach the epithelium, and activate immune responses. Certain A. fumigatus allergens appear more strongly associated with ABPA than A. fumigatus.

5.6.2  Waste industry

Fungal spores have been widely measured in studies of the waste industry (Table A5.8, Appendix 5). A variety of measurement metrics have been used which limits interstudy comparison. It is also probable that the species composition of the aerosol would affect its potential to cause adverse effects. Adverse effects on respiratory health have been generally reported at concentrations of more than 10$^5$ cfum$^{-3}$ (Burger et al, 2000) with limited data suggesting that gastrointestinal effects may arise at concentrations of less than 10$^5$ cfum$^{-3}$ and clearer evidence of adverse gastrointestinal effects at concentrations greater than 10$^5$ cfum$^{-3}$ (Figures 5.6, 5.13). Ivens et al (1999) reported that the relationship between nausea and viable fungi was stronger than for total fungi, total microbes or endotoxin (There are no studies reporting adverse health effects below concentrations of about 10$^3$ cfum$^{-3}$). Mild inflammation of the upper airways has been observed in workers exposed to concentrations of 10$^3$ to 10$^6$ total spores/m$^3$ as assessed by scanning electron microscopy (SEM) and gastrointestinal symptoms have been observed at spore concentrations of 10$^5$ spores/m$^3$ (Ivens et al, 1997, 1999). Heldal et al (2003a) reported a range of symptoms including irritation of the eyes and nose, cough, tiredness, nausea and headache associated with a
median exposure concentration of about $0.3 \times 10^6$ spores/m$^3$ compared with an absence of effects at $10^5$ spores/m$^3$. An investigation of the effects of composting on community health reported respiratory symptoms, irritation of the eyes, nose and throat, long term respiratory illness, nausea and excessive tiredness at microbial concentrations exceeding $10^4$ cfum$^{-3}$, but a specific link to fungi was not established (Herr et al, 2003).
Figure 5.13: Median fungal spore concentrations associated with the presence or absence of symptoms in organic waste collectors (Heidal & Eduard, 2004)

5.6.3 Other workplace studies

Although there have been a large number of investigations on the respiratory health of mould-exposed workers, few investigations have quantified exposure. Some studies have reported an increased risk in respiratory symptoms at exposure concentrations of about $10^6$ cfu m$^{-3}$ whereas others have reported effects at exposure levels of less than $10^4$ cfu m$^{-3}$ (Table A5.9). Factors that are likely to have contributed to this apparent range in potency include:

- Uncertainties in exposure measurement;
- Variability in the species present in different workplace environments, even within single industry sectors;
- Differences in the overall make-up of the bioaerosols particularly with respect to pathogenic bacteria and/or endotoxin.

The Nordic Expert Group (2006) identified a lowest reported effects level of $10^5$ spores/m$^3$ for lung function decline, respiratory symptoms and airways inflammation in studies of woodworkers and farmers. More severe respiratory symptoms including hypersensitivity pneumonitis have been reported at concentrations of $10^6$-$10^9$ cfu m$^{-3}$. A small proportion of cases of Farmers’ Lung (hypersensitivity pneumonitis) may result from exposure to various Aspergillus species (Wild and Chang, 2007 [http://www.emedicine.com/med/topic771.htm]). Hypersensitivity pneumonitis has also been reported in mushroom workers sensitised to the spore (Tanaka et al, 2003). Malmberg et al (1988) suggested $10^6$-$10^9$ spores m$^{-3}$ as a tentative OEL to protect against hypersensitivity pneumonitis. Dahlqvist et al (1992) reported that exposures to concentrations of viable moulds of $3 \times 10^5$ cfu m$^{-3}$ and total moulds of $10^5$ spores/m$^3$ in a sawmill were associated with a decline in lung function parameters over the working week. In contrast, exposure to fungal concentrations of $10^3$-$10^5$ cfu m$^{-3}$ in the cotton industry were unrelated to the development of byssinosis. Eduard et al (1983) observed that exposure to fungal spore counts exceeding $10^5$ m$^{-3}$ in sawmills was associated with respiratory symptoms, mucous membrane irritation and ODTS. Tanaka et al (2003) reported that 71% of mushroom workers developed chronic cough within 3 months of starting work, a small proportion developed ODTS and a slightly greater proportion developed cough variant asthma. The cough tended to improve or disappear after weekends or holidays.

Despite extensive interest in the health effects associated with water damaged buildings, there has been little investigation of symptoms in relation to airborne concentrations of fungal aerosols. For example Park et al (2004) reported visible mould in office buildings was
significantly associated with wheeze, chest tightness and shortness of breath, and nasal and sinus symptoms, and also that mould odour was significantly associated with throat irritation. Although exposure-response relationships were developed on the basis of visible mould, water stains and mould odour, these cannot be related to airborne fungal concentrations.

5.6.4 Respiratory symptoms in children

There have been a large number of studies of respiratory symptoms and asthma in children living in damp homes or attending damp schools. Although there are well-established relationships between moulds and/or dampness and respiratory symptoms and asthma, there are few data linking observed effects to concentrations of airborne fungi (Section A5.5.2, Appendix 5). In a UK study, Strachan et al. (1990) reported a non-significant relationship between higher fungal counts at home and a 10% or greater decline in lung function (FEV1) in children after exercise (geometric mean 354 v 253 cfum⁻³). In an Australian study, Garret et al. (1998) reported that the odds ratio for physician-diagnosed asthma associated with a 100 cfum⁻³ increase in Penicillium spores was 1.43 (95% CI 1.03-2.00) and the odds ratio for atopy (a positive response to at least one skin prick test) associated with a 10 cfum⁻³ increase in Aspergillus spores was 1.48 (95% CI 1.10-1.99).

In a study of IgE antibodies to 24 moulds, Taskinen et al. (2002) reported that antibodies to moulds common in moisture-damaged buildings were associated with allergic diseases, as well as with mould-specific immunoglobulin E (IgE) or skin prick test (SPT) findings. Aspergillus fumigatus and A. versicolor were the moulds with the most consistent findings. In contrast, the association between asthma, wheezing or cough symptoms, and IgG to moulds was not significant and there were no significant differences in mould-specific IgG concentrations between exposed and non-exposed school-children. Similarly in a study of schools with and without mould damage, Hyvarinen et al. (2003) found that children's microbi-specific IgG levels were often higher in the reference school. In a German study, Jacob et al. (2002) found that mould spore counts for Cladosporium and Aspergillus were associated with an increased risk of allergic sensitisation which in turn was associated with symptoms of rhinoconjunctivitis on exposure to high levels of mould spores (> 90th percentile), as defined by concentrations in settled dust.

A few US studies have linked fungal exposures to severe respiratory illness in infants but these findings are disputed and are of little relevance to the UK (Section A5.4.4, Appendix 5).

Overall, the results of studies in children do not provide a clear indication of a threshold level for effects and they also suggest that background levels of fungal exposure, in the absence of waste management sources, are sufficient to give rise to adverse respiratory effects in some children, including an increased risk of developing asthma. Species of both Penicillium and Aspergillus have been identified as being particularly associated with respiratory effects.

5.6.6 Other studies of moulds in indoor air

Bornehag et al. (2001) identified 590 peer-reviewed articles describing the health effects of "dampness" in buildings of which they reviewed 61. They concluded that building "dampness" increases the risk for health effects such as cough, wheeze and asthma. Relative risks were in the range of 1.4-2.2. They also found some evidence of an association between "dampness" and other symptoms such as tiredness, headache and airways infections.

A number of studies have reported an association between exposure to moulds in indoor air including Penicillium, Cladosporium, Aspergillus, Acremoniu and Alternaria and increased risks of developing allergy with or without asthma (Bobbit et al., 2005; Gutaowska et al., 2005; Matheson et al., 2005). Increased mould exposure may lead to an increased risk of asthmatic attacks, of developing atopy and mould sensitisation (Matheson et al., 2005; Thorn et al., 2001a). Mould sensitisation (Alternaria alternata or Cladosporium herbarum, or both) may also be associated with increased asthma severity (Zureik et al., 2002). Mould sensitisation may be associated with allergy to other common allergens. At an individual level, however, no relationship has been found between patients’ presenting symptoms, atopic status, and magnitude of exposure (Bobbit et al., 2005).
A number of studies have demonstrated associations between residential exposure to mould and increased respiratory symptoms (Koskinen et al. 1999; Bruneckreef, 1992; Skorge et al. 2005), although exposure to mould made only a small contribution to the respiratory symptom burden in the population at large (Skorge et al. 2005). Klanova (2000) reported that occupants of rooms with an average concentration of 2.476 cfum\(^{-3}\) reported health complaints such as cough, headache, rhinitis and sore throat. In a small study, Rouppi et al. (2003) found that the respiratory symptoms reported by occupants of mouldy residences were apparently related to non-specific inflammation following irritation rather than mould allergy.

The Nordic Expert Group (2006) identified a NOEL (no observed effect level) of 7x10\(^2\) spores/m\(^3\) for nasal irritation in the single well designed indoor study that were able to identify (Roponen et al, 2003). A more detailed account of these studies is given in Section A5.4.5, Appendix 5 but overall they provide little to link airborne concentrations to effects on health.

5.6.7 Fungi in ambient air

The results of the few available studies suggest an association between short term increases in spore concentrations and an exacerbation of asthma symptoms. In a study of the joint effects of aeroallergens (grass and birch pollen, basidiospores, Didymella, Alternaria and Cladosporium), rainfall, thunderstorms and outdoor air pollutants on daily asthma admissions and Accident and Emergency attendance, Lewis et al (2000) reported that asthma admissions increased with Cladosporium count but there were no statistically significant interactions between effects of any individual aeroallergen and outdoor air pollutant upon either measure of asthma morbidity. In an 8 week study of 22 asthmatics, Dellino et al (1997) reported that an increase of nearly 4,000 spores/m\(^3\) from minimum concentrations to the 90th percentile was associated with an increase in asthma symptom scores of 0.36 (95% CI, 0.16-0.56), an increase in inhaler use of 0.33 puffs (95% CI, -0.02-0.69) and a decrease in evening Peak Expiratory Flow (PEF) of 12.1 l/min (95% CI, -1.8-22.3). These associations were much stronger for specific fungal types (e.g., Alternaria, basidiospores, and hyphal fragments) than for fungi generally and were also much stronger in the 16 asthmatics allergic to tested deuteromycete fungi. There were no significant associations between health effects and low levels of pollen or O\(_3\), but inhaler use was associated with PM\(_{10}\) concentrations. In an earlier study of 12 asthmatic subjects aged 9 to 16 years, Dellino et al (1996) reported that fungal spores were significantly associated with symptoms (scores increased by 0.1 to 0.3/1,000 spores/m\(^3\)) and inhaler use (0.1 to 0.4 puffs/1,000 spores/m\(^3\)), whereas pollen and fine particles (low levels) were not associated with any outcomes. In a study of asthma-related deaths in Chicago, Targonski et al (1995) reported that mean mould spore levels but not tree, grass, or ragweed pollen levels were significantly higher for days on which asthma-related deaths occurred than for days on which no deaths occurred. The odds of a death caused by asthma occurring on days with mould spore counts of 1000 spores/m\(^3\) or greater was 2.16 times higher (95% CI = 1.31, 3.56) than on days on which mould spore counts were <1000 spores/m\(^3\). After controlling for pollens, the odds of an asthma-related death increased by 1.2 times (95% CI = 1.07-1.34) for every increase of 1000 spores/m\(^3\) in daily mould spore levels.

5.6.8 Role of mycotoxins

Mycotoxins are known to produce disease in animals and humans when consumed in contaminated foods but there is no clear evidence of adverse effects following inhalation of indoor air (Nordess et al, 2003). Kelmen et al (2004) modelled the possible dose of mycotoxins that could be inhaled in 24 hours of continuous exposure to a high concentration of mould spores in indoor air. For the maximum reported concentrations in indoor residential, school, and office environments, the predicted intakes of aflatoxins B1 and B2, satratoxins G and H, fumitremorgens B and C, verruculogen, and trichoverrols A and B were lower than the effects data for the same mycotoxins. This suggests that inhalation of mould spores in indoor environments is highly unlikely to give rise to sufficient exposure to mycotoxins in mould-contaminated home, school, or office environments to cause illness. Other reviews have similarly failed to establish that mycotoxins have an important role in the development of mould-associated symptoms (Fung and Hughson, 2003; Hardin et al, 2003).
There is limited evidence that exposure to mould in indoor air may be associated with slight neurological impairment consistent with low levels of exposure to neurotoxic mycotoxins. In a study of 100 patients from mould-affected homes, Rea et al (2003) reported physical signs and symptoms of neurological dysfunction (e.g., inability to stand on the toes or to walk a straight line with eyes closed, as well as short-term memory loss). Objective neuropsychological evaluations of 46 of the patients showed typical abnormalities in short-term memory, executive function/judgment, concentration, and hand/eye coordination. Kilburn (2003) compared 65 patients exposed to mould at home with 202 community subjects who had no known mould or chemical exposures. The mould-exposed group exhibited decreased function for balance, reaction time, blink-reflex latency, colour discrimination, visual fields and grip, and showed reduced scores in some neurophysiological tests: digit-symbol substitution, peg placement, trail making, verbal recall, and picture completion. They also showed elevated mood state scores and symptom frequencies and reduced lung function parameters consistent with airways obstruction. No exposure-response information is available.

5.6.9 Other studies in humans

Several recent studies investigating the role of fungi in chronic rhinosinusitis have failed to conclusively demonstrate a link, and levels of fungi in nasal mucus are similar in patients with chronic rhinosinusitis to those in healthy individuals (Hafidh et al, 2007; Ragab & Clement, 2007; Braun et al, 2003). In a study of chronic hyperplastic sinusitis patients, Kostamo et al (2005) reported that 46% patients reported mould odour or moisture problems in the home or work environment but no association was found between chronic hyperplastic sinusitis or fungal sinusitis and moisture damage.

5.6.10 Experimental studies

A number of experiments in human volunteers have established the potential for airborne fungi to cause respiratory symptoms in those with and without previous exposure and sensitisation or atopy (Section A5.4.5; Appendix 5). These studies do not provide useful exposure-response information.

Other studies have demonstrated the toxicity of inhaled spores of various fungal species in animals and have confirmed the potential of inhaled fungi to cause a dose-dependent inflammatory response in the respiratory system. Animal studies also provide evidence of the variable potency of different fungal species, evidence of lasting respiratory damage and progressive lung disease following a single over-exposure to some fungal species and the potential importance of endotoxin in enhancing the respiratory response to inhaled fungal spores. It is however, important to note that animal responses are not directly comparable to human responses. A more detailed account is given in Section A5.4.6 of Appendix 5.

5.6.11 Conclusions

The exposure-response information available for airborne fungi is highly inconsistent and the use of variety of measurement metrics limits interstudy comparison, as does the variation of the species present in different environments. Adverse effects on respiratory health have been generally reported in workers at concentrations of more than $10^4$ cfum$^{-3}$ with limited data suggesting that gastrointestinal effects may arise at concentrations of less than $10^3$ cfum$^{-3}$. There is also limited evidence of an absence of adverse health effects at workplace exposure concentrations of about $10^3$ cfum$^{-3}$. Mild inflammation of the upper airways has been observed in workers exposed to concentrations of $10^3$ to $10^6$ total spores/m$^3$ as assessed by SEM and gastrointestinal symptoms have been observed at $10^5$ spores m$^{-3}$.

Studies of the general population have demonstrated that a large proportion of individuals are sensitised to one or more common moulds. There is some evidence to link increased levels of IgE to common moulds to asthma and allergic rhinitis but there is little evidence of a close association between these conditions and measured concentrations of fungi in air. It is possible that the measurements of airborne fungi used in epidemiological studies provide only a short term snap shot of exposure that is not closely related to long term exposure levels. There is extremely limited evidence to suggest that indoor mould exposures of more than
2000 cfum⁻³ or outdoor exposures of more than 1000 spores m⁻³ may be associated with increased risks of respiratory symptoms. The results of studies in children suggest that exposure levels as low as 350 cfum⁻³ in indoor air, may be sufficient to cause mild adverse effects on respiratory health. The mould concentrations found in some UK homes are associated with an increased risk of asthma. In reporting an indoor air quality survey, Jo and Seo (2005) refer to guidelines specified by the American Conference of Government Industrial Hygienists (ACGIH), of between 100 and 1000 cfum⁻³ for the total fungi and a Korean indoor bioaerosol guideline of 800 cfum⁻³. Health Canada was reported to have a guideline of 50 cfum⁻³ but this is no longer listed on their website.

The results of experimental studies confirm the potential harmfulness of inhaled fungi but do not provide dose-response information that can be readily translated into human health risks.

5.7 BETA (1→3) GLUCAN

5.7.1 Overview

Beta (1→3) glucan has received much less attention than endotoxin in studies of workplace exposure. Douwes (2005) reviewed the then available epidemiological literature on health effects of beta (1→3) glucan. The observational and experimental studies reviewed suggested some association between beta (1→3) glucan exposure, airway inflammation and symptoms, however, results were mixed and specific symptoms and potential underlying inflammatory mechanisms associated with exposure could not be identified. No conclusions could be drawn regarding the presence (or absence) of an association between environmental beta (1→3) glucan exposure and specific adverse health effects, nor was it clear which specific immunological mechanisms underlie the presumed health effects.

5.7.2 Studies in waste workers

Table A5.10, Appendix 5, summarises the results of studies in the waste industry. The results of several studies suggest that respiratory symptoms and airways inflammation are more prevalent in workers exposed to concentrations exceeding 25 ngm⁻³ than at lower levels of exposure. The results of one study in compost workers, however, suggest that exposures exceeding 29,000 ngm⁻³ may be tolerated without adverse effect (Douwes et al., 2000). It is possible that figure is a transcription error in the units of concentration.

5.7.3 Studies in other workplaces

Mandryk et al (2000) reported a significantly increased prevalence of regular cough, chronic bronchitis, regular blocked nose, regular sneezing, sinus problems, flu-like symptoms, and eye and throat irritation in sawmill workers with high levels of exposure to endotoxin and beta (1→3) glucan, (17.7 ngm⁻³ and 4.69 ngm⁻³ as an arithmetic mean across 3 plants; Table A5.11, Appendix 5). Significant positive correlations were found among beta (1→3) glucan and fungi, and endotoxin and beta (1→3) glucan exposure levels. Significant dose-response relationships were found for personal exposures to beta (1→3) glucan and regular blocked nose, chronic bronchitis and reduced lung function. The prevalence of blocked nose and chronic bronchitis in exposed workers was 36% and 49% versus 12% and 29% in the controls. The relative importance of beta (1→3) glucan in giving rise to effects is difficult to determine.
In a Taiwanese study, Wan & Li (1999) reported an association between beta (1→3) glucan in indoor air and lethargy (OR 1.25; 95% CI 1.05-1.51) and close to significant relationships for eye irritation, headache and difficulties in concentration. No significant relationship was found for respiratory symptoms. Concentrations of beta (1→3) glucan were reported as 5.7, 3.2 and 3.7 ng m\(^{-3}\) in day care centres, offices and homes respectively. Reported concentrations of bacteria, fungi and endotoxin were <8000 cfu m\(^{-3}\), <900 cfu m\(^{-3}\) and <2 EU m\(^{-3}\) respectively and were unrelated to respiratory symptoms or measures of more general health. Given the number of parameters investigated, the relationship between beta (1→3) glucan and lethargy could be a chance finding, although the close to significant relationship with other health endpoints provides limited support for a real effect.

Rylander (1999) summarised exposure response information linking low level exposure to beta (1→3) glucan to respiratory symptoms, headache and fatigue (Fig. 5.13). Rylander (1999) also highlighted a case report where respiratory symptoms and fatigue were reported in children living in a house with mould growth and mean indoor air concentrations of beta (1→3) glucan of 42 ng m\(^{-3}\). Other studies cited by Rylander found an increased prevalence of atopy in the inhabitants of houses where concentrations exceeded 4 ng m\(^{-3}\), increased respiratory symptoms in a school where mean concentrations were 15.3 ng m\(^{-3}\) compared with 2.9 ng m\(^{-3}\) in another school and a reduction in airways responsiveness in staff working at a day care centre following the reduction of beta (1→3) glucan levels from 11.4 to 1.4 ng m\(^{-3}\).

![Figure 5.13: Exposure-response information for beta (1→3) glucan reported by Rylander (1999) based on studies in a day care centre, post office and school](image)

### 5.7.4 Human volunteer experiments

Thorn *et al* (2001b) exposed 21 healthy subjects by inhalation to saline or a dose of 124 ng of beta (1→3) glucan suspended in saline in a random, double-blind, cross-over design. The equivalent concentration would be about 6 ng m\(^{-3}\) for an exposure period of 24 hours. Volunteers were examined before exposure and 24 and 72 hours afterwards with lung function testing, blood sampling and collection of induced sputum. Inhalation of beta (1→3) glucan caused a greater increase in inflammatory markers, eosinophilic cationic protein (ECP) and tumour necrosis factor-alpha (TNF-alpha) in induced sputum than saline but the difference was not statistically significant. In blood, there was a significant decrease in the number of eosinophils and in TNF-alpha production from stimulated blood mononuclear cells 72 hours after challenge with beta (1→3) glucan, but not following inhalation of saline alone.

Beijer *et al* (2002) exposed 17 subjects from homes with high levels of airborne beta (1→3) glucan (G-high) and 18 subjects from homes with low levels of beta (1→3) glucan (G-low), to beta (1→3) glucan suspended in saline and to saline alone. Inhalation challenge with beta (1→3) glucan induced a decrease in TNF-alpha secretion by peripheral blood mononuclear cells in both the G-high group as well as in the G-low group. In the G-high group, the inhalation of beta (1→3) glucan induced a significant increase in blood lymphocytes.
Rylander and Lin (2000) reported that exposure to beta (1→3) glucan was associated with an increased severity of symptoms of nose and throat irritation.

5.7.5 Animal experiments

Animal studies have demonstrated that inhalation exposure to beta (1→3) glucan is associated with respiratory irritation with or without an accompanying inflammatory response. The inflammatory effects of beta (1→3) glucan appear to be less than those of endotoxin. There is some evidence that beta (1→3) glucan may enhance the allergic response to inhaled allergens whereas other authors have reported a reduction in response. Variable interactions between beta (1→3) glucan and endotoxin have been reported with some evidence that combined response to both substances is less than the response to either individual substance. The animal data are described in more detail in Section A5.5.2, Appendix 5.

5.7.6 Conclusions

There are insufficient data to determine whether beta (1→3) glucan is itself a cause of ill health or merely a marker of fungal exposure with observed effects being caused by some other fungal component. The value of beta (1→3) glucan as a marker of fungal exposure may be compromised by its presence in a wide range of plant-derived material in addition to fungi. Additionally beta (1→3) glucan is not a single substance and there may be variability in the biological activity of beta (1→3) glucan from different environments.

The limited data available suggest that adverse health effects may arise at levels of exposure between 1 and 10 ng m\(^{-3}\) in the workplace. Exposure-response relationships have been reported but it is unclear whether beta (1→3) glucan was necessarily the causal agent for effects in these studies. Rylander and Lin (2000) suggest that beta (1→3) glucan may cause a worsening of subclinical inflammation and/or may alter susceptibility to other environmental agents such as allergens. Individuals with atopy appear to be more susceptible to beta (1→3) glucan.

5.8 SUMMARY OF KEY EXPOSURE-RESPONSE INFORMATION

Table 5.3 summarises the exposure-response information described in this chapter.
Table 5.3: Summary exposure-response information for bioaerosols

<table>
<thead>
<tr>
<th>Bioaerosol component</th>
<th>Health endpoint</th>
<th>Exposure-response information</th>
<th>Study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic dust</td>
<td>Irritation of eyes and nose, chest tightness and wheeze, chronic respiratory illness</td>
<td>Symptoms reported at 200 ugm$^{-3}$, reported at 1-2 mgm$^{-3}$, prevalence increases with concentration, may arise at concentrations &gt;0.3 mgm$^{-3}$, but normally associated with concentrations &gt;1.2 mgm$^{-3}$.</td>
<td>Waste workers, various industries, cotton workers</td>
</tr>
<tr>
<td>Fungi</td>
<td>Respiratory symptoms, nausea, headache etc</td>
<td>Symptoms reported at $&gt;10^4$ cfu m$^{-3}$ and between $10^3$-$10^5$ spores m$^{-3}$, increased symptoms associated with concentrations of 2000 cfum$^{-3}$ in indoor air or 1000 spores m$^{-3}$ in outdoor air, mild adverse respiratory effects may arise at concentrations ≥ 350 cfum$^{-3}$ in household air</td>
<td>Waste workers, general community, children</td>
</tr>
<tr>
<td>Total microbes</td>
<td>Respiratory symptoms, nausea, headache etc</td>
<td>Symptoms reported at $10^3$ cfum$^{-3}$, very limited evidence of increase in symptom prevalence with increasing exposure</td>
<td>General community near compost operations</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>Respiratory symptoms, fatigue</td>
<td>Greater prevalence of symptoms at concentrations &gt;50 EU m$^{-3}$, but indications of nasal irritation reported in one study of waste workers at 4.5 EUm$^{-3}$, clear evidence that risks increase with increasing exposure</td>
<td>Workers in various industries</td>
</tr>
<tr>
<td>Beta(1→3) Glucan</td>
<td>Respiratory symptoms, nausea, headache etc</td>
<td>Limited evidence of adverse effects at concentrations 1&lt;0 ngm$^{-3}$, no adverse effects at 1 ngm$^{-3}$</td>
<td>Studies of indoor air quality</td>
</tr>
</tbody>
</table>
6 Exposure-response relationships for sensitive subpopulations

6.1 INTRODUCTION

A high proportion of the exposure-response information available for bioaerosols has been derived in workplace studies. It seems unlikely that workers are representative of the full range of susceptibility to bioaerosols that may exist in the general population. The workforce does not include children, the elderly and long term sick. In addition, individuals who are particularly sensitive to bioaerosols are less likely to remain in employment where they are exposed to bioaerosols than less sensitive individuals. In considering the general population the following groups may potentially have increased sensitivity to bioaerosol exposure:

- Atopics (people with allergic tendencies);
- People with asthma;
- Babies and young children;
- Elderly people;
- Immuno-compromised individuals; and
- Others with pre-existing illness.

In addition, people may vary in their innate genetically programmed sensitivity to individual components of bioaerosols and some important genetic factors may not be clearly aligned to more readily recognisable characteristics such as asthma. This chapter discusses the relative sensitivity of different groups to bioaerosol exposure.

6.2 FACTORS ASSOCIATED WITH INCREASED VULNERABILITY

6.2.1 Atopy

Atopy is a genetic tendency to develop allergic diseases: atopic dermatitis, allergic rhinitis (hay fever), and asthma. Atopy involves an increased capacity to produce IgE in response to common environmental proteins such as house dustmite, grass pollen, and food allergens. Atopy would be expected to predispose towards mould allergies. There is limited evidence that atopic individuals sensitised to specific moulds are also likely to develop allergic symptoms on exposure to other moulds (Savolainen et al., 1999).

Atopy has an important influence on susceptibility to respiratory illness and a tendency towards atopy may be more important than environmental exposures in determining the likelihood of individuals developing respiratory illness. For example, in a study of Taiwanese children, Lee et al. (2003) concluded that parental atopy contributed more to childhood asthma than indoor or outdoor environmental factors.

There appear to be few studies that provide a clear comparison of the effects of bioaerosol exposure in atopic individuals to effects in normal subjects. In a study of the impacts of the indoor storage of organic waste on community health, Herr et al. (2004) reported a significantly greater increase in risk of diarrhoea, fatigue and skin rash in atopic individuals. In contrast, in a study of 135 patients with possible mould-related health effects following prolonged indoor exposure to mould, Bobbit et al. (2005) failed to find associations between the patients' presenting symptoms, atopic status, and magnitude of exposure. The results of several studies suggest that atopy in the form of sensitisation to common fungi is linked to the severity of asthma symptoms. In a study of 1041 children aged 8.9 +/- 2.1 years with mild to moderate asthma, Nelson et al. (1999) reported a strong correlation between increased sensitivity to inhaled methacholine and atopic status as assessed from skin test sensitivity to common allergens including moulds. In a study of 1132 adults aged 20-44 years with asthma, asthma severity was correlated with sensitisation to common moulds (Zureik et al., 2002). In a Spanish study of 194 patients with fatal or near fatal asthma attacks, sensitisation to Alternaria was associated with asthma attacks leading to hospital admission occurring at a significantly younger age, a higher number of deaths and a greater risk of severe neurological effects (Plaza et al., 2003). In contrast, Thorne et al. (2005) reported that the risk of asthma or asthma symptoms was similar in adults with or without allergy. In a small study of eight urban dwelling patients with allergic bronchopulmonary aspergillosis (ABPA) and five atopic
controls, no elevated concentrations of airborne *Aspergillus fumigatus* spores were recorded in the homes of either group. Patients with ABPA had not been more exposed to potentially rich sources of *A. fumigatus* than the atopic control patients. It was concluded that specific host susceptibility was more important in the pathogenesis of ABPA than environmental factors (Vernon & Allan, 1980). However, as the study was of extremely limited size, the wider significance of its findings is unclear.

The results of volunteer experiments have demonstrated that individuals can vary widely in their response to LPS but this variation in responsiveness is not clearly linked to atopy. Neither Nightingale et al. (1998) or Michel et al. (1992b) found evidence that atopy increased sensitivity to LPS. In experiments involving normal and atopic volunteers, Michel et al. (2001) reported that the effects of LPS on lung function and systemic markers of inflammation were inversely related to atopy whereas Peden et al. (1999) reported that 1 ug of LPS increased the percent of eosinophils in nasal lavage fluid in atopic but not normal subjects. In an experiment with endotoxin-rich grain dust, Sigurdarson et al. (2004) found no significant change in pulmonary function in the normal subjects whereas in asthmatics, increased bronchial hyper reactivity and a significant transient decrease in lung function (FEV₁) were induced. In an experimental study of the effects of exposure to *A. fumigatus*, Stark et al. (2006) reported that atopy had no significant impact on response.

There is some evidence from studies conducted in children and young people in Australia and in Germany, that exposure to airborne moulds may be a risk factor for atopy and allergic sensitisation (Garret et al., 1998; Jacob et al., 2002; Matheson et al., 2005).

In conclusion, there is limited evidence that the severity of response to bioaerosols is linked to atopic status with atopic individuals being more susceptible to the effects of bioaerosols than others, particularly in relation to moulds. Atopy does not, however, appear to be an important determinant of sensitivity and atopic individuals vary in their susceptibility to effects.

### 6.2.2 Pre-existing respiratory illness

**Asthma**

Individuals with asthma would be expected to be at greater risk of developing adverse respiratory effects on exposure to bioaerosols but sensitization to environmental allergens is itself an important risk factor for the development of asthma (Zeldin et al., 2006). In general the risks of sensitisation to common allergens in indoor air including fungi, dust mite or cockroach allergen, increase with increasing levels of exposure (eg Garret et al., 1998; Jaakkola et al., 2005). There is some evidence, however, that high levels of exposure to some allergens may lead to a reduced risk of sensitisation. Children living with cats, for example, have reduced risks of sensitisation to cat allergen (although it is less likely that families with cat allergy will own cats). The results of a small number of studies suggest an association between adult-onset asthma and exposure to moulds (Thorn et al., 2001a; Jaakkola et al., 2002) and/or bioaerosols more generally (Sigsgaard et al., 1990). Bornehag et al. (2001) concluded from an extensive review of the published literature that "dampness" in buildings appears to increase the risk for asthma. About 5% of individuals are predicted to have some allergic airway symptoms from moulds over their lifetime (Hardin et al., 2003). There is less evidence of a relationship between endotoxin and asthma. Thorne et al. (2005) reported significant relationships between increasing endotoxin levels, as assessed from concentration in house dust, and diagnosed asthma, asthma symptoms in the past year, current use of asthma medications, and wheezing among the adult residents of surveyed homes.

There appears to be paucity of data to demonstrate that individuals with asthma are indeed more susceptible to the effects of bioaerosol exposure than other individuals. The results of volunteer experiments comparing asthmatic and normal subjects are mixed. Sigurdarson et al. (2004) reported that exposure to endotoxin-rich grain dust (4 mg m⁻³) had no significant effect on lung function in normal subjects but mild asthmatics showed increased bronchial hyper reactivity and a significant transient decrease in FEV₁ (unaffected by concurrent exposure to ammonia). Kitz et al. (2006) reported that an inhalation challenge of LPS at dose of up to 45 ug induced similar reductions in lung function and systemic responses in both healthy and
asthmatic volunteers. Although variable sensitivity to LPS was apparent, sensitivity appeared unrelated to asthma. Alexis et al (2001) reported that asthmatics showed a greater inflammatory response to inhaled LPS associated with a higher level of CD14 – a receptor on cell surfaces that interacts with endotoxin. In a comparison of asthmatic and normal subjects, Nightingale et al (1998) reported slightly different patterns of response to inhaled LPS but their results do not provide evidence of any increased susceptibility to LPS.

There is some evidence that asthma is associated with an increased risk of developing ABPA, a rare hypersensitivity reaction to aspergillosis. The reported prevalences in asthmatics range from 1-8% and there is growing evidence that genetic factors play an important role in determining the susceptibility of individuals with asthma to ABPA (Vinig & Bush, 2007).

**Cystic Fibrosis**

Cystic fibrosis patients are recognised to be at increased risk of developing hypersensitivity in response to *Aspergillus* exposure with reported prevalences of ABPA in patients with cystic fibrosis ranging from 2-25% (Knutsen et al 2006, Vinig & Bush, 2007).

Individuals with cystic fibrosis vary in their susceptibility to ABPA. In a case control study involving 160 cystic fibrosis patients including 11 with ABPA and 20 with evidence of *Aspergillus fumigatus* sensitisation, Ritz et al (2005) reported that sensitisation was associated with significantly higher cumulative doses of inhaled corticosteroids. Sensitisation was also independently associated with a longer duration of *Pseudomonas aeruginosa* colonisation. Additionally, ABPA was associated with bronchial colonisation with *Stenotrophomonas maltophilia*.

Genetic factors may also play an important role in determining the relative susceptibility of individuals with cystic fibrosis to developing ABPA. Knutson et al (2006) describe some genetic markers that they believed to be predictive of an increased risk of ABPA.

**Animal data**

Sorkess et al (2007) demonstrated that the response of sensitised rats to the effects of repeated low-level exposures to inhaled *Alternaria* extract in animals with pre-existing chronic airways disease was enhanced compared to that in sensitized controls with healthy airways.

In a study in calves, Kalina et al (2006) established that infection with bovine respiratory syncytial virus (BRSV) was unaffected by prior exposure to *Alternaria alternata* aerosol but exposure to *Alternaria alternata* did reduce the impacts of re-infection three months later. BRSV infection, however, did enhance the immune response to inhaled *Alternaria*.

**6.2.3 Immunosuppression**

Immunocompromised individuals such as those with HIV infection, patients in intensive care and transplantation units, and cancer patients are at a greatly increased risk of developing fungal infections (Richardson, 2005). These individuals are at risk of developing invasive candidosis (usually as a result of prior infection by *Candida* species), and, of more relevance to the waste industry, Aspergillosis (fungal colonization of scarred lung tissue) and invasive aspergillosis (infection with pneumonia which can affect the heart, lungs, brain and kidneys). Much more rarely, HIV patients have developed ABPA most commonly in response to *Aspergillus fumigatus* (Jain, 2000). Specific risk factors for invasive aspergillosis include underlying lung disease, prolonged neutropenia (reduced white blood cell count), immunosuppressive therapy, corticosteroid therapy, allogenic haematopoietic stem cell transplantation and graft-versus-host disease and its treatment. In lung transplant patients, important risk factors include pre-transplant airways colonisation by *Aspergillus*, cytomegalovirus disease, immunosuppressive therapy and the contact between the lung allograft and the external environment. In kidney transplant patients important risk factors include the cadaveric donor, prolonged pre-transplant dialysis, end-stage renal disease due to diabetes, rejection and maintenance tacrolimus treatment. Other risk factors for aspergillosis
include deep skin damage, burns, some viral and bacterial infections, chemotherapy and radiotherapy.

A number of case studies have demonstrated the potential of *Aspergillus fumigatus* to infect debilitated or immunosuppressed subjects. Sessa *et al* (1996) and Arnow *et al* (1978) for example, describe cases of *A. fumigatus* infection occurring in kidney transplant patients associated with building renovation work in the proximity to the wards where patients were housed. Alberti *et al* (2001) investigated the incidence of invasive nosocomial aspergillosis (INA) in a bone marrow transplantation unit and two haematology wards, over a four year period. A total of 79 cases of INA were diagnosed, of which 64 were probably or possibly INA and a significant relationship was found between the incidence of INA and the degree of fungal contamination of air and surfaces in conventional patient rooms (not equipped with high efficiency particular air filters; HEPA) and common sites.

The Health Protection Agency (HPA, 2006) have estimated the risks of developing invasive aspergillosis for different patient groups (Table 6.1). The greatest number of cases is predicted to arise in cancer patients whereas those at greatest risk are bone marrow transplant, leukaemia and HIV/AIDS patients.

**Table 6.1: Invasive aspergillosis infection – estimated number of cases, 2002 (HPA, 2006)**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number of patients</th>
<th>Risk estimates</th>
<th>Expected number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allogeneic bone marrow transplant</td>
<td>793</td>
<td>10%</td>
<td>79</td>
</tr>
<tr>
<td>Solid organ transplant</td>
<td>2956</td>
<td>1.9%</td>
<td>56</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>16269</td>
<td>6%</td>
<td>976</td>
</tr>
<tr>
<td>Solid tumour (neutropenic)</td>
<td>28955</td>
<td>2%</td>
<td>579</td>
</tr>
<tr>
<td>Advanced cancer</td>
<td>131678</td>
<td>1.5%</td>
<td>1975</td>
</tr>
<tr>
<td>Intensive Care</td>
<td>210130</td>
<td>0.2%</td>
<td>420</td>
</tr>
<tr>
<td>Burns</td>
<td>378</td>
<td>1.9%</td>
<td>7</td>
</tr>
<tr>
<td>Renal dialysis</td>
<td>24536</td>
<td>0.02%</td>
<td>5</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>661</td>
<td>4%</td>
<td>26</td>
</tr>
</tbody>
</table>

### 6.2.4 Children and babies

There is considerable evidence that young children may respond differently to bioaerosols than adults. A large number of studies suggest that elevated levels of exposure to endotoxin in house dust during early childhood may be associated with reduced risks of developing asthma. In a review, Eder and von Mutius (2004) concluded that the results of cross-sectional surveys, prospective cohorts, and experimental studies *in vitro* and in rodents suggest that exposure to house dust endotoxins in early life protects from atopic sensitization and IgE-mediated diseases, but is a risk factor for wheezing in infancy. The results of subsequent studies generally support this conclusion as outlined below.

In a study of the relationship between exposure to dust mite allergen and endotoxin at age 2 to 3 months and asthma, wheeze and atopy in children with a parental history of atopy, Celedon *et al* (2007) established that early endotoxin exposure was associated with a reduced risk of atopy but an increased risk of wheeze. High levels of dust mite allergen were associated with increased risks of asthma and late-onset wheeze. In a study of New York children, Perzanowski *et al* (2006) reported that domestic endotoxin exposure was inversely associated with eczema at age 1 year, but positively associated with wheeze at age 2 years. In another study in children, Douwes *et al* (2006) reported that levels of exposure to biocontaminants and dust at 3 months in age were inversely associated with the development of doctor-diagnosed asthma between the ages of 1 and 4, with the association being most pronounced for endotoxin in floor dust. Persistent wheeze was also consistently less common in the high-exposure group. In a UK study, Tavernier *et al* (2006) reported that asthma in children was significantly associated with household levels of endotoxin, living in a single-parent family, redecoration in the living room and self-reported absence of dampness. There
was no difference between asthmatic and healthy children in their exposure to dust mite allergen, objective measurements of dampness, guardian's smoking habits, pet ownership, house type or age, time in residence, central heating systems, insulation types, glazing systems, floor types, and age and measurements of several indoor pollutants. In contrast, cross sectional studies in German and Swiss children have also demonstrated that exposure to higher levels of house dust endotoxin is associated with lower prevalence of allergic sensitization and asthma in children (Gehring et al, 2003; Braun-Fahrländer et al, 2002).

There is little data from animal experiments relevant to understanding the relative susceptibility of children to the adverse effects of bioaerosols. Carl et al (2004) exposed mice ages 2, 4, 7, 10, 14, 28, and 56 days to LPS by inhalation with an estimated deposited dose of 26 EU. Two hours after exposure, the proinflammatory cytokine interleukin (IL)-6 was not detected in 2- and 4-day-old mice; however, 8- to 10-fold increases were measured in 7-, 14-, and 28-day-old mice and approximately 20-fold in 56-day-old mice. Similar effects were seen in some but not all other investigated markers of inflammation.

6.2.5 Pregnancy and reproduction

The results of a limited number of studies in animals suggest that pregnant and lactating animals may show an enhanced inflammatory response in the airways following inhalation exposure to endotoxin. There is also limited evidence that extremely high levels of exposure to endotoxin may have serious adverse effects on the unborn child with foetal death, reduced foetal weights and skeletal deformations arising in animals. There are differences in physiology and metabolism between species however, that can give rise to uncertainties about the relevance of effects seen in some animal experiments for the prediction of human health effects.

Huffman et al (2004) exposed pregnant Sprague-Dawley female rats (17 days of gestation) or age-matched virgin female rats to air or endotoxin (LPS) by inhalation for 3 hours. At 18 hours following exposure to endotoxin, lactate dehydrogenase activity levels in bronchoalveolar lavage (BAL) fluid samples from pregnant rats were 1.5-fold greater than those from endotoxin-exposed virgin rats. BAL polymorphonuclear leukocyte (PMN) numbers were also approximately twofold greater in pregnant rats than in virgins following the inhalation of endotoxin.

Xu et al (2006) investigated the effects of maternal LPS exposure on intra-uterine foetal growth and skeletal development mice. An intraperitoneal injection of LPS (75 ug/kg) resulted in 63.2% foetal death, significantly lowered foetal weight, reduced crown-rump and tail lengths, and retarded skeletal ossification in caudal vertebrae, anterior and posterior phalanges, and supraoccipital bone. Given that the experimental dose of LPS was several orders of magnitude greater than likely levels of environmental exposure to endotoxin, the findings are not relevant to understanding the effects of human environmental exposure to bioaerosols.

Gordon et al (1993) demonstrated that lactating rats were considerably more susceptible to the effects of inhaled endotoxin than virgin rats, with inflammatory markers in BAL being generally 1.5- to 3-fold greater in lactating than in virgin female rats. Markers of lung inflammation were detected in BAL in lactating rats exposed to 1.3 ug m⁻³ endotoxin.

6.2.6 Older people

A number of studies have demonstrated that older people are more sensitive to air pollution, particularly PM₁₀, than younger adults. There are no specific data that suggest that older people may be particularly susceptible to adverse effects following exposure to bioaerosols.
6.2.7 Genetic factors

Endotoxin

The response to LPS exposure is highly variable and might be a result of genetic diversity between individuals. The toll-like receptor 4 (TLR4) is the principal receptor for LPS on cell surfaces. Schwartz (2002) demonstrated that the common, cosegregating missense mutations (Asp299Gly and Thr399Ile) in the extracellular domain of the TLR4 receptor are associated with a significantly blunted response to inhaled LPS in 83 humans. However, it was also apparent that genes other than TLR4 may be playing a role in the biological response to LPS. Mutations in the TLR4 receptor may predispose people to develop septic shock with gram-negative microorganisms (Lorenz et al., 2002). In a study in which 91 patients with septic shock were compared with 73 healthy blood donor controls, the TLR4 Asp299Gly allele occurred exclusively in patients with septic shock and patients with septic shock with the TLR4 Asp299Gly/Thr399Ile alleles had a higher prevalence of gram-negative infections. In a separate study of 116 healthy subjects challenged with 20 ug LPS by inhalation, subjects heterozygous for either TLR-4+/896 or TLR-4+/1196 had significantly lower numbers of white blood cell counts and lower levels of C-reactive protein and LPS-binding protein compared with homozygotes with the common allele. Only 1 of the 18 heterozygous subjects was a high responder to LPS (defined as a rise in C-reactive protein > 10 mg/L), whereas 36 of 98 homozygous subjects were high responders (P < .02). No association was observed between the TLR4/-2026 and TLR4/-1607 polymorphisms and LPS responsiveness.

In contrast, in a study of 57 volunteers exposed to LPS (2 ng/kg) by intravenous injection, the inflammatory response in subjects with hTLR4 mutations (Asp299Gly and Thr399Ile) (8/57) was indistinguishable from that in subjects without the mutations (Calvano et al., 2006).

Togbe et al (2006) investigated the influence of the TLR4 gene in acute respiratory response to endotoxin in transgenic mice. Mice expressing three, six, or 30 copies of TLR4, control, and TLR4-deficient mice received intranasal administration of 10 ug LPS. Overexpression of TLR4 lead to a dose-dependent enhanced LPS-induced bronchoconstrictive effect, as well as increased levels of pro-inflammatory cytokines and increased alveolar epithelial injury with protein leak in the airways.

The receptor CD14 which is mainly expressed on the surface of white blood cells (monocytes, macrophages and neutrophils) has specificity for endotoxin. Genetic variation in a functional single nucleotide polymorphism in the 5 genomic region of CD14 (CD14/-159) has been widely tested in relation to asthma and associated traits. In a study of the interaction of exposure house-dust endotoxin or domestic sources of microbial exposure with CD14/-159 variation, Martinez (2007) reported that the C-allele is a risk factor for allergic phenotypes at low levels of exposure, whereas the T-allele is a risk factor at high levels of exposure. Simpson et al (2006) established that increasing endotoxin exposure as assessed from the endotoxin content of house dust is associated with reduced risk of allergic sensitization and eczema but with increased risk of nonatopic wheeze in children with the CC genotype at -159 of the CD14 gene.

Fungi

There is evidence of variable genetic susceptibility to fungi-induced inflammation in animal experiments, but no clear information about genetic susceptibility in humans. Intratracheal instillation of Stachybotrys chartarum spores suspended in saline in three different strains of mice gave rise to significantly different levels of inflammatory response. Additionally in OVA-sensitised mice, OVA-induced airway inflammation produced a protective effect against some S. chartarum-induced pulmonary responses (Rosenblum et al, 2006).

6.3 EXPOSURE RESPONSE RELATIONSHIPS

There is no specific exposure-response information for sensitive subgroups. Most study populations will have included a range of susceptibility, however, and what exposure-
response information does exist for workplace or community studies would be based on effects in some relatively susceptible individuals. The wide disparity in exposure-response functions reported from different workplaces may partly reflect the relative susceptibility of the workforce. The availability of alternative work will have affected whether workers suffering from mild respiratory illness have remained in post or moved on. It is probable that the proportion of susceptible individuals in the general population is greater than in workplace populations but it may not be substantially greater. The general population will include some particularly vulnerable individuals who are unlikely to be in active employment, or if in active employment, are unlikely to be employed in industries where substantial bioaerosol exposure would occur.

A substantial minority of the population are sensitised to different fungal species in ambient air (or are genetically susceptible to becoming sensitised) and will have been represented in studies of bioaerosol-exposed populations. Individuals with asthma may be more likely to experience adverse effects on exposure to bioaerosols than other individuals, but most study populations will have included some individuals with asthma. It is also likely that study populations have included a range of genetic susceptibility to the effects of endotoxin.

A small number of immuno-compromised individuals are susceptible to fungal infection at extremely low levels of exposure. There is no clear threshold below which no adverse effects are likely to arise. Some of the most vulnerable individuals in the population will be undergoing treatment in hospital but there are also other vulnerable individuals who are likely to be living in the general community including those with HIV infection and transplant patients.

6.4 CONCLUSIONS

The limited exposure-response information available for bioaerosols is largely based on studies where the study population has included a range of sensitivity including people with asthma, atopy, specific sensitisation to bioaerosol components and an increased reactivity towards endotoxin. It is unlikely that most study populations will have included particularly sensitive individuals including those with severe immunosuppression such as transplant patients, HIV-infected individuals or cancer patients. Some of these individuals may be susceptible to serious adverse effects at exposure levels commonly encountered in the general community in the absence of a specific bioaerosol source such as a waste management operation. It is not clear whether thresholds will exist for adverse effects arising in the most sensitive individuals.

There is no clear evidence that children or the elderly are particularly susceptible to the effects of bioaerosol exposure. There is very limited evidence from animal experiments that women may have an increased susceptibility to effects during pregnancy and lactation, and that high levels of exposure to endotoxin may be damaging to the unborn child. There are no data that suggest that adverse effects are likely to arise in humans at environmental levels of exposure to endotoxin.
7 Use of exposure-response information to support regulation

7.1 INTRODUCTION

This chapter discusses different issues about the use of health data to support the regulation of bioaerosol emissions from waste sites. In principle, exposure-response information could be used to predict the level of risks to health associated with exposures arising as a result of different practices associated with different waste management options. In turn this could help inform the approach taken to regulating different waste management options in order to minimise the risks to human health. The quantification of health benefits/disbenefits using exposure-response relationships combined with estimated exposures could also be used within the context of cost-benefit analysis to inform regulatory impact analysis. Cost benefit analysis may be an important tool in the selection of the most appropriate approaches to regulating bioaerosol emissions from waste management.

The main regulatory options considered in this chapter include:

- Environmental Quality Standards;
- Emissions limits;
- Best Practice Guidance; and
- Stand off distances.

For each option a variety of approaches is possible. The output from this project is particularly relevant to the setting of environmental quality standards and a consensus view on acceptable environmental levels may also be required in order to set emissions limits. Exposure-response information could also be used to inform the development of Best Practice Guidance, if the exposures arising from best practice can be measured or modelled. A quantitative health impact assessment based on the available exposure-response information could demonstrate whether current best practice is sufficient for the purposes of health protection and/or the extent of reduction in exposures that is desirable. Advice on stand off distances could be based on the results of the few limited published studies and/or the use of exposure-response information coupled with exposure modelling in order to predict likely health impacts at differing distances from different types and sizes of waste operations. Doing nothing is a further regulatory option that is not discussed in detail here but is likely to be included in any future regulatory impact analysis. The use of exposure-response information to estimate the current impact of bioaerosol emissions on community health is likely to play an important role in the decision on the necessity for greater regulation.

It is important that officers of the regulatory agencies are provided with clear, publicly available guidance in order to ensure that bioaerosol emissions from waste processes are regulated in a consistent fashion. It is also important that the regulatory approach adopted is proportionate to the risk of adverse health effects arising in local communities. Ideally regulatory policy should be underpinned by a quantification of the health benefits for local communities versus the costs for operators and the regulators. It should be demonstrated that the health benefits of controlling bioaerosol emissions from waste sites outweigh the costs of developing and implementing regulatory policy. There are a number of difficulties in quantifying the benefits associated with different regulatory options:

- Uncertainties as to the impact of different options on community exposure to bioaerosols;
- Uncertainties about the health impact of community exposure to bioaerosols arising from waste processes;
- Uncertainties in the exposure-response information from which health impact might be assessed; and
- The relatively non-specific nature of many of the health complaints associated with bioaerosol exposure.
Symptoms such as headache, nausea and fatigue, that are often reported to be associated with bioaerosol exposure, are also common complaints in communities exposed to odour. These symptoms would contribute to a loss of well-being in affected communities but not necessarily to more tangible disbenefits such as sickness absence from work. Although it seems probable that well-being is linked to long term life expectancy, there are no hard data to enable the conversion of ill defined effects on well-being to quantifiable impacts on life expectancy or other health endpoints to which monetary values can be readily attached. Although an approximate estimate of impact could be derived by considering the impact of airborne particulate, using methodologies that have been developed within the Clean Air for Europe programme (Hurley et al., 2005), this approach will not capture specific effects associated with bioaerosols. It is not known whether bioaerosol exposure is likely to give rise to cardiovascular impacts similar to those seen with combustion particulate and the relative importance of particulate versus specific bioaerosol attributes in governing overall impact has not been determined. Adverse effects on well-being may arise in odour-exposed populations even where bioaerosol concentrations are negligible (Schiffman & Williams, 2005). The benefits of introducing specific measures to control bioaerosol emissions are likely to be limited, unless odour nuisance is also appropriately controlled.

The results of workplace studies indicate that the development of respiratory symptoms as a result of exposure to bioaerosols is likely to lead to long term respiratory illness following prolonged exposure. Given a relatively high background prevalence of respiratory illness, particularly among older members of the population (between 10 and 20% of adults report chronic cough or phlegm and the prevalence of Chronic Obstructive Lung Disease is believed to exceed 1.5%; LAIA, 2003), a relatively small increment in risk (for example, a 0.7% annual increase in new cases of chronic bronchitis per $\text{ugm}^{-3}$ increase in long term mean exposure concentrations of organic dust) is likely to give rise to a relatively large number of additional cases of illness. From this perspective, the mean exposure levels of the general population to bioaerosol emissions from waste operations could be associated with a relatively substantial burden of ill health. The risk of developing long term respiratory illness is likely to be an important consideration in comparing the benefits associated with different regulatory options for controlling bioaerosol exposure.

The development of an appropriate strategy for the regulation of bioaerosol emissions from waste processes should take account of the Government's Better Regulation initiative (summarised in Appendix 6). Of particular relevance are the requirements for a risk based approach to inspection, consistency of enforcement, a reduction in targets and inspection burden imposed on frontline staff, a streamlining of data requirements and the use of information technology to minimise reporting burdens for industry and regulators.

### 7.2 ENVIRONMENTAL QUALITY STANDARDS/GUIDELINES

#### 7.2.1 Introduction

Environmental quality standards or guidelines are widely used to inform regulation as they allow the easy benchmarking of impacts. The Environment Agency (EA), for example, sets Environmental Assessment Levels (EALs) for air pollutants against which the modelled impacts of process emissions can be compared. Typically process permit conditions would take account of both EALs and identification of Best Available Techniques (BAT).

Environmental quality standards are generally set at a level at which the risks to health are believed to be negligible. Standards are intended to protect most people most of the time. Given the vast range of susceptibility to some pollutants that exists within the general population, it may not be possible to set standards at sufficiently low levels to provide complete protection for the most susceptible groups.

Where threshold levels of exposure associated with adverse effects can be established, then standards would normally be set at levels below the threshold. Where it is not possible to establish a threshold, for example for genotoxic carcinogens, standards are set at a level that

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1 Based on information for PM$_{10}$ (Hurley et al., 2005)
controls risks to a low level. In the UK, standards/guidelines for environmental exposure are normally set at levels that are intended to control the excess lifetime risk of cancer or premature mortality to \(10^{-5}\). Appendix 7 outlines the process by which environmental quality standards are set.

The concept of thresholds is usually applied to chemical or physical agents rather than biological agents. It is probably reasonable to seek to identify threshold levels of exposure to bioaerosols for effects such as mucous membrane irritation and sensitisation that may also be caused by chemical agents. The concept of threshold may be less appropriate in considering effects such as invasive fungal infection.

### 7.2.2 Issues that need to be considered in standard setting

Air quality guidelines normally include definition of the substance, a reference concentration and averaging time. The supporting documentation is likely to describe the health outcomes that the guideline is intended to protect against and a description of the evidence used in the development of the guideline. It may also include a defined measurement method.

For bioaerosol exposure, the relevant health outcomes are likely to include acute and chronic respiratory effects, nausea, headache and fatigue. Unlike health outcomes such as mortality and cancer, these outcomes are relatively poorly defined, largely subjective (ie no objective measurement of effect is possible) and have multiple causes. Nausea, headache and fatigue and, to a lesser extent, reporting of respiratory symptoms may be influenced by psychological factors. Some objective measures of respiratory impairment are available (eg lung function) but these are difficult to interpret in terms of community health. In addition, respiratory health and function are substantially affected by age, smoking habits, workplace exposure to air contaminants and exposure to environmental tobacco smoke. It is possible to make objective measures of the development of immune response in terms of the development of specific Ig antibodies. However, although the development of specific Ig antibodies is linked to the development of an allergic response, it is not a clear predictor of health effects.

High quality exposure-response data is required to set a defensible guideline. Section 7.2.3 discusses the data availability and quality and assesses the feasibility of setting guidelines for relevant bioaerosol components: organic dust, endotoxin, bacteria, fungi and beta\((1\rightarrow3)\) glucan.

For a guideline to be used as part of the regulatory process, it is necessary to be able to assess compliance (Section 7.2.4). As bioaerosols are emitted from various sources, regulation of bioaerosol emissions from the waste industry must take account of bioaerosol exposure from other sources. Any health effects are likely to be related to total exposure and in some areas background bioaerosol concentrations resulting from agricultural sources are likely to be substantial. In addition, individual exposure to bioaerosols may be heavily influenced by lifestyle with exposures arising from living and/or working in premises affected by damp, handling domestic waste, gardening, composting and outdoor recreational activities.

### 7.2.3 Feasibility of developing health-based exposure standards by pollutant

**Organic dust**

*Relevance to health:* There are substantial workplace data that demonstrate that exposure to organic dusts is associated with the development of serious respiratory illness. Workplace exposure to organic dusts at concentrations of 200 \(\mu g m^{-3}\) or greater is associated with irritation of the eyes and nose. The development of respiratory symptoms in the workplace occurs at concentrations of 1-2 \(\mu g m^{-3}\), equivalent to an environmental exposure of 200-400 \(\mu g m^{-3}\), if time-averaged for continuous exposure (200x8 hour working days versus 365x24 hour days per year). Long term workplace exposure to concentrations exceeding 0.3 \(\mu g m^{-3}\) (300 \(\mu g m^{-3}\)) is associated with the development of respiratory illness with serious chronic conditions such as byssinosis generally arising at concentrations exceeding 1.2 \(\mu g m^{-3}\). Workplace exposure over 40 years to 300 \(\mu g m^{-3}\) is approximately equivalent to lifetime (24 hours x 365 days x 80 years) environmental exposure to 30 \(\mu g m^{-3}\) (as an annual average). In
comparison, the air quality standard for PM\(_{10}\) in ambient air is 50 \(\mu g m^{-3}\) (24 hour mean) with a 2010 objective of 20 \(\mu g m^{-3}\) as an annual mean (UK except Scotland and London). The apparent potency of organic dusts varies in different workplace environments.

**Measurement:** Concentrations of organic dust can be determined by collection onto filter and determination of the mass loss following plasma ashing, although this method is subject to interference from diesel fume and other combustible dusts which would also be removed by plasma ashing. It would be feasible to make 24 hour gravimetric measurements using a sampling regime similar to that required for PM\(_{10}\) sampling. In addition, for routine process monitoring, PM\(_{10}\) (including organic and inorganic dust) can be measured on a continuous basis. The relative proportion of organic/inorganic dust in PM\(_{10}\) could be determined by gravimetry combined with plasma ashing for a number of typical samples to inform the interpretation of results.

**Specificity to waste processes:** organic dust can be emitted from a wide range of environmental sources including agriculture, food processing, textiles, forestry, forestry products and so on. Dusts emitted from these sources are likely to have different compositions and potentials to cause adverse effects. In addition, a simple measurement method based on plasma ashing will not distinguish between organic dusts and soot from vehicle and other combustion sources. Combustion-generated particulate plays an important role in the association between PM\(_{10}\) and adverse health effects (EPAQS, 2001 and the relative toxicity of organic dusts versus combustion particulate has not been established.

**Potential to develop a guideline value:** The results of epidemiological studies performed in the waste, agriculture and textile industries could inform the development of a standard. The variable potency of dusts in different workplace environments means that there would be uncertainty as to the level of protection associated with any guideline. The results of a number of studies suggest that the harmfulness of organic dusts is related to their endotoxin content. It may be desirable to develop a standard for endotoxin rather than organic dust but organic dust may still prove a useful measure of exposure to bioaerosols that is relevant to health. If the mean endotoxin content of organic dust from a specific process is determined, the measurement of organic dust may provide a useful marker of endotoxin exposure.

**Reference period:** Exposure to high concentrations of organic dust over an 8 hour working shift may give rise to irritation of the eyes and respiratory system and it seems appropriate to set a guideline to minimise these effects. The data would support the setting of an 8 hour guideline, although it may be desirable to take account of the 24 hour averaging time for the PM\(_{10}\) standard as it is likely that the control of organic dust emissions from waste sites will be integrated with the control of other dust emissions including PM\(_{10}\). Long term exposure to organic dust in the workplace is associated with the development of chronic respiratory illness and it would be appropriate to set a guideline for annual mean exposure in ambient air.

**Preliminary assessment of guideline values:** Based on the development of mucous membrane irritation in workers exposed to concentrations \(\geq 200 \mu g m^{-3}\), it may be appropriate to control short term exposure in ambient air to organic dust arising from waste processes to between 10 and 20 \(\mu g m^{-3}\) as an 8 hour or 24 hour guideline. The margin of uncertainty allows for interindividual differences in susceptibility and the potentially greater susceptibility of some members of the general population than the workers in the source studies.

Based on the development of chronic respiratory illness in cotton workers at concentrations \(\geq 300 \mu g m^{-3}\), it may be appropriate to control annual mean concentrations of organic dust arising from waste processes to about 3 -6 \(\mu g m^{-3}\). This allows a factor of 10 to allow for differences in the duration of exposure and a further factor of 5-10 to allow for interindividual differences in susceptibility and the potentially greater susceptibility of some members of the general population than the workers in the source studies.

**Endotoxin**

**Relevance to health:** Endotoxin is the most consistently reported measure of workplace exposure to bioaerosols, although there has been little investigation of environmental
exposure to endotoxin. Both workplace studies and studies in human volunteers have demonstrated a clear link between elevated exposures to endotoxin and a range of adverse health effects. Some healthy individuals will experience respiratory symptoms on exposure to concentrations of endotoxin of about 50 EUm$^{-3}$ in the workplace and a small proportion of individuals may experience symptoms at concentrations of <10 EUm$^{-3}$. Long term workplace exposure to concentrations exceeding about 20 EUm$^{-3}$ is associated with an increased risk of developing chronic respiratory illness.

**Measurement:** It is not possible to make continuous measurements of endotoxin but collected dust samples can be analysed using the LAL assay (Section 2.4.5). Although the measurement of endotoxin is less precise and associated with greater uncertainty than that of dust or organic dust, it is probably more precise than any of the methods that have historically been used to determine microbial concentrations. A British Standard method is available.

**Specificity to waste processes:** Endotoxin is released from processes in the agricultural, food and waste water treatment industries and is not specific to bioaerosols from waste processes.

**Potential to develop a guideline value:** Endotoxin is the only bioaerosol component for which the exposure-response information provides a strong evidence base for a defensible exposure limit.

**Reference period:** Most workplace studies of endotoxin exposure have focussed on effects such as respiratory irritation that could develop over a single 8 hour working shift. There is limited evidence from the cotton industry that repeated exposure on successive days may lead to short term tolerance, although this reduces following a couple days of no exposure. Given the range of acute effects associated with endotoxin, there does not appear to be strong evidence to support the development of a guideline based on an 8 hour reference period as opposed to a 24 hour reference period. Long term exposure to organic dust containing endotoxin in the workplace, particularly in the cotton industry, is associated with the development of chronic respiratory illness. It may be appropriate to set a guideline for annual mean exposure in ambient air but it may be difficult to demonstrate compliance using existing measurement methodologies.

**Preliminary assessment of guideline values:** Based on the development of respiratory symptoms at concentrations exceeding about 50 EUm$^{-3}$ in workers exposed to organic dust in various industries, it may be appropriate to control exposure to endotoxin in ambient air to levels less than about 2.5-5 EUm$^{-3}$ as an 8 hour average or to a lower level (perhaps between 1 and 3 EU m$^{-3}$) as a 24 hour standard to protect against acute effects. The margin of uncertainty allows for interindividual differences in susceptibility and the potentially greater susceptibility of some members of the general population than the workers in the source studies. Given that some studies have reported possible adverse effects at workplace exposures to < 10 EUm$^{-3}$, it is unclear whether an environmental guideline of 5 EUm$^{-3}$ would provide adequate protection for the general population. It may be appropriate to set a lower annual mean guideline in order to protect against the development of chronic respiratory illness. Based on the development of long term respiratory illness in some workers exposed to more than 20 EUm$^{-3}$, it might be appropriate to set a guideline of 0.2 EUm$^{-3}$ as an annual guideline. The margin of uncertainty allows for differences in the duration of exposure and the interindividual differences in susceptibility and the potentially greater susceptibility of some members of the general population than the workers in the source studies. The results of other workplace studies suggest a much higher threshold for the development of long term respiratory illness and a slightly higher guideline might be adequately protective.

**Comments:** Endotoxin is a good general marker of bioaerosol exposure although as it is derived only from gram negative bacteria, it is unlikely to account for all of the biological activity of a mixed bioaerosol. It has not been widely measured in ambient air and there are no reports of elevated concentrations in the vicinity of waste operations. The results of studies in the waste industry suggest that although endotoxin contributes to the overall harmfulness of bioaerosol emissions, the relationship between endotoxin concentration and the potential harmfulness of emissions is variable. If endotoxin were to be used as a single representative
marker of bioaerosol exposure for the purposes of regulation, the extent of protection provided would be highly variable.

Microbial concentrations

Relevance to health: Airborne microbes are associated with adverse health effects, particularly respiratory illness, but also headache, fatigue and nausea. There are limited data suggesting exposure to microbial concentrations exceeding $10^5$ cfum$^{-3}$ in the vicinity of a composting facility may be associated with increased risks of respiratory and gastrointestinal symptoms, headache and fatigue in the local community (Herr et al., 2004). Adverse effects on respiratory health and more general health (excessive tiredness) have been reported in waste workers exposed to bacterial concentrations exceeding $10^3$ as total bacteria/m$^3$ or $10^5$ cfum$^{-3}$, although no specific relationship between bacterial exposure and health has been established (section 5.4.2). Adverse effects on respiratory health have also been reported in waste workers exposed to concentrations of airborne fungi exceeding $10^3$ cfum$^{-3}$ with limited data suggesting that gastrointestinal effects may arise at concentrations of less than $10^3$ cfum$^{-3}$. There is also limited evidence of an absence of an association between workplace exposure to a fungal concentration of about $10^3$ cfum$^{-3}$ and adverse effects on health (section 5.6.2). Mild inflammation of the upper airways has been observed in waste workers exposed to concentrations of $10^3$ to $10^6$ total spores/m$^3$ as assessed by SEM and gastrointestinal symptoms have been observed at spore concentrations of $10^5$ spores/m$^3$ (section 5.6.2). Studies in other industries have found limited evidence of adverse effects at lower levels of exposure, although the similarity of the species make up of the bioaerosol to that found in the waste industry is uncertain (section 5.6.3). The results of studies of microbial exposure in indoor air suggest that a relatively substantial proportion of individuals (perhaps >10% of the population) are likely to be susceptible to developing respiratory symptoms at levels of bioaerosol exposure (microbial counts of $10^2$-$10^3$ cfum$^{-3}$) that are encountered in the general community, in the absence of any specific waste management source. A UK study (Strachan et al., 1990) found evidence of reduced lung function in children with asthma exposed to mean concentrations of 354 cfum$^{-3}$ in indoor air, although the findings were not statistically significant and it is possible that effects were largely associated with exposures that were much greater than 354 cfum$^{-3}$. A US study reported that the risk of asthma related death was increased on days when spore concentrations in outdoor air exceeded 1000 spores/m$^3$ (Targonski et al., 1995). The most vulnerable members of the general community who have severe immunosuppression arising from drug treatment (transplant patients), infection or cancer may be susceptible to developing fungal infections at levels of exposure that are at or below the detection limits for available measures of bioaerosol exposure.

Measurement: A wide range of methods have been used for the sampling and analysis of microbial aerosol. There has been considerable effort invested in method development but relatively little interest in method harmonisation. Although most epidemiological studies have characterised exposure in terms of viable microbes, these measurements may be particularly sensitive to errors arising from the impacts of the sampling method on organism viability. It would clearly be feasible to develop a standardised measurement protocol based on existing EA guidance (section 2.5) to support any proposed guideline value. It would be desirable that samples are collected over minimum periods of several hours in order to provide a representative measurement of community exposure.

Specificity to waste processes: The microbial mix emitted from different processes or from an individual process at different times is very variable and is likely to have a variable potential to cause adverse health effects. Given that the processes of microbial degradation that occur in waste are similar to those occurring in soil and other damp materials, many of the species present are likely to have other environmental sources.

Potential to develop a guideline value: Studies of community exposure to bioaerosols have used various indices of exposure to viable microbes. The available exposure-response information from community studies is too weak to set a defensible exposure standard. There are limited data describing the lowest levels of fungal exposure associated with adverse effects in waste workers but differences in measurement protocol limit the extent of comparison between studies and the derivation of clear lowest and/or no effects levels. In
addition some individuals are so sensitive to specific fungal species that any level of exposure might potentially give rise to adverse effects. The uncertainties in identifying threshold levels of exposure associated with effects would give rise to considerable uncertainty as to the appropriateness of any guideline value that may be derived. There are insufficient data on which to base a guideline value for bacteria. Although it might be desirable to specifically set guidelines and monitor for known pathogenic species, there are insufficient epidemiological data to permit the determination of key marker species on health grounds. Although the identity of some key pathogens such as Aspergillus species is well established, the relative importance of other species in the microbial mix has not been established.

Reference period: Most studies of the effects of microbial exposure in the waste industry have focussed on acute effects such as respiratory irritation and nausea that could develop over a single working shift, although it is possible that repeated exposure may lead to an enhanced or reduced response. Similarly the limited data about community health in the vicinity of waste processes does not provide clear evidence about the effects of repeated exposure. The data underlying any guideline value is likely to take account of experience from the workplace where exposures would be typically 8 hours in duration and community studies where exposure would be continuous. Repeated exposure to certain microbial species is likely to be associated with increased risks of sensitisation and respiratory illness. There is no information about the effects of transient high exposures as opposed to effects developing over an 8 hour shift and whether the effects observed in workers could be largely ascribed to short term peak exposures during the working day as opposed to the shift mean exposure.

Preliminary assessment of guideline values: Based on the reported development of respiratory symptoms, nausea, headache and other effects in waste workers exposed to airborne fungi at concentrations of \(10^4\) cfum\(^{-3}\), and effects on lung function in sawmill workers at 3000 cfum\(^{-3}\), it might be appropriate to control concentrations in ambient air to between 300-1000 cfum\(^{-3}\) as an 8 hour or 24 hour average. The margin of uncertainty allows for interindividual differences in susceptibility and the potentially greater susceptibility of some members of the general population than the workers in the source studies. Given that it is probable that individuals who develop allergies to airborne fungi are unlikely to remain in jobs in the waste industry if they become unwell, it may be appropriate to allow a relatively high margin of uncertainty in order to provide an acceptable level of protection for the general population. The weak evidence linking mean exposures to 350 cfum\(^{-3}\) in indoor air to small effects on respiratory function in children with asthma suggests that the lower end of the suggested range is likely to be appropriate, although as the relationship was not statistically significant, it would be inappropriate to use it as the basis for standard setting.

There are limited data that suggest workplace exposure or exposure in outdoor air to fungal concentrations of 1000 spores/m\(^3\) may give rise to adverse effects. An appropriate guideline for spores might be as low as 50-100 spores/m\(^3\).

Based on the evidence of the adverse effects of microbial exposure on community associated with microbial concentrations of about 1000 cfum\(^3\), a suitable guideline value for viable microbes might be of the order of 100-1000 cfum\(^{-3}\) as a 24 hour mean. This takes account of uncertainties arising from the limited data base and data quality, but as the study population would be expected to be representative of the general population, no differences in susceptibility would be anticipated.

Other comments: The extreme sensitivity of some individuals to certain fungal species means that it is unlikely to be feasible to set a guideline concentration that will fully protect these individuals. The Nordic Expert Group (2006) were unable to determine an OEL for fungal spores and recommended that further research was undertaken to better understand the allergic response to fungal spores and the effects of pathogenic species and mycotoxins.

Beta (1→3) glucan

Relevance to health: The results of several studies in waste workers suggest that respiratory symptoms and airways inflammation are more prevalent in workers exposed to concentrations exceeding 25 ngm\(^{-3}\) than at lower levels of exposure (section 5.7.2). There is
limited evidence from studies in other workplaces and indoor air quality (including residential properties) of increased risks of respiratory symptoms, headache, fatigue and atopy at concentrations less than 10 ngm$^{-3}$ but no evidence of effects below 1 ngm$^{-3}$ (section 5.7.3). Human volunteer studies do not give clear evidence that beta (1→3) glucan causes adverse health effects (section 5.7.4). It is unclear whether effects attributed to beta (1→3) glucan are caused by beta (1→3) glucan or whether it is merely a marker for fungal exposure.

**Measurement:** Several assays are available of which the glucan-specific LAL assay has been more widely used in studies of the waste industry than ELISA based assays (section 2.4.5). The intercomparability of these methods is uncertain and a standardised method would be required if beta (1→3) glucan were to be used in regulation.

**Specificity to waste processes:** There is no information about typical concentrations of beta (1→3) glucan in ambient air. Beta (1→3) glucan is found in the cell walls of some plants and is not specific to fungi or waste. It is unclear whether particular beta (1→3) glucan molecules are more strongly associated with waste than others. It would be technically demanding (probably impossible) to determine concentrations of (a) specific beta (1→3) glucan(s) in ambient air.

**Potential to develop a guideline value:** It is tempting to propose the use of beta (1→3) glucan as a simple marker of fungal exposure that would not be subject to issues of viability during and subsequent to collection. Unfortunately it is not clear that beta (1→3) glucan concentrations would represent a simple marker of fungal exposure as it may also be released from some plant material. Even in the absence of other sources, the relationship between beta (1→3) glucan concentrations and the potential harmfulness of fungal aerosols is likely to be variable. In addition, there is only weak evidence linking beta (1→3) glucan to adverse health effects. Overall, there is not a strong case for setting a guideline for beta (1→3) glucan. However, it may be desirable to undertake some measurements to determine typical concentrations in ambient air proximal to waste facilities and at more distant locations in order to determine whether it may be a potentially useful marker of bioaerosol exposure.

**Reference period:** Studies of workplace exposure to beta (1→3) glucan have identified effects such as headache that could arise from a single exposure to a toxic substance and effects such as fatigue that might be more likely to result from repeated exposure. The data underlying any guideline value would be largely based on experience from the workplace where exposures would be typically 8 hours in duration on successive days but would take account of the limited data from studies of residential indoor air. Given the relatively weak database that would underlie any guideline set for beta (1→3) glucan, it might be appropriate to set a short term guideline based on the same reference period as that for endotoxin.

**Preliminary assessment of guideline values:** Based on the limited evidence that suggests that a range of symptoms may be associated with workplace or residential exposure to less than 10 ng$^{-3}$ and an absence of effects at 1 ngm$^{-3}$, an appropriate guideline for beta (1→3) glucan in ambient air may be less than about 1 ngm$^{-3}$ as a 24 hour standard. The limited margin of uncertainty takes account of the inclusion of exposure in residential indoor air in the source studies. The overall justification for setting a guideline would be weak.

### 7.2.4 Demonstration of compliance

**Measurement**

Measurement data are an important tool in the demonstration of compliance with environmental standards. Without the evidence provided by measurement data, it is impossible to demonstrate that standards are achieved. Compliance with regulation may depend on what is measured and how, when and where the measurement is made. Bioaerosol emissions from waste processes vary through time in composition, in release rate and in the exact location on site from which releases occur. In the surrounding area, the interaction of variable weather conditions with local topography will further enhance the inhomogeneity of bioaerosol concentrations in time and space. Any measurement programme must take account of this variability and should include sufficient sampling effort to
demonstrate compliance with relevant standards. In practice, however, monitoring is likely to be constrained by the cost and practicality of monitoring. Given the temporal fluctuation in bioaerosol levels, it may be difficult to demonstrate that a standard is consistently achieved (unless a proxy like PM$_{10}$ that can be measured continuously is used). There are also uncertainties in the levels of bioaerosol exposure that may arise from other sources, particularly for waste processes located in rural locations where subject to substantial bioaerosol emissions from agriculture. It might be desirable to use key marker species to distinguish the contribution of waste versus other sources to total bioaerosol concentrations.

In order to incorporate monitoring into the regulatory regime, it would be necessary to adopt a standard approach to determining what to measure at individual sites, how to make the measurements, the location of sample points and the frequency/duration of sampling. At any one site it would be appropriate to regularly review monitoring results and to adjust the monitoring regime in the light of experience. For example, there is no value in making frequent measurements of a component consistently found at concentrations below the level of detection at a given location. It may be appropriate to continue to make less frequent check measurements in order to provide reassurance that no major changes have occurred.

**What to measure**: will depend both on the selection of substances for which guidelines are set and also on local factors. A risk-based approach should be taken to determining the range of bioaerosol components that should be monitored in the vicinity of any single site.

**Measurement method**: a range of measurement methods exist with only limited method harmonisation in the UK, although a CEN method may eventually become available (Chapter 2). Reported results depend on the measurement method used. It would be desirable to involve the laboratories who provide monitoring services in the development of standardised protocols. It is important that measurement methods are affordable, reliable (easily reproducible) and readily available from a number of providers.

**Sample locations**: A risk based approach should be taken and ideally measurements should be made in one or more local communities depending on proximity to the waste site and taking account of local topography and weather conditions.

**When**: monitoring must be sufficient to demonstrate that relevant standards are achieved and take account of the variability in background concentrations and in species mix through time at any individual location. Samples must be representative, relevant and cover all process variations and seasonal variation. In addition, simultaneous determination of background concentrations will be necessary (eg simultaneous monitoring upwind and downwind) unless measurements are based on source specific marker species or substances.

If it were possible to characterise the relationship between PM$_{10}$ and concentrations of key bioaerosol components for samples representing the range of processes and weather conditions at any particular site, continuous monitoring of PM$_{10}$ may provide a proxy for monitoring bioaerosol exposure. Background levels of PM$_{10}$ do, however, vary considerably depending on the weather and emissions from different sources and it is likely to be difficult to characterise the impact of waste sites on local concentrations of PM$_{10}$. At some sites, PM$_{10}$ monitoring may already be in place to address dust emissions from waste processes.

**Modelling**

In other situations where air quality standards are used in regulation and where it is not practical to demonstrate compliance by measurement, compliance may be demonstrated by the use of appropriate air dispersion models. The modelling is based on estimated emission rates and characteristics that can ideally be validated by appropriate emissions monitoring.

ADAS/SWICEB (2005) undertook dispersion modelling for composting sites where they had also measured bioaerosol concentrations. The emission rate was calculated from measured downwind concentrations using the dispersion model and taking account of the weather conditions at the time of sampling. The microbial counts collected using an Andersen sampler were converted to mass concentrations that could be used in dispersion modelling. The
authors concluded that their confidence in the model predictions was low. Although the outputs of dispersion modelling could be informative, they indicated that considerably more weight should be given to measurement results. The difficulty in establishing suitable emission rates taking account of the variation in emissions through time resulting from process and seasonal factors is likely to prove a major constraint in the development of dispersion modelling techniques for waste processes.

7.2.5 Discussion and conclusions

Air quality guidelines provide a clear benchmark against which regulatory decisions can be determined. There must however be sufficient data to support a defensible guideline, demonstration of compliance must be feasible and guidelines must be attainable through the use of practical means or they become meaningless. Bioaerosols are a mixture and it is desirable that sufficient of components are monitored to be confident that exposure to the mixture is adequately controlled. Equally it is important to avoid an unnecessary monitoring burden arising from trying to monitor too many components with no clear benefit for health.

There are sufficient health data to set ambient air quality guidelines for endotoxin or organic dust but insufficient exposure data to determine whether these are likely to be relevant and useful measures of community bioaerosol exposure. Measurement methods are available and it would be technically possible to determine the endotoxin content of dust samples collected over 24 hour (or shorter periods). Exposures to organic dusts and endotoxin have both immediate effects on health and long term effects following repeated exposure such that both short term (8 or 24 hours) and annual mean guidelines would be appropriate.

There are few data on which to base guidelines for other bioaerosol components. Where data are available, lowest effect levels are of similar magnitude to background concentrations in ambient air. Scaling for differences in duration of exposure and allowing a margin of safety to take account of the potentially greater sensitivity of general population is likely to lead to the derivation of guidelines that represent levels of exposure well below background levels (Table 7.1). The existence of substantial natural sources of bioaerosols may severely limit the extent to which any health-based guidelines could be achieved.
Table 7.1: Comparison of threshold levels and possible guideline values (24 hour means unless otherwise indicated) for bioaerosol components and background concentrations

<table>
<thead>
<tr>
<th>Bioaerosol component</th>
<th>Health endpoint</th>
<th>Threshold</th>
<th>Study population</th>
<th>Possible guideline values*</th>
<th>Background concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic dust</td>
<td>Mucous membrane irritation Chronic respiratory illness</td>
<td>200 ug m(^{-3}) 300 ug m(^{-3})</td>
<td>Waste workers Cotton workers</td>
<td>10-20 ug m(^{-3}) 3 ug m(^{-3})***</td>
<td>&lt;&lt;20 ug m(^{-3})</td>
</tr>
<tr>
<td>Fungi</td>
<td>Respiratory symptoms, nausea, headache etc</td>
<td>10(^{6}) cfu m(^{-3}) 1000 spores m(^{-3})</td>
<td>Waste workers</td>
<td>500 -1000 cfum(^{-3}) 50-100 spores m(^{-3})</td>
<td>Usually &lt;1000 cfum(^{-3}) Usually &lt;4000 spores m(^{-3})</td>
</tr>
<tr>
<td>Total microbes</td>
<td>Respiratory symptoms, nausea, headache etc</td>
<td>10(^{7}) cfum(^{-3})</td>
<td>General community</td>
<td>100 -1000 cfum(^{-3})</td>
<td>May exceed 1000 cfum(^{-3})</td>
</tr>
<tr>
<td>Endotoxin</td>
<td>Respiratory symptoms</td>
<td>50 EU m(^{-3})</td>
<td>Workers in various industries</td>
<td>2.5-5 EU m(^{-3})</td>
<td>2 EU m(^{-3})</td>
</tr>
<tr>
<td>Beta(1→3) Glucan</td>
<td>Respiratory symptoms, nausea, headache etc</td>
<td>10 ng m(^{-3})</td>
<td>Studies of indoor air quality</td>
<td>1 ng m(^{-3})</td>
<td>&gt;1 ng m(^{-3})***</td>
</tr>
</tbody>
</table>

*See section 3.3
**Annual mean
***No data for outside air

There is a broad consensus among standard setting authorities that it is not possible to set standards that will protect absolutely everybody all of the time. It is well established that very young children, the elderly and asthmatics are more sensitive to air pollution than others within the population. Asthematics constitute a growing proportion of the population (some estimates of prevalence in children are as high as 30% and the prevalence in adults is between 10 and 20%; Burr et al., 2006; Asthma UK, 2004). Air quality standards in the UK are set at levels intended to protect the very young, the elderly and asthmatics. There is good evidence that asthmatics are more sensitive to bioaerosol exposure than others and it would be appropriate to take account of mould-sensitised asthmatics in setting guidelines. It may not be possible to set guidelines that will protect some individuals with asthma or cystic fibrosis who develop hypersensitivity to _Aspergillus_ or severely immunocompromised individuals who may be at risk of infection by moulds at extremely low levels of exposure. There are no existing criteria to determine what an acceptable increment in bioaerosol exposure and risk arising from waste derived bioaerosols may be.

Demonstration of compliance with air quality guidelines is best achieved through measurement. Measurement strategies should be risk based and are likely to involve sample locations within communities proximal to waste sites. Sample duration should be consistent with the averaging period specified in the guideline. In determining the frequency with which sampling should be performed, consideration should be given to the likely variation of concentrations through time as a function of weather and process operations. There are likely to be substantial measurement difficulties in demonstrating compliance with an exposure limit based on bioaerosol concentrations, particularly if based on some measure of microbial concentration. The endotoxin or organic dust component of airborne particulate is more amenable to measurement but there would be substantial costs involved in undertaking sufficient sampling and analysis to demonstrate compliance. A major difficulty in demonstrating compliance with air quality guidelines for bioaerosols emitted from waste processes is that bioaerosols are emitted from a variety of sources. Standard measures of bioaerosols are not specific to waste emissions and suggested guideline values may be
exceeded even in the absence of waste processes (Table 7.1). Background concentrations of bioaerosols may show substantial hour to hour variability. It may be appropriate to undertake continuous monitoring for PM$_{10}$ at one or more of the selected sample locations. This would allow assessment of probable typical levels and maximum levels of bioaerosol concentration based on a relatively small number of bioaerosol samples (see section 7.3 below).

7.3 ALTERNATIVE APPROACHES TO SETTING A GUIDELINE

7.3.1 Use of marker species to determine exposure to bioaerosol emissions specific to waste processes

Measured microbial concentrations in close proximity to waste sites may not be significantly higher than background concentrations measured at other locations, although it is possible or even probable that the mix of species would be different. If it were possible to identify marker species that were specific to waste processes, these could be used in the development of an exposure index that is specific to microbes derived from waste processes. Ongoing research at NPL (National Physical Laboratory) indicates that exposure measurement could be based on quantitative polymerase chain reaction (PCR) techniques that determine the presence and quantity of specific target DNA sequences that can be linked to individual fungal or bacterial species. Extensive development work would be required in order to link measurements of key marker species using PCR techniques to measures of microbial bioaerosol concentrations for which exposure-response information is available from epidemiological studies.

The variation in species mix in emissions from waste processes through space and time is likely to be a major barrier to this approach. For example, the species make up of emissions from composting change as the process progresses. There is unlikely to be a consistent relationship between concentrations of selected marker species and the nature and severity of health effects. Currently there are too few epidemiological data to support the development of health-based guidelines based on specific marker species. Existing health studies of waste workers do not provide information that would allow the linkage to effects to concentrations of marker species. It is unlikely that this will be resolved through studies of communities living in the vicinity of waste facilities because the effects of bioaerosol exposure are likely to be small in relation to other influences on health. The likely health impacts (respiratory symptoms, headache, fatigue, nausea) are such that it would be impossible to determine a specific link to bioaerosol exposure as opposed to some other local factor associated with the local area such as employment status or deprivation. If it were possible to link measured concentrations of some key marker species to measures of microbial exposure used in workplace health studies, it might be possible to derive approximate lowest and no effects levels for these key marker species. The uncertainties in this process would be substantial and given the uncertainty in the NOELs and LOELs (lowest observed effects level) reported in different studies, it seems unlikely that this approach will provide a defensible guideline value.

An alternative approach that is not directly based on health information would be to use marker species simply to determine whether significant exposures to waste derived bioaerosols are occurring. Given that some individuals appear to experience adverse effects following very low levels of exposure to bioaerosols, it is arguable that any significant increase in bioaerosol concentrations could give rise to adverse effects in more vulnerable members of the population. Significance could be defined in relation to typical levels of microbial exposure experienced by the UK population. For example, it might be desirable to control exposure to total/viable bioaerosol emitted from a waste process to less than 10% (or lower) of typical levels of microbial exposure experienced in the UK. In order to develop this approach, it would be necessary to identify appropriate marker species specific to waste processes and to evaluate the relationship between these species and total microbial emissions. It would also be necessary to establish the typical level of exposure to microbial aerosol in UK outdoor air.

Ideally selected marker species would have a strong association with waste, be consistently present in waste emissions and have a consistent relationship with total bioaerosol concentration, however defined. In addition marker species should not be strongly associated
with other bioaerosol sources such as farming and should be easily measured. The variability in processes leading to bioaerosol emissions means these criteria are unlikely to be met.

It may also be difficult to derive a consensus view on the typical level of microbial exposure in outdoor air and the most relevant measure of exposure: viable versus total organisms, bacteria versus fungi. The variability in bioaerosol composition from different sources is likely to give rise to a variable potential to cause adverse health effects. The relationship between concentrations of the marker species and any increased risk to health is likely to vary in space and time as a function of the variability of emissions from waste processes.

The major advantage of basing an air quality guideline on marker species would be that emissions could be attributed specifically to waste. The absence of a direct link to health is a disadvantage, although it is arguable that any increase in exposure levels will be associated with an increased risk of adverse effects in vulnerable individuals. The main difficulty in developing this approach is the identification of appropriate marker species that will be a reliable marker of bioaerosol exposure arising from waste processes.

7.3.2 Use of index substances as a proxy measure of bioaerosol emissions specific to waste processes

Given that it may be difficult to identify and reliably measure microbial markers of waste-generated bioaerosols, it may be possible to use a limited number of organic compounds as proxy markers of bioaerosol exposure. Ideally the selected substances would be:

- Readily measurable using well established techniques (e.g., gas chromatography);
- Specific (or reasonably specific) to waste processes;
- Show a reasonably consistent relationship with bioaerosol emissions; and
- Present at measurable concentrations in ambient air around waste sites.

Although it is likely to be impossible to select an individual marker substance that is specific to the waste industry, it may be feasible to select a characteristic group of substances. Development work would be required to relate concentrations of these marker substances to bioaerosol components for which exposure response information is available.

A number of recent studies have tried to establish chemical markers of bioaerosol exposure including the use of GC-MS for the identification of characteristic compounds associated with specific groups or specific species of microbe or characteristic compounds emitted by specific microbial species (e.g., Mayrhofer et al., 2006). Initial findings suggested these are variable in time and space and this approach to monitoring bioaerosol exposure has not been further developed. Although this approach could lead to the development of an exposure index specific to waste, extensive development work would be required in order to understand how measured concentrations of proxy substances related to concentrations of bioaerosol components for which exposure-response data are available.

7.3.3 Development of a modified air quality objective for PM$_{10}$ in areas where bioaerosol concentrations may be elevated

There is a recognised air quality standard for PM$_{10}$, agreed air quality objectives and an existing obligation to achieve these objectives through Local Air Quality Management and the wider regulatory regime. The air quality objectives are supported by well established measurement methods. It may be desirable to control bioaerosol exposures indirectly through the control of PM$_{10}$. It is apparent from studies of dust-exposed workers that organic dusts are associated with an increased risk of adverse effects relative to those associated with inert inorganic dusts (Chapter 5). This implies that it may be appropriate to set more rigorous guidelines for PM$_{10}$ in the vicinity of waste sites, although the importance of the waste site versus other local sources in determining local concentrations of PM$_{10}$ would have to be taken into account. Although the adverse effects of exposure to PM$_{10}$/PM$_{2.5}$ in ambient air are well established, the relative harmfulness of bioaerosols versus urban PM is unclear, as is the potential role of bioaerosols in giving rise to the effects observed with PM. It would be
possible to make a preliminary assessment of the relative harmfulness of bioaerosols versus urban PM through comparison of the exposure-response relationships found in workplace studies for organic dust with those found for low toxicity mineral dusts and assessment of the relative toxicity of low toxicity mineral dusts and urban PM. This assessment could be based on the results of published experimental studies and epidemiological investigations in communities exposed to PM from different sources. It would be necessary to take account of differences in the airborne dust fraction measured in different studies (PM$_{10}$ versus inhalable or respirable dust). Despite some uncertainties, the data should be sufficient to support the derivation of a defensible guideline value for PM$_{10}$ in the vicinity of waste processes.

7.4 EMISSIONS LIMITS

7.4.1 Introduction

Emissions limits for industrial processes are usually derived through reference to environmental quality standards. Dispersion modelling is used to assess the relationship between site emissions and the impact on local air quality. Site specific limits are set that take account of the sensitivity of the receiving environment and the availability and suitability of technologies to reduce, control and abate emissions. For many industrial processes, guidance exists that indicates the emission levels that can be achieved or bettered through the use of available technology.

Emissions limits are readily applicable where emissions are well defined and measurable (typically stack emissions or aqueous discharge). They are likely to be less suitable for fugitive emissions from waste processes. Some benefits, limitations and possible approaches that could be taken to establishing emission limits for biowaste are outlined below.

7.4.2 Benefits and limitations

In general terms, the major benefits of setting emissions limits are:

- They can be used to inform process design;
- On-site monitoring is easier to perform and manage than offsite monitoring;
- Emissions can be directly controlled by the process operator; and
- The measurement of emissions is often simpler than that of small concentrations at the site boundary or offsite.

These benefits primarily apply to pollutants emitted from well defined sources and are less likely to apply to diffuse emissions of bioaerosols that are difficult to define for the purposes of dispersion modelling. The major limitation to setting emissions limits for bioaerosols from waste processes is that it would be extremely difficult to define, model and measure emissions in a quantitative fashion for most waste management processes. This would make both establishing limits and assessing compliance extremely difficult. Even where a waste process does incorporate discrete point sources that would be amenable to measurement, these sources may be small in comparison to fugitive sources elsewhere on site and be of limited relevance to overall emissions of bioaerosols from the site.

7.4.3 Possible approaches

Given that there are no readily available methods for measuring emissions from most waste sites, alternative non-instrumental methods may have a role. The visual assessment of aerosol/dust emissions is unlikely to be sensitive enough to provide an effective method of minimising off site concentrations of bioaerosols. If, however, emissions are visible, this does provide a clear indication that emissions are being inadequately controlled and that action is required to reduce emissions levels. The detection of odour on site is a considerably more sensitive marker of emissions, but it may be too sensitive to be of real value in assessing offsite impacts. Given, however, that no odour should be discernable at the site boundary by agency officers, the detection of odour at the site boundary would provide an indication that better emissions control was required. Odour assessments are highly subjective and there is considerable interindividual variation in odour perception and sensitivity.
Air quality measurements at site boundary may provide an indirect but quantitative measure of emissions. It would be desirable to make measurements at more than one location along the site boundary, taking account of the proximity of local receptors and local weather conditions. Measurements could be made of dust/organic dust, microbial concentrations and endotoxin, taking account of relevant air quality guidelines.

7.4.4 Conclusions

In conclusion it would not be practical to continuously quantify bioaerosol emissions from most waste processes. Some quantification of selected species at site boundaries may be helpful as an indirect measure of site emissions. Appropriate boundary monitoring could be incorporated into the regulatory regime as part of the operating conditions for individual sites, where such monitoring is likely to be effective as a means of controlling local levels of exposure to bioaerosols. Given that the costs of monitoring at site boundaries could be onerous if there is a requirement for continuous measurement at more than one location; careful consideration should be given to the measurements to be made. The cost of monitoring should be proportionate to the health benefits for local communities arising from the proper control of bioaerosol emissions. Some simple assessment of emissions using qualitative criteria such as the visual assessment of dust emissions or subjective odour assessment might be usefully incorporated into good practice guides but it is not likely to be effective as a main route of regulation.

7.5 BEST PRACTICE GUIDELINES

7.5.1 Overview

Best practice guidelines offer an attractive approach to good regulation that complies with the Government’s Better Regulation initiative. Given that best practice should already be followed under the permitting/licensing regime, there should be no additional cost burden for operators. The development of best practice guidance could be integrated with the development of environmental quality standards/guidelines and emissions limits, or could be effective as a stand alone approach to regulation. The scope and likely nature of guidance, benefits and limitations are discussed below.

Best practice guidance might cover collection and transport of waste to site; unloading; storage, treatment and subsequent processing/disposal. It should address the:

- Prevention of emissions – eg moisture content, elimination of processes leading to dust release, process containment;
- Minimisation of emissions where emissions cannot be prevented; and
- Reduction in harmfulness of emissions, for example, through the elimination of pathogenic species.

7.5.2 Components of good practice

Waste collection

Waste collection is not a major source of community bioaerosol exposure although the exposure of waste collection workers may be substantial. Factors affecting worker exposure will include vehicle design, domestic waste storage arrangements and collection regime. Modern systems employing wheelie bins and automated loading should help to reduce worker exposure but may not be sufficient for exposure control where unwrapped organic wastes are collected. There is limited evidence to suggest that worker exposure is lower under a weekly versus fortnightly collection regime (Sections 4.2, 4.3). The kerbside collection of other segregated wastes for recycling may also lead to elevated bioaerosol exposures if workers manually sort paper, cans, glass and other dry recyclate during the collection process. There is a need to introduce guidelines to minimise worker exposure.
The waste collection regime will affect public exposure to bioaerosols. There is some evidence of increased symptom prevalence consistent with higher levels of exposure to waste-derived bioaerosols in households where waste, particularly organic wastes are stored indoors (Herr et al, 2004). In addition, policies such as the encouragement of composting by householders will influence public exposure to bioaerosols. The separate storage of kitchen waste prior to addition to a domestic compost heap and the management of the heap will both lead to increased bioaerosol exposure. There is scope to reduce public exposure to bioaerosols through ensuring that, where organic wastes are separately collected, appropriate storage containers are provided and householders are encouraged to keep stored wastes cool. The extensive publicity given to the benefits of composting at a domestic scale should include information on practices that will minimise bioaerosol exposure (eg the use of closed containers for the indoors storage of organic waste).

Transport

Under normal circumstances, waste transport should not constitute an important source of community exposure to bioaerosols. Existing requirements to fully contain wastes during transport should adequately control bioaerosol emissions. Measures such as wheel washes may be appropriate to minimise emissions arising from vehicle deliveries to waste sites. Such measures are likely to have already been implemented in order to control emissions of dust.

On-site handling

The disturbance/handling of waste is a major cause of bioaerosol emission. Processes should be designed to minimise waste handling in the open in order to minimise off-site emissions. Where wastes are handled in the open, appropriate measures should be taken to minimise emissions. These might include ensuring wastes are kept moist, minimising drop heights and using wind breaks to minimise windspeeds across open sites. The design of any waste handling processes should take account of the requirement to minimise worker exposure. It is important to ensure that measures designed to minimise offsite impacts such as the increased enclosure of processes do not result in increased workplace exposure.

Storage

The potential for bioaerosol emissions increases with storage times and therefore it is desirable to minimise the time delay between waste collection and treatment.

Stored wastes should be appropriately contained.

Treatment

*Materials recycling facilities:* these are often enclosed and provided that appropriate air handling and treatment measures are in place, bioaerosol emissions to outside air should be negligible. There is a requirement for good site management to prevent the outdoor storage of wastes that may be bioaerosol sources and to ensure that the emissions abatement systems are adequately maintained. Although bioaerosol emissions to outdoor air from an enclosed facility should be negligible, significant workplace exposure is possible and there is a need to develop a code of good practice to minimise these exposures. Where materials recycling facilities have developed as a secondary activity on a landfill site or at an existing waste transfer or civic amenity site and are currently operating in an outdoor environment, all practical measures should be taken to contain wastes and to minimise emissions. Measures to minimise emissions may include optimising moisture content (eg garden wastes), minimising storage times, imposing strict rules on waste acceptability, minimising waste disturbance and reducing the potential for dust pick up arising from wind disturbance.

*Composting:* the Composting Association has produced a good practice guide. Elements of good practice include the containment of wastes, ensuring that a good mix of wastes are used in order to attain an appropriate nutrient balance and ensuring that moisture and aeration are optimised. Other issues include the requirement to ensure that wastes reach an appropriate
temperature for the elimination of pathogens and good separation of materials at different stages in the process in order to prevent re-infection of composted material by pathogens.

Landfills: Landfills are already subject to strict permitting conditions that enforce good practice to reduce emissions of dust and odour. Required measures such as the use of daily cover, restriction of tipping to a specific area of site and minimisation of the tipping area will also be effective in minimising bioaerosol emissions. In addition good environmental management including frequent inspection of site boundaries for evidence of dust/odour emission coupled with a prompt response on the discovery of problems will also minimise bioaerosol impacts.

Other processes: other waste treatment processes such as anaerobic digestion, pyrolysis or incineration are mostly in vessel or otherwise fully contained. Provided appropriate emissions treatment measures are in place, then the emission of bioaerosols to the wider environment should be negligible. In common with all waste handling facilities, systems should be in place to minimise bioaerosol emissions during waste delivery, on-site storage and handling prior to treatment (above). Similarly there is a requirement to ensure that worker exposures are minimised through good workplace design and operating systems.

7.5.3 Benefits

There are a number of benefits of regulating processes through the implementation of required good practice. It is easy to determine compliance during site inspection. There should be no extra cost to operators as best practice should be required by process permits and there should be no monitoring costs beyond those involved in achieving best practice.

The development costs that may be incurred in the development of formal good practice guidelines are likely to be modest as there are some existing good practice guidelines that could be used to inform the development of appropriate guidance such as those published by the Composting Association (2004). In addition other guidance on controlling particulate emissions from waste processes is likely to be relevant (EA, 2004).

7.5.4 Limitations

Best practice may appear to be implemented during site inspections but there are no objective measures that can be used to demonstrate compliance at other times. This limitation may be unimportant if site inspections are typically unannounced but it is more likely that sites will have plenty of notice of inspections and the opportunity to get their housekeeping up to scratch before the inspector arrives. A requirement to keep a daily environmental log recording information such as evidence of dust nuisance/odour at site boundaries may help to encourage continuing implementation of good practice (although forgery is possible). The creation of a local liaison group involving the local community may also help to encourage the perpetuation of good practice as the community will be quick to complain about lapses leading to dust or odour nuisance that are likely to be markers of bioaerosol exposure.

Regulators may wish to consider requiring operators to develop and implement environmental management systems accredited to ISO 14001. This would ensure that all sites are regularly inspected by a third party with the additional benefit that operators would be committed to continual improvement. It has not yet been demonstrated however that ISO 14001 accreditation offers an adequate level of environmental protection.

The requirements of best practice will not be specific to controlling bioaerosol exposures. Other considerations will include compost quality, leachate/run off, noise emissions, minimisation of residue and so on. Economic considerations will also impact on process design and operation. It is possible that these other considerations may lead to conflicting process requirements and the impact of bioaerosol emissions will have to be balanced against other environmental impacts and economic considerations.

There is no certainty that exposures to bioaerosols experienced by local residents can be controlled to an appropriately low level through the application of current best practice. Even if
sites commission measurements intended to demonstrate the effectiveness of their control measures, the presence of other bioaerosol sources in the general environment may make the interpretation of measurement data very important. Despite extensive existing data for bioaerosol concentrations around waste sites, relatively few studies have been able to demonstrate that sites have a measurable impact on local air quality. Very similar issues are faced in the minerals industry where new developments often face substantial local opposition because of concerns about dust nuisance, but there are very few historical data to demonstrate that existing minerals sites have a substantial impact on local air quality.

7.5.5 Other comments

The development of best-practice guidelines should be risk-based and take account of the extent of potential exposure and the harmfulness of emissions. The measures required to control emissions should be proportional to risk as assessed through the estimation of exposure and risk. Guidelines should provide adequate detail for the individual stages of processes while being based on a holistic view of the entire process. Control measures should be simple to implement and operate, and it should be possible to monitor both that control measures are being implemented as described in process guidelines and also the effectiveness of any control measures. In practice, it is unlikely to be practical to monitor effectiveness on a routine basis but consideration should be given to the benefits of undertaking regular measurements of relevant parameters at local receptors. There should be consistency in the guidance developed for different waste processes.

In order to inform planning decisions and the rigour of control measures it may be desirable to include stand off distances as a component of best practice guidelines (see section 7.6 below). It seems reasonable to assume that stand off distances could be minimised if best practice is maintained to minimise emissions.

The most that could be achieved through the implementation of best practice guidelines based on current best practice is current best practice. There are no data to demonstrate whether this is likely to provide adequate control of bioaerosol exposure in local communities or for exposed workers. In developing best practice guidelines, it would be desirable to evaluate impact on bioaerosol exposure relative to background exposure and also in relation to levels associated with adverse effects in epidemiological studies. The exposure data reviewed in Chapter 4 do not provide much specific information about practices giving rise to elevated exposure or the effectiveness of different control measures. It may be possible to draw more from existing unpublished measurement data.

The published data that are available suggest that there is likely to be a greater need to implement measures to reduce worker exposure than measures targeted at reducing the exposure of the general population. Although the general population would be exposed for longer to emissions from any one site (5x on annual basis, 10x on lifetime basis) than workers and will include some individuals that are more sensitive to bioaerosols than will be found in the workforce, the levels of exposure of the general population are likely to be trivial in comparison to those experienced by workers.

7.5.6 Conclusions

The development of a regulatory approach based on best practice guidance represents least additional cost for the industry or for regulators. Given the probable uncertainties in monitoring compliance with any proposed air quality standards for bioaerosol components, the implementation of best practice is likely to be at least as effective in controlling bioaerosol exposure in local communities. Regulators may wish to consider mandatory accreditation to ISO 14001 for regulated processes as a route to driving improvements in environmental performance. It would also ensure that sites are subject to independent inspections regardless of the resource available to the regulator.
7.6 STAND OFF DISTANCES

7.6.1 Introduction

There is already a requirement for a site specific human risk assessment to be undertaken if operators apply for a licence/permit to develop new composting facilities or modifications of existing processes where the boundary of the facility is within 250 m of a workplace or boundary of a dwelling (EA, 2007). The risk assessment must provide clear scientific evidence demonstrating that bioaerosol levels can be maintained at appropriate levels at the dwelling or workplace. Stand off distances have also been used to inform regulatory decisions in relation to other types of industrial process. For example, there is a presumption against the granting of planning permission for opencast coal operations within 500m of the end of any community in Scotland2. This policy was based on the findings of a study that were consistent with a small effect on children’s respiratory health associated with exposure to PM$_{10}$ in communities close to opencast coalmines (Pless-Mulloli et al, 2000), although a clear relationship between opencast coalmines and adverse respiratory effects in children was not found (Pless-Mulloli et al, 2001). Also in Scotland, government advice discourages the development of large livestock buildings within 400m of residential areas in order to minimise the risk of odour nuisance, although this is coupled with guidance indicating that these facilities should be sited downwind of residential areas3.

7.6.2 Benefits and Limitations

The major benefit of using a stand off distance is the minimal measurement or monitoring uncertainty around its application. Stand off distance provides a simple unambiguous criterion for decision making that is easy to apply prospectively to new sites or to proposed developments (eg housing) within the vicinity of existing sites.

The implementation of a stand off zone intended to limit bioaerosol exposure is likely to help control exposure to noise, odour and other pollutants in local communities.

The implementation of guidance on stand off zones is likely to severely limit the availability of new sites for waste operations. The publication of any guidance indicating that stand off distances are appropriate will create concern around existing sites in communities that are already sites within what would be the “stand off zone”.

The development of a simple distance criterion does not take account of protection offered by local topography, the particular effects of local weather (eg channelling of wind) or the impacts of other pollutant sources.

The existing 250 m guideline for compost sites is based on a relatively small evidence base and does not take account of potential differences in emission rates or dispersion at different sites. Ideally more tailored guidelines for different sizes and types of operation should be developed coupled with guidance on how to take account of local weather and topography. For recommended stand off distances to be defensible, they are likely to be highly protective. A presumption against development within a specified distance of a waste site could severely restrict landuse in large areas where real risk of illness arising from bioaerosol exposure is very small. Although, the Sewerage Nuisance (Code of Practice) (Scotland) Order 2006 does not set a specific distance cordon around waste water treatment plants, it does require Planning Authorities to take account of possible odour nuisances when considering new developments in the surrounding area and this has led to restrictions on development in areas around sewage treatment works.

This approach provides no incentives for operators to improve their performance. In the absence of explicit monitoring requirements, it is possible that community exposures to bioaerosols could be higher than those that might arise under alternative regulatory regimes.


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7.6.3 Comments

The current requirement to undertake a risk assessment for proposed composting operations that are less than 250m from a receptor is based on limited scientific evidence. The area influenced by emissions from individual waste sites will depend on the size and nature of operation, the safeguards in place to minimise emissions, how well the site is run and local weather and topography. A simple rule that limits the development of waste facilities within a given distance from communities or limiting other developments within a given distance of waste facilities would offer a very variable level of protection for the general population.

There is a need for any advice on stand off distances to be rigorously supported by scientific evidence linking proximity to source to exposure and in turn to health risk. There is a need to develop guidelines that take account of the size and nature of regulated processes eg open windrows versus in-vessel systems. There is also a need to better understand the impact of other types of waste management process (eg anaerobic digestion) on local air quality and associated risks to health in order that appropriate guidance on stand off distances can be developed. In addition to uncertainties in exposure levels, there are uncertainties in the exposure-response information. The development of guidance on stand off distances would effectively have to take account of the same data that are available for standard setting and similarly determine what constitutes an acceptable level of public exposure. At present there are insufficient data from which the impacts of different sizes and types of process on local bioaerosol concentrations can be inferred. This is likely to prove a major barrier to the development of scientifically based guidance on stand off zones.

The exposure-response information described in Chapter 5 could be used to inform a site specific risk assessment as currently required for composting facilities within 250 m of potential receptors. A defensible exposure estimate would be required in order to utilise the exposure-response information.

7.6.4 Conclusions

The development of guidance on stand off distances represents a simple pragmatic approach to regulation that at one level would be simple to implement for new sites. It is not, however, applicable to existing sites. Levels of bioaerosol exposure are likely to be minimal in a substantial proportion of land within any stand off zone and it is likely that publication of guidance on stand off zones would create unnecessary anxiety for those living in relatively close proximity to existing sites, within what would be the stand off zone for a new site. The implementation of stand off zones is likely to severely restrict development in the vicinity of waste sites and to make it exceedingly difficult to identify new waste sites.

7.7 DISCUSSION

7.7.1 Recommended regulatory approaches

In developing a strategy for the regulation of bioaerosol emissions from waste processes it is appropriate to consider the:

- Quality of evidence on which recommendations are based;
- Development work required to implement alternative approaches;
- Multiple sources of bioaerosol exposure including non-waste significant sources;
- Health impacts of co-exposure to bioaerosols and other pollutants; and the
- Protection of abnormally sensitive members of the population.

It would also be appropriate to consider the costs of implementation and the benefits arising from each regulatory option including the “doing nothing” option.

Table 7.2 below summarises the advantages and disadvantages of alternative approaches to the regulation of bioaerosol emissions from waste sites. The development of best practice guidelines currently appears the most promising. The major deficiency in this approach is the
difficulty in demonstrating that the health of local communities is adequately protected. Environmental quality guidelines can provide communities with considerable reassurance about the efficacy of emissions control measures, provided that they are met. Given that bioaerosols are emitted from a variety of commercial and domestic sources, it is unlikely that health-based guidelines for bioaerosols derived from currently available data would be met. In addition, the only bioaerosol component for which there are sufficient data to be able to derive a defensible guideline is endotoxin and this has not been widely measured in ambient air. The development of exposure guidelines based on more specific markers of bioaerosol exposure arising from waste processes would require a substantial development work. An attractive alternative approach would be to introduce modified PM$_{10}$ objectives in areas subject to elevated bioaerosol concentrations. There are probably sufficient data to support the development of such objectives and there are well established methods for the assessment of compliance.
### Table 7.2: Summary of benefits, limitations and development work required to implement different regulatory approaches

<table>
<thead>
<tr>
<th>Approach</th>
<th>Benefits</th>
<th>Limitations</th>
<th>Development work required</th>
<th>Other Comments</th>
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<tbody>
<tr>
<td>Development of health-based air quality guidelines for bioaerosol components for which adequate epidemiological data are available</td>
<td>Allows easy differentiation between acceptable and unacceptable levels of exposure; Ensures consistency in regulation across different sites; Provides public reassurance that health is adequately protected</td>
<td>Strong possibility that recommended guideline values will be lower than background concentrations; Practical difficulties in assessing compliance; Difficulty in source attribution for measured bioaerosol concentrations</td>
<td>Health-based guidelines could be derived from the information summarised in Chapter 5. Stakeholder consultation would be required to finalise guidelines and to agree on what to measure and appropriate methods of measurement and assessing compliance.</td>
<td>Strong evidence base on which guideline for endotoxin could be based; Weaker evidence base for organic dust and very weak evidence base for fungi, total microbes, beta (1→3) glucan. Inadequate evidence for bacteria; Possibility that guidelines could be met while the site remained a major source of odour nuisance giving rise to continuing community concerns about health</td>
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<tr>
<td>Development of health-based air quality guidelines based on marker species specific to waste processes</td>
<td>General advantages of health-based guidelines plus specificity to waste</td>
<td>Emissions of marker species likely to be variable in time and space; Relationship between marker species and bioaerosol components for which exposure-response information is available is likely to be inconsistent both at individual sites and across different sites; Health relevance of marker species unknown</td>
<td>Extensive measurement campaign in order to understand the relationship between marker species and bioaerosol components for which exposure-response information available; development of health based guidelines based on these data</td>
<td>Likely to be precluded by extent of development work required; Even if development work undertaken, the scientific evidence underlying any proposed guidelines would be extremely weak. There would only be a tenuous link between measured concentrations of marker species and measures of bioaerosol exposure employed in published epidemiological studies, combined with the underlying uncertainty in the epidemiological data linking microbial exposure to effects</td>
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<td>Approach</td>
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<td>Development of air quality guidelines based on waste specific components of bioaerosol to limit impact of waste processes on total population exposure to bioaerosols</td>
<td>Given that population exposure to bioaerosols may exceed health-based guidelines in the absence of emissions from waste processes, this pragmatic approach would limit the health impact of waste emissions to non-significant levels;</td>
<td>Guidelines only indirectly linked to health being based on the assumption that any reduction in exposure is likely to be beneficial</td>
<td>Identification of key marker species that are specific to waste processes; collection of sufficient monitoring data to establish typical baseline bioaerosol concentrations in the UK; quantitative assessment of likely health benefit; stakeholder agreement of definition of significant increase in bioaerosol concentrations</td>
<td>It is by no means certain that appropriate marker species that can be widely identified in emissions from waste processes will be developed. Although this approach has a number of attractions, given the uncertainty in the exposure-response information and the development work required, serious consideration as a potential regulatory approach is probably precluded</td>
</tr>
<tr>
<td>Development of air quality guidelines based on proxies for waste bioaerosols</td>
<td>Measurement of marker substances would allow assessment of the specific contribution of waste processes to bioaerosols in ambient air; measurement of marker substances likely to be technically easier than measurement of bioaerosols (no issues about organism variability or counting errors, use of straightforward instrumental techniques rather than biological assays)</td>
<td>Relationship between proxy measures of exposure and bioaerosol harmfulness likely to be variable leading to inconsistencies in regulation across different sites as well as practical difficulties in assessing compliance at an individual site; Measurement of compliance may be almost as difficult as direct measurement of bioaerosols; Guidelines likely to be based on very weak evidence base; No guarantee that marker substances are unique to emissions from waste processes</td>
<td>Development of appropriate proxy measures of bioaerosol exposure (substances, measurement methods) which is likely to involve an extensive measurement campaign in order to relate these measures to parameters for which exposure-response information is available (Chapter 5). Development of exposure guidelines based on the relationship between the proxy for bioaerosol concentrations and bioaerosol harmfulness</td>
<td>Extensive development work required with little certainty of success; likely that relationship between nonbiological components of emissions and bioaerosols very variable; this is unlikely to be a useful regulatory approach</td>
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<td>Approach</td>
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<td>Development of modified objectives for PM$_{10}$ in the vicinity of waste operations</td>
<td>Easy to monitor compliance; Readily harmonised with existing requirements to manage population exposure to PM$_{10}$; Well established and accepted measurement methods</td>
<td>The measured particulate concentrations will represent particles from waste and nonwaste sources and include inorganic dust</td>
<td>Examination of existing data to establish likely impact of bioaerosols on concentrations of PM$<em>{10}$ within the vicinity of waste operations; use of exposure-response information from Chapter 5 in the estimation of likely harmfulness of bioaerosols within the PM$</em>{10}$ size range.</td>
<td>The relatively easy implementation of this approach is attractive, although it is uncertain that waste sites will have any demonstrable impact on concentrations of PM$_{10}$ in local communities. Unlike any of the other suggested approaches, it provides the possibility of a continuous objective measure of the risk to health associated with bioaerosol exposure.</td>
</tr>
<tr>
<td>Quantitative emissions standards</td>
<td>Approach widely used in regulation of other processes where it is easier to monitor quantities discharged to air (or water) than impact on receiving environment</td>
<td>Impossible to quantitatively measure emissions from open air operations on a routine basis; link between emissions and impact on bioaerosol concentrations in local communities not established</td>
<td>Development of methods to quantitatively assess emission rates; Improved understanding of relationship between emissions and impact on local air quality; Development of air quality guidelines against which impacts can be assessed</td>
<td>Unlikely to represent a useful approach for waste processes (unless entirely enclosed with a limited number of emission sources) although daily (or hourly) observation of visible dust emissions or odour at site boundary may form part of routine good practice</td>
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<tr>
<td>Qualitative emissions standards (visible dust, odour)</td>
<td>Addresses the issues of greatest public concern with the incidental effect of reducing bioaerosol exposure; existing legislation requires proper control of these impacts</td>
<td>Difficult to apply; Unlikely to provide adequate control of bioaerosol exposure in the absence of other measures</td>
<td>Improved technologies for preventing emissions</td>
<td>Although unlikely to be completely effective in controlling bioaerosol exposure, the absence of dust and odour nuisance is likely to lead to reduced public concern about waste sites with a related real benefit for health</td>
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<td>Development of best practice</td>
<td>Easy to assess compliance during site inspections; Best practice would</td>
<td>Not directly linked to health; Difficult to objectively assess compliance</td>
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<td>guidance</td>
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<td>and maximise benefits may not lead to minimisation of health impacts of</td>
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<td>achieved to meet any air quality guidelines or emissions limits</td>
<td>bioaerosol emissions (guidance would have to take account of water and soil</td>
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<td>adoption of best practice will control the contribution of waste processes</td>
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<td>required to develop best practice guidelines; It would be desirable to</td>
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<td>quantitatively estimate the health benefits arising from the implementation</td>
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<td>of best practice; Acceptability to local communities is likely to require</td>
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<td>against ISO 14001 may improve the effectiveness of a best practice approach</td>
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- Development of best practice guidance:
  - Easy to assess compliance during site inspections; Best practice would have to be achieved under the Pollution Prevention Control regime regardless of other considerations; Best practice would have to be achieved to meet any air quality guidelines or emissions limits.
- Limitations:
  - Not directly linked to health; Difficult to objectively assess compliance between site inspections; Requirement to minimise overall adverse impact and maximise benefits may not lead to minimisation of health impacts of bioaerosol emissions (guidance would have to take account of water and soil quality, nuisance, compost quality etc); Difficult to demonstrate adequate protection of health in local communities.
- Development work required:
  - Compilation of existing knowledge on best practice; demonstration that adoption of best practice will control the contribution of waste processes to community bioaerosol exposure to an acceptably low level.
- Other Comments:
  - Best practice guidelines are likely to prove a key tool in regulation whether or not other approaches are also adopted; Relatively little work required to develop best practice guidelines; It would be desirable to quantitatively estimate the health benefits arising from the implementation of best practice; Acceptability to local communities is likely to require excellent control of dust, odour and noise; A requirement for accreditation against ISO 14001 may improve the effectiveness of a best practice approach to control.
<table>
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<tr>
<th>Approach</th>
<th>Benefits</th>
<th>Limitations</th>
<th>Development work required</th>
<th>Other Comments</th>
</tr>
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<tr>
<td>Stand off distances</td>
<td>Unambiguous, extremely simple to apply</td>
<td>Not directly based on health; Can only be readily applied at planning stage; Likely to greatly restrict site availability for waste process; Prevention of new development (e.g., housing) in stand off zones around existing or new sites is likely to sterilise large areas of land where risks to health posed by bioaerosols are negligible; Creation of unnecessary anxiety about waste sites in communities sited within what would become the stand off zone around existing sites; No incentive for operators to improve performance</td>
<td>A stronger evidence base is required to support recommended stand off distances; Exposure-response information from this study could inform development of guidelines if combined with an improved understanding of the impact of different types of waste process on bioaerosol concentrations in local communities; Guidelines should take account of nature of process and quantity of waste handled/likely emission rates</td>
<td>Advice on stand off distances may form part of best practice guidance but disadvantages likely to outweigh advantages; Stand off distances are unlikely to provide an effective regulatory approach in the absence of other measures.</td>
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<tr>
<td>Do nothing</td>
<td>No cost</td>
<td>No benefit; this option is unlikely to inspire public confidence</td>
<td>There is a need to establish the current burden of ill health associated with bioaerosol emissions from waste processes; the reduction in ill-health that could be achieved by active regulation and the costs of that regulation</td>
<td>It is important that the costs of implementing other regulatory approaches are proportionate to the risks associated with bioaerosol exposure; there are existing regulatory powers to deal with dust and odour nuisance which are both likely to be associated with increased levels of bioaerosol exposure.</td>
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</table>
7.7.2 Quality of evidence on which recommendations based

The studies reviewed in Chapter 5 provide strong evidence linking bioaerosol exposure in the workplace to adverse effects on health including long term respiratory illness. The evidence linking mould exposure in indoor air to adverse effects is weaker, but still convincing. The evidence linking bioaerosol exposure in ambient air arising from waste management processes to adverse effects is extremely weak. The workplace data indicate a real need to control worker exposure to bioaerosols in order to protect their health. Although by implication, this would suggest that there is also a need to control community exposure to bioaerosols in order to minimise health impacts. However, in practice community exposure to bioaerosols is likely to be a small fraction of that experienced in the workplace so there may not be any important risk to community health. It may be difficult to determine whether constraints put on the waste industry with the intention of minimising public bioaerosol exposure are effective. The uncertainties in the exposure-response information available for low level exposure to bioaerosol mean that it is likely to be difficult to predict or detect the public health benefits.

The exposure-response information available for endotoxin is considerably better than for other components of bioaerosols and the strength of evidence of any air quality standards or other guidance based on endotoxin concentrations would be considerably greater than that underlying recommendations based on other measures of bioaerosols. There are, however, considerable uncertainties in the exposure-response information available for endotoxin because of expected measurement inconsistencies in different studies and the unknown importance of other components in the bioaerosol mix in contributing to observed effects.

There are considerable uncertainties in the epidemiological data linking measures of exposure to airborne microbes to adverse effects. There would be considerable uncertainties as to the level of protection that might be afforded by any air quality guidelines based on fungi or total microbes (viable or including the nonviable fraction). Normally such uncertainties would be accommodated in standard setting by the use of substantial uncertainty factors but this would have the effect of reducing proposed guidelines to levels that are a small fraction of the background levels of exposure. There are very few data to set exposure guidelines for beta (1→3) glucan and considerably uncertainty as to whether beta (1→3) glucan is the causal agent of effects observed in epidemiological studies. There are insufficient data to set exposure guidelines based on bacterial counts in air.

There are some measurement data to support the development of good practice guidelines intended to limit bioaerosol emissions and bioaerosol exposure, but these are primarily data that indicate what practices give rise to particularly high levels of emission. There are no data that specifically demonstrate the effectiveness of good practice in controlling exposure levels. There are also no data that demonstrate that good practice will control exposures to levels at which no adverse effects on health would be anticipated.

The evidence underlying any proposals on stand off zones would be weak. There are few data that describe the impact of composting processes on community exposure to bioaerosols and no data that describe how the size and type of waste process impact on bioaerosol concentrations in local communities. The data on community health around composting facilities are weak and there are no data describing the impacts of bioaerosol emissions from other waste processes on community bioaerosol exposure or health.

7.7.3 Additional development work required to implement alternative approaches

The gaps in the epidemiological database are a major limitation on the development of air quality guidelines based on existing widely used measures of bioaerosol exposure. Despite extensive investigation of bioaerosol concentrations around composting facilities, there is still a relatively poor understanding of how these facilities impact on community exposure to bioaerosols and the related impact on health. It seems unlikely that these issues will be resolved without extensive research and thus the regulatory approach taken to bioaerosols should be based on existing knowledge.
One of the difficulties in determining the importance of waste management in giving rise to bioaerosol exposure is that there are multiple sources of the components typically used to characterise bioaerosols. The development of techniques to determine bioaerosol exposures that are more specific to waste may be technically feasible, but the weakness of the epidemiological database would preclude the derivation of health-based guidelines based on these exposure markers. It would however be possible to use marker components to demonstrate the importance or otherwise of waste-derived bioaerosols in determining total bioaerosol exposure. This would indicate where the priorities for regulation should lie and could also be used to measure the effectiveness of regulatory measures in reducing exposures.

The use of modified PM$_{10}$ objectives in the vicinity of bioaerosol sources as a tool for regulating bioaerosol emissions would require some review work in order to inform the setting of the objectives at appropriate levels to provide a similar level of protection of health as would be provided by the PM$_{10}$ objectives in typical urban air.

### 7.7.4 Mixed sources of exposure

Bioaerosols are ubiquitous and waste is not the sole source of bioaerosols in ambient air. Currently used indices of exposure do not differentiate between bioaerosols from different sources. Personal exposure may be dominated by factors such as the indoor storage of biowastes prior to collection, home composting, gardening or occupation.

Given that exposure to bioaerosols arises from a wide variety of sources, the regulation of bioaerosol emissions from waste processes needs to be proportionate to the associated risk to health. It may be appropriate to develop monitoring strategies that are based on specific markers of waste-derived bioaerosols.

### 7.7.5 Exposure to other pollutants

There has been little investigation of the effects of co-exposure to bioaerosols and other respiratory irritants such as ozone or acid aerosol. It seems plausible that the irritative effects of bioaerosols might be enhanced by the presence of other potential irritants such as ozone. It would be prudent to take account of the potential interaction with ozone on high pollution days in the regulation of bioaerosol emissions. For example, additional measures to limit emissions might be required under certain weather conditions and this should be reflected in any good practice code. If air quality standards or stand off zones are adopted, their determination should include consideration of co-exposure to other pollutants. Some research may be required to determine whether the conditions leading to elevated ozone exposures are also associated with impacts on bioaerosol exposure.

### 7.7.6 Protection of the abnormally sensitive

Environmental guidelines should protect nearly everybody, nearly all of the time, but it may not be possible to provide complete protection for the most sensitive individuals including those with severe immunosuppression. Some members of general population are at risk of developing adverse health effects following exposure to concentrations of bioaerosols that have been reported as background environmental levels. For those individuals, any bioaerosol exposure arising from waste processes would be highly undesirable but they would be at substantial risk of illness even in the absence of waste-derived bioaerosols. It may, in fact, be difficult to protect these individuals using any regulatory approach which raises difficult ethical issues. A relatively substantial minority of population, perhaps 10%, are susceptible to developing allergic sensitisation to microbial species. It seems reasonable to include these individuals within the definition of “nearly everybody” who should be protected but many of these individuals may develop symptoms at background levels of exposure. The regulation of bioaerosol emissions from the waste industry should take account of sensitive individuals through ensuring that the contribution of the waste industry to their total bioaerosol exposure and the associated risk to health is appropriately small.
7.7.7 Odour

Odour has been specifically excluded from the scope of this project but it will be difficult to convince communities that bioaerosol emissions from waste facilities are being adequately controlled, if a substantial odour nuisance exists. In a recent review for Scottish Environment Protection Agency we identified several studies that have found an association between self-rated odour exposure and subjective symptoms such as respiratory irritation, headache, nausea and fatigue (Schiffman and Williams, 2005). These symptoms are consistent with those that are widely reported to be associated with bioaerosol exposure. It is not clear whether odour is merely a good marker for bioaerosol exposure or whether odour plays an important role in the causation of effects. Similar associations have also been found around plants where bioaerosols are unlikely to have been important, although effects could plausibly have been due to chemical exposure (Luginaah et al, 2000, 2002; Steinheider, 1999). The results of experimental studies (for example, Dalton, 1997, 1999) suggest that it is possible that the subjective symptoms reported in epidemiological studies could be simply a reaction to odour rather than the result of toxicity of the compound/organism causing the odour (or a co-pollutant present in an odorous mix). The severity of response to odour in part depends on offensiveness and on an individual’s beliefs about its harmfulness (Sucker et al, 2001). Some individuals, for example those of an anxious disposition, are considerably more sensitive to the effects of odour than others (Carlson et al, 2005; Bell et al, 1996). “Chemical odour intolerance” is a relatively common condition in which people feel ill simply as a result of exposure to a “chemical” smell. The prevention of odour nuisance is important for community health and well being. At many waste sites, the control of odour to levels that are not discernible beyond the site boundary is likely to prove a greater challenge than the control of bioaerosol emissions to levels that are below those that could cause adverse effects in local communities. Odour may be a good tracer for bioaerosol exposure, and it seems unlikely that excessive exposure to bioaerosols will arise without concurrent exposure to odour.
8 Conclusions and Recommendations

8.1 NATURE OF BIOAEROSOLS EMITTED FROM WASTE HANDLING PROCESSES

Bioaerosol consists of a complex mix of viable and dead organisms and fragments of organisms. Airborne concentrations may be described in terms of organic dust concentration, total or viable microbial/bacterial/fungal counts, endotoxin or beta(1→3)glucan.

Thermophilic bacteria have been identified as being of particular importance in emissions from composting operations and various Aspergillus species, a pathogenic group of fungi, are emitted from a wide range of waste processes.

8.2 METHODS USED TO MEASURE BIOAEROSOL EXPOSURE ARISING FROM WASTE MANAGEMENT ACTIVITIES

8.2.1 Overview of available methods

There are no standard methods of bioaerosol measurement in the UK. A wide range of instruments have been used to capture bioaerosols on filter, in fluid or onto gel. Side by side comparisons of sampling devices have demonstrated considerable variability in performance, particularly in respect to viable microorganisms that are destroyed to greater or lesser degrees by different sampling devices and protocols. Because of the difficulties in preserving organism viability during sampling, sample times for bioaerosol measurement tend to be very short from a few minutes to an hour and half whereas bioaerosol concentrations can vary dramatically in the space of a few hours (see section 3.2.2). The EA (2004) highlight the importance of acquiring large number of samples over an extended period, in order to gain a representative picture of bioaerosol concentrations.

The analysis for viable organisms involves culturing the sample in a suitable medium and at an appropriate temperature to support the growth of the organisms of interest and then counting the number of colonies that develop. Counts of total viable and nonviable organisms are made microscopically using fluorescence staining to aid identification.

A number of recent studies have explored alternative approaches to the characterisation of airborne microbial samples. These include the use of gas chromatography – mass spectroscopy (GC-MS) for the identification of characteristic compounds associated with specific groups or specific species of microbe and the use of quantitative polymerase chain reaction (PCR) to determine the presence and quantity of specific target DNA sequences that can be linked to individual fungal or bacterial species.

Endotoxin is most commonly measured using a chromogenic Limulus amebocyte lysate (LAL) assay but has also been assessed using GC-MS to determine the LPS content of samples. LPS levels reported using GC-MS are not directly comparable with endotoxin levels determined in the LAL assay. There has been very little investigation of endotoxin concentrations in ambient air and methods have primarily been developed for the sampling and analysis of workplace air. Beta (1→3) glucan has been analysed using:

- Glucan-specific LAL assay;
- Inhibition Enzyme-Linked ImmunoSorbent Assay (ELISA); and
- Sandwich ELISA.

8.2.2 Towards method standardisation

A CEN working group on the “Measurement of bioaerosols in ambient air and emissions”(CEN/TC 264/WG 28) has recently been formed but this group is still at the stage of investigating current practice in different member states and no draft protocols have been proposed for wider adoption (see section A1.8 of Appendix 1).

Currently the EA recommends that bioaerosols are collected by active impaction onto agar using Andersen or split samplers or liquid impingers followed by analysis for viable micro-
organisms based on cultivation of the sample and colony counting or counting of total microorganisms using optical microscopy and staining (EA, 2004). A draft EA report (EA, 2007) indicates that in the future samples may be collected onto filter and analysis is likely to focus on thermophilic actinomycetes and Aspergillus fumigatus.

The Composting Association (1999) recommends the use of the Andersen sampler to collect bioaerosols and sampling at a minimum of three locations: upwind of the site, downwind of the site, and adjacent to the nearest sensitive receptor (occupied building), requires the collection of relevant weather data and stipulates that sampling should not be performed at temperatures less the 5°C or during precipitation.

The American Society for Testing and Materials (ASTM) method E884-82 (ASTM, 2006) recommends use of a multistage impactor (eg Andersen sampler) and all-glass liquid impingers with sampling times of 30 minutes and up to 1.5 hours, respectively. It is recommended that sampling is undertaken at at least one site 300 m upwind and one site 100 m downwind of the site (three replicates upwind and five downwind).

In addition to standardisation of measurement methods there is a need for a harmonised approach to sampling strategy.

8.2.3 Biomarkers of exposure

There are no widely used biomarkers of exposure to bioaerosols. Specific IgG antibodies to moulds and actinomycetes do not appear to be an effective measure of exposure in waste workers in comparison to unexposed controls. Various studies have assessed inflammatory markers in nasal lavage and sputum, but these markers are not specific to bioaerosol exposure.

8.2.4 Measurement of bioaerosol exposure in published epidemiological studies

Uncertainty in bioaerosol measurement is a major issue affecting the interpretation of exposure-response information. The comparability of measurements of individual measures of bioaerosol exposure made by different groups is likely to be limited. The use of slightly different measures by different groups further limits interstudy comparison. It is unclear how well published data in epidemiological studies represent actual levels of exposure.

8.3 BACKGROUND LEVELS OF EXPOSURE TO BIOAEROSOLS

Background levels of exposure to bioaerosols are hugely variable.

Fungal concentrations in outdoor air vary by location and season and there is little readily available information for the UK. Both UK data and the results of studies elsewhere suggest that concentrations in urban air are typically less than 1000 cfu m⁻³ but may be considerably higher, particularly during the autumn. There is even less information available about concentrations of bacteria. Allowing for uncertainties in the measurement data and the small quantity of data available, it seems probable that typical bacterial concentrations in urban air are less than 1000 cfu m⁻³.

Most studies of bioaerosol exposure in rural areas have focussed on occupational exposure associated with intensive livestock rearing and there has also been some interest in the impacts of sewage sludge spreading. There is very little information about typical concentrations of bacteria or fungi in rural areas.

Both external air quality and building dampness play an important role in governing exposure to airborne moulds in indoor environments with reported concentrations in indoor air ranging from <100 to 20000 cfu m⁻³. Concentrations of bacteria in indoor environments are typically about 100 cfu m⁻³ but may be considerably higher in some environments.

Typical endotoxin levels in outdoor urban air are less than 1 EU m⁻³, but slightly higher concentrations may arise in some rural locations where agricultural activities emit endotoxin. Indoor concentrations of endotoxin are heavily influenced by environmental tobacco smoke.
Background concentrations of bacteria and fungi reported in studies of the impacts of waste management processes tend to be higher than those typically found in indoor air or outdoor air in urban areas but are comparable to levels reported in some buildings with damp problems. Background concentrations of endotoxin reported in studies of waste management processes are about 2 EU m\(^{-3}\), comparable with those found in urban air.

**8.4 LEVELS OF WORKPLACE AND COMMUNITY EXPOSURE ARISING FROM WASTE MANAGEMENT ACTIVITIES**

Elevated levels of workplace exposure to bioaerosols are found widely throughout the waste industry including waste collection, materials recovery, composting and the storage of waste material prior to incineration. Exposure levels in most sectors tend to be higher during the summer but are not clearly linked with waste composition. There is limited evidence that waste storage may lead to increased exposure concentrations and more substantive evidence that increased activity levels are associated with increased bioaerosol exposures. Exposure levels vary within individual sectors suggesting that there is potential to reduce exposures through good practice.

In studies of waste collection workers, reported mean or median concentrations of viable fungi vary from 30 to 10000 cfu m\(^{-3}\), mean/median concentrations of bacteria range from 1700 to 80000 cfu m\(^{-3}\), mean/median dust concentrations range from 0.4 to 8 mgm\(^{-3}\), mean/median endotoxin concentrations from 13 to about 370 EUm\(^{-3}\) and mean/median beta (1→3) glucan concentrations range from about 10 to more than 1000 ngm\(^{-3}\).

Reported mean/median workplace exposure concentrations for composting are similarly variable. For dust, they range from about 0.5-5 mgm\(^{-3}\), for bacteria from about 5 x10\(^3\) to 10\(^7\) cfu m\(^{-3}\) and for fungi from about 20 to 10\(^7\) cfu m\(^{-3}\). Reported mean/median endotoxin concentrations range from less than 100 to over 700 EUm\(^{-3}\) and mean/median beta (1→3) glucan concentrations range from about 0.5-5 ugm\(^{-3}\).

There have been relatively few studies of bioaerosol exposure at waste transfer stations, materials recovery facilities (MRFs), landfills or other waste processes. Reported median/mean dust concentrations for MRFs are between about 2 and 8 mgm\(^{-3}\) and are associated with endotoxin levels of about 50 – 600 EU ngm\(^{-3}\) In other studies that have examined microbial exposures, mean total viable bacterial counts have ranged from less than 10\(^{2}\) to more than 10\(^6\) cfu m\(^{-3}\) and mean fungal counts have ranged from about 10\(^2\) to about 10\(^5\) cfu m\(^{-3}\). Waste sorting is associated with particularly high exposures in some plants with reported exposures to moulds that exceed 10\(^5\) cfu m\(^{-3}\) and exposures to bacteria exceeded 10\(^4\) cfu m\(^{-3}\). Extremely high levels of bioaerosols were reported to be associated with waste storage at an incinerator with mean concentrations of 3.3 mgm\(^{-3}\) for dust, 24500 and 2670 cfu m\(^{-3}\) for mesophilic and thermophilic bacteria, 118225 and 5235 cfu m\(^{-3}\) for mesophilic and thermophilic fungi and 39500 EUm\(^{-3}\) for endotoxin.

Several groups have investigated community exposure to bioaerosols emitted from composting. Concentrations of >10\(^5\) cfu m\(^{-3}\) of thermophilic actinomycetes, moulds, and total bacteria have been reported 200 m from a large composting site, dropping to near background concentrations within 300 m. The results of several other studies also suggest that microbial counts generally drop to background levels within 300 m although raised microbial concentrations may occasionally arise at distances of about up to about 0.5 km from composting operations.

**8.5 IMPORTANCE OF WASTE MANAGEMENT ACTIVITIES IN DETERMINING POPULATION EXPOSURE TO BIOAEROSOLS**

Waste management operations represent locally important bioaerosol emission sources but it is apparent that elevated levels of bioaerosol exposure also occur in the absence of any important contribution from waste management operations. Raised levels of community exposure to bioaerosols may arise within 250 m downwind of a composting facility and under rare circumstances at distances of up to 0.5 km. In a UK study, elevated concentrations arose...
intermittently at 100 m from the site boundary but on other occasions, on-site concentrations and concentrations close to the site boundary were close to zero. There are insufficient data describing the geographical variation of bioaerosol concentrations in the UK to determine the relative importance of the waste industry as opposed to other industrial or domestic sources in governing the overall population exposure to bioaerosols. The agricultural industry is a substantial source of bioaerosol emission, waste water treatment and food processing are also locally important sources. Most people spend most of their time indoors and building dampness can have a massive influence on personal exposure to bioaerosols. In some areas of low quality housing, building dampness is likely to be the overwhelming factor in determining population exposure to bioaerosols.

In addition to the direct impact of commercial/municipal waste management activities on population exposure to bioaerosols; waste management policy and delivery may impact on individual bioaerosol exposure resulting from individual behaviours. For example, the encouragement of domestic composting may lead to increased exposures and the storage of organic or unseparated wastes indoors may also lead to increased exposure, particularly if waste is not properly contained in sealed containers. Increased levels of microbial activity in stored waste may arise during prolonged storage in warm conditions. It is important that householders are clearly informed on how to store and handle waste in a manner that will minimise potential bioaerosol exposure.

8.6 HEALTH EFFECTS ASSOCIATED WITH BIOAEROSOL EXPOSURE

There is strong evidence linking workplace exposure to bioaerosols in the waste industry to adverse effects on health including long term respiratory illness. This is consistent with the findings of studies of bioaerosol exposure in other sectors. There is much less information about the effects of community exposure to bioaerosols with only weak evidence that suggests that a range of respiratory symptoms, fatigue and gastrointestinal symptoms may be linked with residential proximity to composting operations. There is also evidence that mould exposure in indoor air (resulting from building dampness) is linked to increased risks of respiratory symptoms.

8.7 EXPOSURE-RESPONSE INFORMATION FOR BIOAEROSOL EXPOSURE

Exposure-response information for bioaerosol exposure is available from studies of workers in the waste and other industries. The results of workplace and experimental studies suggest that the risks of illness increase with increasing levels of exposure and that the effects of exposure to a mixture of bioaerosol components is more harmful than exposure to individual agents. The exposure-response information available for endotoxin is considerably better than for other components of bioaerosol but there are large uncertainties because of expected measurement inconsistencies in different studies and the unknown importance of other components in the bioaerosol mix in contributing to observed effects. The exposure-exposure information available for beta (1→3) glucan is particularly uncertain and it is unclear whether beta (1→3) glucan is harmful to health or merely a marker for more harmful components of bioaerosol exposure. Table 5.3 summarises the exposure-response information reviewed in this report.

8.8 BIOAEROSOL COMPONENTS WITH GREATEST POTENTIAL IMPACTS ON HEALTH AND NO AND/OR LOWEST EFFECTS LEVELS

Endotoxin is the most widely used metric of bioaerosol exposure in published investigations of health effects. There is some evidence of mild adverse effects associated with workplace concentrations of < 10 EUm⁻³, and stronger evidence of effects at concentrations > 50 EUm⁻³.

Workplace exposure to fungal concentrations greater than 10⁴ cfum⁻³ is associated with an increased risk of respiratory symptoms. Severe respiratory symptoms including hypersensitivity pneumonitis have been reported at concentrations of 10⁹-10⁴ cfum⁻³ and also in some workers with high exposures to specific Actinomycetes species. A relatively substantial proportion of individuals (perhaps >10% of the population) may be susceptible to developing respiratory symptoms at levels of exposure (microbial counts of 10⁵-10⁷ cfum⁻³)
encountered in the general community, in the absence of any specific waste management source. There are few studies of the effects of bacterial exposure that are relevant to the waste industry. Adverse effects on respiratory health and more general health (excessive tiredness) have been reported at concentrations exceeding $10^6$ as total bacteria/m$^3$ or $10^5$ cfu/m$^3$. There are limited data that suggest the threshold for adverse effects arising from beta (1→3) glucan exposure is between 1 and 10 ng m$^{-3}$.

On consideration of all the available studies, it is apparent that there are no clear thresholds of effect for different bioaerosol components. This may partly reflect the susceptibility of some individuals in the population to adverse effects (below), the importance of sensitisation in governing some health effects and the potential for infection. It is also worth noting that the concept of a no effects level comes from chemical toxicology and arises from the conduction of animal experiments with a limited gene pool and a limited number of animals. Virtually all the health information for bioaerosols arises from studies in human populations with a range of susceptibility to effects.

### 8.9 POTENTIALLY SUSCEPTIBLE SUBGROUPS OF THE POPULATION

There is good evidence that asthmatics are more sensitive to bioaerosol exposure than others and it would be appropriate to take account of mould-sensitised asthmatics in the regulation of bioaerosol exposure. Given the prevalence of moulds in indoor air, it is unlikely that the control of bioaerosol levels in outdoor air would lead to any significant reduction in the risk of individuals becoming mould sensitised. However, it may help in the reduction of respiratory symptoms.

A small proportion of individuals with asthma or cystic fibrosis develop hypersensitivity to *Aspergillus* and may develop severe adverse reactions on exposure to concentrations of *Aspergillus* that may be found in many indoor environments in the absence of any specific bioaerosol sources. Similarly severely immunocompromised individuals (for example, transplant patients, some cancer patients or individuals with AIDS) are at risk of developing serious illness resulting from invasive fungal infection at levels of fungal exposure found in many indoor environments. It would not be possible to prevent such infections arising simply through better regulation of bioaerosol emissions from the waste industry and any reduction in risk arising from reduced emission levels may be small because of the potential importance of indoor sources in governing exposure.

### 8.10 USE OF EXPOSURE-RESPONSE INFORMATION TO INFORM REGULATION

The exposure-response information that is available for bioaerosol exposure could be used in a number of ways to help inform the regulation of bioaerosol emissions from the waste industry, including assessing the relative costs and benefits of any proposed regulatory actions. Of the various regulatory options that have been reviewed in this study the following approaches appear most promising at present:

- Development of good practice guidelines, possibly supported by a requirement for sites to become ISO 14001 accredited; and

- Consideration of modified objectives for PM$_{10}$/PM$_{2.5}$ in the vicinity of waste processes.

There is sufficient exposure-response information for endotoxin to support the development of air quality guidelines that might be used to inform process regulation but the data for the various microbial indices of exposure or beta (1→3) glucan are less extensive. Major difficulties in the development and application of air quality guidelines include the fluctuation in background bioaerosol concentrations, the emission of bioaerosol from multiple sources other than the waste industry, the likelihood that any recommendations based on standard methods of deriving guidelines are likely to be lower than background and measurement issues.

This report has focussed on the control of community exposure to bioaerosols but the data reviewed in chapters 3 and 4 demonstrate that workers in the waste industry are at risk of...
developing a range of acute symptoms and long term respiratory illness. The substantial change that has occurred in the waste industry over the last few years has led to greatly increased numbers of workers employed in materials recycling facilities and in composting. There is a need for the Health and Safety Executive to ensure that the bioaerosol exposure of these workers is adequately controlled in order to prevent a future increase in the prevalence of respiratory illness in waste workers.

8.11 KNOWLEDGE GAPS AND RECOMMENDATIONS FOR FURTHER RESEARCH

8.11.1 Long term health risks associated with bioaerosol emissions from waste processes

Our understanding of the health effects of bioaerosol exposure is dominated by the results of studies focussed on short term impacts, particularly in the waste industry. The risks of developing chronic respiratory illness as a result of long term exposure to bioaerosols arising from waste management are unknown. Given the increasing evidence suggesting that the long term effects of exposure to air pollution are of greater importance than the short term response to high pollution events, it is clearly important to establish whether a similar situation exists for bioaerosols. It is unlikely to be feasible to investigate the effects of long term exposure to waste derived bioaerosols in the general community because of the difficulties in characterising exposure and in separating the effects of waste-derived bioaerosols from those attributable to bioaerosol from other sources, other types of environmental pollution or other causes of ill-health. The most effective approach to investigating the long term effects of exposure is likely to involve studying workers with long term employment in the waste industry. The quantification of both effects and exposure are likely to be key challenges. It is recommended that consideration is given to the design and funding of an appropriate long term study of the health of waste workers in relation to bioaerosol exposure.

8.11.2 Development of exposure guidelines for bioaerosols

The most cohesive information that is available for the effects of bioaerosol exposure is from workplace studies of the effects of exposure to endotoxin. This information could be used as the basis for developing guidelines on bioaerosol exposure concentrations in ambient air but there are very few data describing concentrations of endotoxin in ambient air. It is recommended that a comprehensive survey of endotoxin concentrations in outdoor and indoor air is undertaken in order to assess feasibility of setting a future guideline. This should include investigation of the endotoxin concentrations arising around waste processes. The extent to which endotoxin concentrations are raised in ambient air in the areas around waste processes could also be informative as to necessity for the improved regulation of bioaerosol emissions.

Given that the measurement of bioaerosol concentrations in ambient air is likely to remain technically demanding and relatively expensive, it would be desirable to investigate the feasibility of managing bioaerosol exposure through the management of ambient particulate levels. There are existing methods for PM$_{10}$/PM$_{2.5}$ measurement and also an existing requirement to manage population exposure to these pollutants. It is recommended that the feasibility of setting a modified PM$_{10}$/PM$_{2.5}$ standard for areas in the vicinity of waste management processes is investigated and proposals made for appropriate guideline values.
9 Acknowledgements

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10. References

ADAS/SWICEB, 2005. Bioaerosol monitoring and dispersion from composting sites. 2. SWICEB report, August


DSTL Characterisation of the UK biological aerosol background. Dstl Chemical and Biological Sciences, Porton Down (supplied by DR Rob Kinnerley of the Environment Agency)


Eduard W. Measurement methods and strategies for non-infectious microbial components in bioaerosols at the workplace. Analyst. 1996 Sep;121(9):1197-201


EPAQS (2001) Airborne Particles. What is the appropriate measurement on which to base a standard? Department for the Environment, Food and Rural Affairs and the Devolved Administrations Expert Panel on Air Quality Standards


Hines CJ, Milton DK, Larsson L, Petersen MR, Fisk WJ, Mendell MJ. Characterization and variability of endotoxin and 3


Knop M, Pohle H, Bergmann A. [Sanitation of biowaste compost by using Salmonella enteritidis as a pathogen indicator and survival of Salmonella in seepage water] Berl Munch Tierarztl Wochenschr. 1996a Nov-Dec;109(11-12):451-6. [Article in German]


Newsom RA, Strachan D, Cordenc J, Millington W. Fungal and other spore counts as predictors of admissions for asthma in the Trent region Occup Environ Med 2000;57:786-792.


Senkpiel K, Trepkau HD, Ohgke H. [Fungal allergen (antigen) burden of refuse disposal workers in biological waste and garbage collection within the scope of a work site analysis] Schriftenr Ver Wasser Boden Lufthyg. 1999;104:163-82. [Article in German]


Syzdek L, Haynes HH. Monitoring Aspergillus fumigatus aerosols from a composting facility. Aerobiologia. 1995; 11, 87-93


Vinneras B. Comparison of composting, storage and urea treatment for sanitising of faecal matter and manure. : Bioresour Technol. 2006 Aug 22; [Epub ahead of print]


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Appendices
APPENDIX 1

Measurement methods

A1.1 MICRO-ORGANISMS

A1.1.1 Overview

A range of devices has been used to collect the microbial component of bioaerosols. Currently there is no standardised method for the measurement of bioaerosols in the UK. Most published studies of bioaerosol exposure have focussed on the viable (culturable) component of bioaerosol, although fragments of pollen and microbial material in air may also promote toxic or allergic responses (Menetrez et al., 2001). Key issues influencing sampler design have been the consideration of the size fractions most relevant to human health (inhaled, thoracic/PM$_{10}$ and respirable) and conservation of organism viability during sampling and within the sample. The need to conserve organism viability has led to the use of relatively short sampling times and therefore a requirement to collect a large volume of air within a short space of time in order to provide a reasonable detection limit. Samplers may collect particles onto a gel, into liquid, onto filter or within foams. The gel and liquid collection media are intended to help maintain organism viability after capture and if filters or foams are used as collection media, then they must be stored in a suitable liquid medium immediately after sampling for transport to the laboratory. Once samples are returned to the laboratory, they are cultured and then a count made of the number of colony forming units present. The analysis may or may not include optical identification of key genera eg Aspergillus and species eg Aspergillus fumigatus. It is essential that samplers and sampling media are sterilised prior to sampling and transported under sterile conditions. Eduard (1996) highlighted the poor precision that is typically associated with measurements of viable bioaerosol components and the lack of interlaboratory comparability due to the unavailability of standard reference materials.

A1.1.2 Sampling

**Personal samplers:** A number of samplers have been developed that are small and light enough to be used as personal samplers, although they could also be used for static monitoring. The bioaerosol sampler developed by Kenny et al. (1998, 1999) is probably of greatest relevance in the UK. It is an adaptation of the IOM gravimetric head designed to collect the inhalable dust fraction and further subdivide the sample into thoracic and respirable fractions through the use of size selective foams. Field trials showed that for sampling periods lasting several hours, micro-organism survival within the sampler was adequate for culture and identification of the organisms present. Analysis could be carried out with similar efficiency either with all three fractions together for a total count, or separately for size selective data. The IOM head may also be used without the foam inserts to capture inhalable dust with no further size selection.

Although most personal samplers are designed to collect onto filter on foam, one of the drawbacks of collecting bioaerosol onto a filter or foam is that there may be a loss of microbial viability prior to incubation of the sample and analysis. In a study of waste incineration workers, Tolvanen et al. (2005) reported that the six-stage impactor gave higher (presumed more accurate) results for viable fungi and actinomycetes than were obtained for samplers collected on filter. Gomer et al. (2006) describe an adaptation of the CIP10 sampler for bioaerosol collection that would overcome the need to collect onto foam. The CIP10 sampler is lightweight personal sampler that incorporates the sampling pump and sample head into a single unit to be worn in the breathing zone. Normally the CIP10 sampler collects dust onto a foam. The CIP 10-M (M-microbiologicis) collects biological particles in 2 ml of liquid medium inside a rotating cup fitted with radial vanes to maintain an air flow rate of 10 l min$^{-1}$ at a rotational speed of approximately 7,000 rpm. The rotating cup is made of sterilisable material. Three particle size selectors allow sampling of the respirable, thoracic and inhalable fractions.

At least two other novel personal samplers for bioaerosol have been developed, although not yet used in epidemiological studies. Graham et al. (2000) describe a personal aerosol
A sampling device worn just inside the nostrils, driven by the wearer's respiration. The sampler comprised a slot impactor with a detachable impaction plate covered with either a specially developed medical adhesive or a protein-binding membrane. Sampler performance was validated by rig tests of aerodynamic resistance and collection efficiency for different sized particles at various flow rates. In field trials with human subjects showed that the sampler could be comfortably worn for periods of up to 4 hours.

Passive samplers have gained wide popularity for other atmospheric contaminants because of ease of use – no pump to fail or to be carried by the operator, no pump noise and no substantial capital investment is required. Nasman et al (1999) tested glycerol as a collection substrate for passive bioaerosol sampling. Filters (mixed cellulose acetate and nitrate) were soaked in glycerol and exposed for an aerosol from three different fungal species: *Penicillium commune*, *Aspergillus versicolor* and *Aecilomyces variotii*. The passive sampling method was compared with a closed-face polycarbonate filter sampling method. The glycerol soaked filter demonstrated a good correlation with the closed-face sampler with regard to the total count. Spores stored in a pumped filter cassette were not affected by storage for up to 7 days. On the other hand, the culturability of the spores was markedly decreased after 1 day when stored on glycerol soaked filters.

Static samplers: The Andersen sampler is commonly used for bioaerosol sampling and has been the sampler most widely used in published studies. Samples are collected onto the surface of agar plates placed under stacked sieve plates with holes of defined size. The Composting Association (1999) guidelines for bioaerosol monitoring recommend a single stage version but a six-stage version is also available that can be used to collect particle size data.

Many studies have used impingers to collect samples of bioaerosol in liquid and the all-glass impinger (AGI-30) has been widely used in North America. In a study of viable microorganisms in dairy barns, Thorne et al (1994) compared the performance of three sampling methods in summer and in winter: (1) the AGI-30 used with peptone solution in both seasons and (2) betaine solution in winter; and (3) the collection of samples onto nuclepore filters followed by elution. Pairwise comparison of the sampling methods in winter yielded no significant differences in airborne concentrations for the yeasts, mesophilic bacteria, and thermophilic bacteria. Both the AGI betaine and filter methods yielded significantly greater concentrations of moulds than the AGI used with peptone. In summer, apparent concentrations of yeasts and mesophilic bacteria were significantly greater with AGI peptone, as were moulds collected on filter.

SKC market a sampler specifically designed for the collection of viable bioaerosol that was developed in co-operation with the University of Cincinnati (www.skcinc.com/prod/225-9594.asp). The SKC BioSampler is a glass collection device that requires a high-volume sonic flow pump to trap airborne microorganisms in the inhalable size fraction. The sampler can be used with non-evaporating liquids that have viscosities higher than water allowing the collection of samples over an 8 hour period as compared with typical sampling times with standard impingers and impinger liquids of only 1 to 1.5 hours. Externally the BioSampler resembles an AGI-30 but the BioSampler's nozzles eject particles at an angle to the sampler's inner wall significantly reducing particle bounce and preserving aggregates of organisms whereas in standard impingers, microorganisms are typically damaged from collision with the impinger base-plate. The nozzles also create a swirling airflow that maintains microorganism viability by gently moving particles to the collection surface without re-aerosolisation. The collection liquid in standard impingers tends to bubble violently, causing collected particles to become re-aerosolised.

An et al (2004) investigated the physical and biological performances of a portable centrifugal sampler for viable bioaerosols, the RCS High Flow. The performance of the test sampler in the laboratory and field environments was compared with that the SKC BioSampler. The test sampler's physical performance when collecting the spores and vegetative cells of *Bacillus subtilis var. niger* (BG) was similar to that when collecting non-biological particles of the same size. In the laboratory tests, the RCS High Flow sampler was found to enumerate approximately only 40% of BG spores and cells relative to the BioSampler. A similar ratio was
found during testing in an indoor environment. This ratio decreased to below 10% when testing was performed in an outdoor environment.

Lee et al (2004a) used a new design of electrostatic precipitator (ESP) for bioaerosol sampling to determine the polarity and relative strength of the electrical charges on airborne micro-organisms in several laboratory and field environments. The ESP differentiated between positively and negatively charged micro-organisms, and in most of the tested environments the airborne micro-organisms had a net negative charge. Pfirrmann and van den Bossche (1994) evaluated an ESP for the collection of airborne viruses.

Cage et al (1996) tested four bioaerosol samplers in an outdoor environment: a Rotorod, a Kramer-Collins suction trap, an AGI-30, and a high-volume cyclonic liquid impinger (SpinCon). The SpinCon collected a larger number of spores than the other devices and the number of spores collected per volume of air sampled was comparable to the AGI-30. The Rotorod and Kramer-Collins collected a lower number of spores per unit of air but collected a larger number of pollen grains per volume sampled. The Alternaria allergens Alt a 1 and GP70 were collected by both liquid impingers; however, the SpinCon collected more Alt a 1 and the AGI-30 collected more GP70.

Jensen et al (1992) evaluated 8 types of bioaerosol sampler as alternatives to the Andersen six-stage sampler and the AGI-30 that had been suggested as the samplers of choice for the collection of viable microorganisms by the International Aerobiology Symposium and the American Conference of Governmental Industrial Hygienists. The study found a wide variation in collection efficiency for free bacteria. The six stage impactor oversampled relative to the AGI-30 whereas single and two stage Andersen impactors and a Mattson-Garvin Slit to Agar Air Sampler all undersampled. There may have been some loss of microbial viability in samples collected by the AGI-30 and the authors suggested that most vegetative bacteria were likely to be too fragile to survive collection in the Slit sampler. It was concluded that the relative performance of the samplers would vary with the conditions of use as some microbial species would be more prone to damage during sample collection and storage than others.

Lee et al (2004b) used four bioaerosol samplers (Reuter Centrifugal, Andersen N6 Single Stage, Surface Air System Super 90, and Air-o-Cell) to take around 300 side-by-side measurements at 75 public building sites. Regression models demonstrated that measurements from these instruments were not directly comparable and inter-instrument calibration was required. Sampling location (indoor vs. outdoor) was a confounder in all the pairwise comparisons between samplers. In addition, the slopes of the relationships between all method pairs except one differed in indoor vs. outdoor locations. Differences in size selection parameters, the make up of the bioaerosol being selected and the impact of sampling on organism viability are all likely to have contributed to the observed differences between samplers.

Bellin and Schillinger (2001) compared the performance of a Surface Air Systems (SAS) high flow portable sampler to an Andersen N6 single stage impactor in several office buildings in Southern California. Data collected on five occasions throughout the year showed that the SAS sampler recovered consistently lower levels of colony forming units than the Andersen impactor whereas there was no statistically significant difference in the measured concentrations of Cladosporium. The SAS sampler recovered about half the number of colony forming units for three other fungal categories, i.e. non-sporulating species, Aspergillus and Penicillium and others.

A1.1.3 Transport and storage

Conditions of storage and transport may promote growth or destruction of viable microorganisms in samples. The Composting Association (1999) recommend storage of samples at a temperature of less than 4°C and the initiation of laboratory processing within 12 hours.

In a study of viable microorganisms in dairy barns in summer and in winter, Thorne et al (1994) reported that concentrations of all microorganisms collected into peptone using the AGI were unaffected by mailing in winter, but mesophilic bacteria increased in summer. AGI
samples collected into betaine were unchanged except that mailing was associated with increased concentrations of moulds in winter. There was a significant decrease in yeasts and mesophilic bacteria after mailing in samples collected onto nucleopore filters.

Palmgren et al (1986) noted that the viable fraction of the total microbial numbers varied significantly when actinomycete and fungal spores from different environments were stored on the filter surface for 1 week, although the microflora composition was not altered.

A1.1.4 Analysis

Viable microbes

Samples are typically cultured in agar and the number of colony forming units enumerated using microscopy. A variety of growth media and conditions have been used. Different organisms require different conditions to thrive and culture conditions are designed in order to investigate the abundance of particular subgroups of organisms. For example, during the composting process there is a gradual build up in temperature and the fungal population of the compost evolves to include a greater proportion of high temperature species (thermophilic). Samples may be cultured at two or more temperatures in order to determine the abundance of species with differing heat sensitivities eg mesophilic (medium temperature) versus thermophilic. Fungal colonies may be classified by their characteristics in culture and appearance and morphology as observed using microscopy, although typically only to Genus rather than Species level. Bacteria can be identified by morphology, Gram staining, growth on specific substances and under special conditions, the production of specific metabolites or sequencing RNA (Eduard & Heederik, 1998).

Terzieva et al (1996) compared four methods for the detection and enumeration of aerosolized bacteria collected in an AGI-30 impinger. Changes in the total and viable concentrations of Pseudomonas fluorescens in the collection fluid with respect to time of impingement were determined. Two direct microscopic methods (acridine orange and BacLight) and aerodynamic aerosol-size spectrometry (Aerosizer) were employed to measure the total bacterial cell concentrations in the impinger collection fluid and the air, respectively. These data were compared with plate counts on selective (MacConkey agar) and nonselective (Trypticase soy agar) media. The percentage of viable bacteria, determined as a ratio of BacLight live to total counts was only 20% after 60 minutes of sampling. High counts on Trypticase soy agar indicated that most of the injured cells could recover. On the other hand, the counts from the MacConkey agar were very low, indicating that most of the cells were structurally damaged in the impinger. The percentage of injured bacteria obtained by the traditional plate count showed good agreement with the data on percentage of nonviable bacteria obtained by the BacLight method.

Total microbial count

Direct microscopic counting using fluorescent staining of cells may be used to enumerate the fungal spore content of samples. Samples are collected onto glass slides and a count made of the number of organisms per unit volume air collected. This is a relatively time consuming technique but it provides much more information about total microbial concentration than counting colony forming units as these may only account for a small proportion of the cells present.

The CAMNEA method described by Palmgren et al (1986) determines the total number of airborne micro-organisms collected on Nuclepore filters by acridine orange staining and epifluorescence microscopy. A high correlation between viable and total counts was noted in environments where the airborne flora was dominated by fungal spores, while a low correlation was found for airborne bacteria.

Scanning electron microscopy offers much higher resolution than light microscopy but has not been widely used in the analysis of bioaerosol samples (Eduard & Heederik, 1998).
Eduard and Heederik (1998) concluded that although microorganisms are most readily identified using culture-based methods as nonviable microorganisms and microbial constituents and products also may cause health effects; nonculture-based methods probably provide results that are more relevant to health. The disintegration of nonviable components of bioaerosol in air prior to sampling, during sampling and/or transport prior to analysis means that the relationship between viable and nonviable components of bioaerosol is complex and the apparent relationship in simultaneously collected samples is likely to be subject to artefacts caused by the sampling and analytical protocols.

**Flow cytometry**

Flow cytometry is a method for counting cells suspended in a liquid medium that uses light scattering to determine the numbers of particles in a given size range. It has not been widely used in the analysis of environmental bioaerosol samples. Chen and Li (2005) evaluated flow cytometry combined with a fluorescent technique (FCM/FL) as a technique to quickly and accurately determine and quantify the total concentration and viability of bioaerosols. They determined the optimal conditions of five fluorescent dyes (acridine orange, SYTO-13, propidium iodide, YOPRO-1, and 5-cyano-2,3-ditolyltetrazolium chloride) used in FCM/FL for laboratory samples of bacterial aerosols (Escherichia coli, and endospores of Bacillus subtilis) and fungal aerosols (Candida famata and Penicillium citrinum spores). Based on the measured cell concentration, fluorescence intensity, and staining efficiency as indicators for dye performance evaluation, SYTO-13 was found to be the most suitable fluorescent dye for determining the total concentration of the bioaerosols, whereas YOPRO-1 was the most suitable for determining viability. The technique was further validated using air and water samples from the aeration tank of hospital wastewater treatment plant.

**Molecular based methods of analysis**

**Gas chromatography-Mass spectrometry (GC-MS):** Several investigators have investigated the use of GC-MS to characterise markers of fungal contamination in air, although this approach has yet to be adopted as a technique for the routine monitoring of bioaerosol exposure. Szponar and Larsson (2001) investigated the use of mass spectrometry in chemical marker analysis for characterising microbial communities in organic dust samples collected in a home and a swine confinement building, respectively. The chemical markers studied included 3-hydroxy fatty acids (markers of endotoxin), ergosterol (marker of fungal biomass), and muramic acid (marker of peptidoglycan/bacterial biomass). Samples were hydrolysed and subjected to various chemical manipulations for rendering the markers suitable for gas chromatography-tandem mass spectrometry analysis. GC-MS may offer a faster method of sample analysis than traditional microscopy methods for the future evaluation of bioaerosol exposure but there is currently insufficient information to fully understand the relationships between the chemical parameters measured by CG-MS and the results of traditional evaluation by microscopy. There has been little epidemiological investigation of the potential links between bioaerosol components as determined by GC-MS and health effects.

**Polymerase chain reaction (PCR):** A number of investigators have investigated the use of quantitative PCR to determine the presence and quantity of specific target DNA sequences that can be linked to individual fungal or bacterial species. Zhou et al (2000) describe the development of a fungus-specific PCR assay for detecting indoor fungi. Wu et al (2002a) undertook further method development for thirty-six fungal strains, representing 26 species from 14 genera of commonly occurring fungi, and 16 different bacterial strains, representing both gram-negative and gram-positive species. Wu et al (2002b) used specific PCR amplification and probe hybridization techniques to examine the compositions of airborne fungi in samples from three different environments in comparison to traditional microscopy and colony counting methods. The detection sensitivity for PCR amplifications was 9 to 73 spores and 1.3 to 19.3 cfus per PCR reaction. The hybridization detection limit was 2 to 4 spores and 0.2 to 1.2 cfu. The hybridization method was more sensitive than PCR amplification and showed less variation among samples. Using specific PCR primers and probes, the presence of several fungal groups and species was determined in air samples. In the future this approach may provide a rapid method of obtaining detailed information about...
bioaerosol composition and concentration. At present there is little available information to link the results of PCR with health effects as the technique has not been widely used in epidemiological investigations.

Characterisation of volatile organic compounds (VOCs) emitted by fungal colonies: VOC emissions from fungi may provide a tool for monitoring the potential for fungal emissions. Mayrhofer et al (2006) investigated the relationship between microbial species in biowastes and composition of emitted VOCs. The succession of microbial communities during 16 days of storage in organic waste collection bins was studied by denaturing gradient gel electrophoresis (DGGE) of amplified 16S ribosomal DNA in parallel with a classical cultivation and isolation approach. Approximately 60 different bacterial species and 20 different fungal species were isolated. Additionally, some bacterial species were identified through sequencing of excised DGGE bands. Proton transfer reaction mass spectrometry (PTR-MS) was used to detect VOCs over the sampling periods, and co-inertia analyses of VOC concentrations with DGGE band intensities were conducted. Positive correlations, indicating production of the respective VOC or enhancement of microbial growth, and negative correlations, indicating the use of, or microbial inhibition by the respective compound, were found for the different VOCs. Measurement of the VOC emission pattern from a pure culture of Lactococcus lactis confirmed the positive correlations for the protonated masses 89 (tentatively identified as butyric acid), 63 (tentatively identified as dimethylsulfide), 69 (likely isoprene) and 73 (likely butanone).

A1.1.5 Sources of uncertainty

Key quality issues arising during the collection and analysis of the viable component of bioaerosols include the need for sterility of sampler, the avoidance of damage to micro-organisms during the collection process and the preservation of viability during the remainder of the sampled period and subsequently until culture and analysis. The use of counting to enumerate microbes introduces an inherent uncertainty as organisms will be spread randomly across a slide and the random choice of fields for counting means that the number of organisms present in a different set of selected fields would probably be different. For a minimum colony count of 30, the coefficient of variation might be greater than 18% (Eduard & Heederik, 1998). The reported coefficient of variation in laboratory and field studies has varied from 9 to 52% (Eduard & Heederik, 1998).

Chang et al (1994) investigated the potential for colony counting error to arise due to indistinguishable colony overlap (i.e. masking) using a computer simulation and experimentally through the collection of aerosolized Bacillus subtilis spores and examining micro- and macroscopic colonies. Colony counting efficiency decreased (i) with increasing density of collected cultivable microorganisms, (ii) with increasing colony size, and (iii) with decreasing ability of an observation system to distinguish adjacent colonies as separate units. Counting efficiency for 2-mm colonies, at optimal resolution, decreased from 98 to 85% when colony density increased from 1 to 10 micro-organisms cm$^{-2}$, in contrast to an efficiency decrease from 90 to 45% for 5-mm colonies. No statistically significant difference between experimental and theoretical results was found when colony shape was used to estimate the number of individual colonies in a cfu. Experimental colony counts were 1.2 times simulation estimates when colony shape was not considered, because of nonuniformity of actual colony size and the better discrimination ability of the human eye relative to the model. Colony surface densities associated with high counting accuracy were compared with recommended upper plate count limits and found to depend on colony size and an observation system's ability to identify overlapped colonies. Correction factors were developed to estimate the actual number of collected micro-organisms from observed colony counts. In a follow up study, Chang et al (1995) investigated the effects of spore density, concentration of nutrients in the culture medium, sample incubation time, and ability of an observation system to distinguish overlapped colonies on colony masking.
A1.2  ENDOTOXIN

A1.2.1 Sampling

Samples may be collected onto filter or into a liquid medium. Most commonly, sampling for endotoxin has been combined with sampling for viable fungi or dust with samples being split for analysis (fungi) or analysed for endotoxin subsequent to the gravimetric determination of the mass of dust collected. Sampling for endotoxin can be undertaken following methods based on standard methods for the monitoring of exposure to respirable and inhalable dust in the workplace such as MDHS 14/3 (Health and Safety Executive, 2000). It is essential that samplers are sterile prior to sampling and that samplers and filters are kept sterile during and subsequent to sampling. Endotoxin concentrations are typically measured in terms of biological activity rather than as a mass concentration of LPS.

The sample collection method may have an impact on apparent endotoxin concentrations. The apparent quantity of endotoxin may increase if the collection method breaks up bacteria to release endotoxin that would have otherwise remained invisible during analysis. The sampling media is also important as the filter type used will influence the ease of recovery of endotoxin from the filter for analysis. Endotoxin may bind more strongly to some filter types than others. Several studies have investigated the impact of sampling method on the apparent concentration of endotoxin. Stephenson et al (2004) compared side-by-side endotoxin sampling using a liquid impinger, a glass fibre filter, and a polycarbonate filter in a wastewater treatment plant. The levels of detected endotoxin appeared to be highest with the impinger whereas the results for the glass fibre filter showed the least variability when sampling was conducted at the highest endotoxin levels. There was an apparent correlation between endotoxin levels obtained with the impinger and the glass fibre filter. Duchaine et al (2001) compared concurrent, triplicate, side-by-side endotoxin measurements using air sampling filters and bioaerosol impingers. In sawmills, impinger sampling yielded significantly higher endotoxin concentration measurements and lower variances than filter sampling with IOM inhalable dust samplers. In swine barns endotoxin concentrations were 10-fold higher on average than in sawmills and comparable endotoxin concentration estimates were derived using impinger and filter methods although the variability was lower using the impinger method. In both occupational settings, side-by-side replicates were more uniform for the impinger samples than for the filter samples.

In conclusion a variety of approaches have been used in the collection of bioaerosols and survey results acquired using one type of sampler are unlikely to be directly comparable to those acquired using a different type of collection device. Key issues include sampling efficiency for different size categories and the preservation of micro-organism viability during particle capture and subsequently within or on the sampling media.

A1.2.2 Storage

Conditions during the transport and storage of samples can have a profound effect on apparent endotoxin concentrations. There is potential both for bacterial growth under damp conditions or desiccation under dry conditions. Either eventuality could give rise to an erroneously high measurement of endotoxin. Poor sample storage can also lead to a loss of apparent activity. Douwes et al (1995) reported that repeated freeze (-20 °C)-and-thaw cycles with commercial LPS reconstituted in pyrogen-free water had a dramatic effect on the detectable endotoxin level. A 25% loss in endotoxin activity per freeze-thaw cycle was observed whereas storage of LPS samples for a period of 1 year at 7 °C had no effect on the endotoxin level. House dust extracts showed a decrease of about 20% in the endotoxin level after they had been frozen and thawed for a second time. The use of different container materials (borosilicate glass, “soft” glass, and polypropylene) had no apparent effect on measured endotoxin levels.

A1.2.3 Analysis

Endotoxin is most commonly measured using a chromogenic Limulus amebocyte lysate (LAL) assay based on blood derived from the horseshoe crab. There are several commercial
suppliers of kits for the analysis and there is considerable variation in the reliability and sensitivity of kits originating from different suppliers (IOM experience). Two different approaches have been used with the LAL assay. The reaction can either be allowed to progress for a fixed time period and the concentration of endotoxin assessed from the colour intensity at the end of that period (endpoint assay) or the concentration can be assessed from the rate of change of colour during the reaction (kinetic assay). The British Standard (BSI, 2001) stipulates use of the kinetic method, but many published studies have used the endpoint method. Endotoxin is generally quantified in terms of Endotoxin Units (EU) with 1 ng of endotoxin being approximately equivalent to 10 EU, although as endotoxin is not a uniform substance, there is no exact equivalence.

A smaller number of studies have used GC-MS to determine the LPS content of samples. Typical LPS contains 3-hydroxy fatty acids (3-OHFs) that can be used as chemical markers in GC-MS (Szponar & Larson, 2001).

There is considerable uncertainty as to the comparability of endotoxin measurements made in different studies. Inter-laboratory variability of up to four orders of magnitude has been reported for replicate samples (Jacobs & Chun, 2004).

The first stage of the analytical process involves eluting filters to recover endotoxin. Both the filter material and the eluting medium affect the efficiency with which endotoxin is recovered from filter. Thorne et al. (1997) found there was no significant difference in extraction efficiency for polycarbonate or glass filters using two aqueous filter extraction methods: 120 minutes extraction at 22 °C with vigorous shaking and 30 minutes extraction at 68 °C with gentle rocking. Using airborne dusts sampled in a potato processing plant, Douwes et al. (1995) established that the endotoxin extraction efficiency of 0.05% Tween 20 in pyrogen-free water was seven times higher than that of pyrogen-free water only. In addition twice as much endotoxin were extracted from glass fibre, Teflon, and polycarbonate filters than from cellulose ester filters. The temperature and shaking intensity during extraction were not related to the extraction efficiency. In a round robin study using cotton dust in which laboratories used their normal extraction procedure; a common extraction procedure; and a common extraction procedure and the same type and lot of a commercially available endotoxin kit, Jacobs and Chun (2004) demonstrated that both intra- and inter-laboratory variability is reduced by using a common extraction protocol and a common assay kit. They found, however, that significant differences remained between the laboratories.

A small number of studies have compared the endpoint and kinetic analytical methods. In samples collected from swine and poultry confinement buildings, Thorne et al. (1997) found that whereas both the endpoint and kinetic methods gave similar estimates for endotoxin activity from polycarbonate filters, glass fibre filters analyzed by the endpoint method yielded significantly higher endotoxin activity estimates, suggesting enhancement of the endpoint assay or inhibition of the kinetic assay with glass fibre filters. In a study performed in five laboratories, Reynolds et al. (2002) compared the kinetic and endpoint methods for the analysis of endotoxin in dusts collected from chicken barns, swine barns, and corn processing facilities. For chicken dust, labs using the endpoint method reported higher results than those using kinetic methods whereas for swine and corn dusts, labs using the kinetic method reported the highest endotoxin values.

Several studies have investigated the comparability of endotoxin measurements made using the LAL assay with those made using GC-MS to quantify LPS as 3-OHFs. Walters et al. (1994) compared endotoxin measurements made using the kinetic-LAL assay and using GC-MS to determine the 3-OHFA content of samples collected from air contaminated by recycled washwater at a fiberglass plant. Samples were collected on polycarbonate filters and extracted into a buffer solution or directly methanolysed. Samples were then lyophilised and methanolysed before analysis by GC-MS. Direct methanolysis of filter samples and methanolysis of buffer extracts of the filters yielded similar 3-OHFA contents. Analysis of buffer extracts for endotoxin content in the LAL assay and by GC-MS for 3-OHFA content produced similar results (P = 0.23); the average difference was 0.88%. In aerosols collected and generated from chicken and swine barns, and corn processing, Reynolds et al. (2005) compared the LPS content of lyophilized aliquots of filter extracts as determined using GC-
MS and 3-OHFA as a marker of LPS with endotoxin measurements made using the LAL assay. They found significant interlaboratory differences in the LAL assay and GC-MS (LPS) results between laboratories for all dust types with the patterns of differences varying by dust type. The relationship between the apparent concentrations of endotoxin measured in the LAL assay and LPS concentrations measured by GC/MS also depended on dust type. The percentages of individual 3-OHFA chain lengths varied across labs suggesting that each lab recovered a different fraction of the LPS available. The presence of large amounts of particle associated LPS and absence of a freezing thawing cycle during storage were associated with lower correlations between LPS and LAL, consistent with a lower LAL response to cell-bound endotoxin. The authors concluded that LAL methods may be most suitable when comparing exposures within similar environments whereas GC-MS offers additional information helpful in optimizing sample treatment and extraction and may be of use when comparing across heterogeneous environments.

A1.3 BETA (1→3) GLUCANS

A1.3.1 Sample collection and analysis

Beta (1→3) glucan has not been widely measured in studies of the waste industry. Samples can be collected onto filter using standard protocols for dust measurement. Due to the insolubility of beta (1→3) glucan, sodium hydroxide is used to extract samples. Several assays have been developed for the measurement of beta (1→3) glucan, in medicine because of its clinical importance in the detection and quantification of fungal infections:

- Glucan-specific limulus amebocyte lysate (LAL) assay;
- Inhibition ELISA; and
- Sandwich ELISA.

Douwes et al (1996) describe an Enzyme-Linked ImmunoSorbent Assay, or ELISA, developed for the quantification of beta (1→3)-glucans in dust samples from occupational and residential environments. Immunospecific rabbit antibodies were produced by immunization with bovine serum albumin-conjugated laminarin [beta (1→3) glucan] and affinity chromatography on epoxy-Sepharose-coupled beta (1→3) glucans. Milton et al (2001) describe a similar specific enzyme immunoassay developed to quantify (1→6) branched, (1→3)-beta-D-glucans in environmental samples. The assay was based on the use of a high-affinity receptor (galactosyl ceramide) specific for (1→3)-beta-D-glucans as a capture reagent and a monoclonal antibody specific for fungal cell wall beta-D-glucans as a detector reagent. The assay was highly specific for (1→6) branched, (1→3)-beta-D-glucans (such as that from Saccharomyces cerevisiae) and did not show any response at 200 ng/ml to curdlan, laminarin, pustulan, dextran, mannan, carboxymethyl cellulose, and endotoxins. The detection level was 0.8 ng/ml for baker’s yeast glucan and Betafectin.

Glucan-specific LAL is commercially available for use in the colorimetric determination of beta (1→3) glucan in dust samples. Foto et al (2004) report the development and application of a method for the analysis of the glucan in indoor air samples extracted in 0.5 N NaOH solution based on commercially available LAL. Beijer et al (2002) used a glucan specific LAL assay to determine the beta (1→3) glucan content of indoor air.

Although some method comparison has been undertaken for clinical samples, there is no information about measurement uncertainty in air samples and it is not possible to judge the comparability of measurements made by different groups.

A1.4 PEPTIDOGLYCAN

Peptidoglycan has not been widely measured in studies of the waste industry, although it is potentially a useful marker of bacterial mass (both viable and nonviable). Samples can be collected onto filter using standard protocols for dust measurement.

Muramic acid is a unique marker of peptidoglycan. At least two different methods have been
developed for the analysis of muramic acid in dust samples by GC-MS. Szponar and Larsson (2001) extracted samples using heated methanolic hydrochloric acid, the extract was dried, then acetylated, washed in dichloromethane and analysed by CG-MS. Mielniczuk et al (1992) hydrolysed samples in hydrochloric acid and then extracted with hexane to remove hydrophobic compounds. The aqueous phase was evaporated, heated in a silylation reagent to form trimethylsilyl derivatives that could be analysed by GC-MS.

A1.5 DATA HANDLING

Spicer and Gangloff (2000) undertook an evaluation of recommended methods for interpreting bioaerosol sampling data. Their simulations indicated that the nonparametric statistical treatment of bioaerosol data which has been recommended in the US for building assessment purposes has limitations. An inordinately high Type II error (failure to reject a null hypothesis which is actually not true) is especially apparent when there are small numbers of samples. For example, in applying this methodology to clearance air sampling, a work zone subjected to removal of all mouldy materials and a thorough particulate cleaning would still have a significant chance of failure solely due to the variability of the data, if individual samples are evaluated to identify “localised” contamination.

A1.6 BIOMARKERS OF EXPOSURE TO BIOAEROSOL

Biomonitoring has been widely used in the assessment of exposure to conventional pollutants in the workplace and wider environment eg lead in blood, mercury in hair or pesticide metabolites in urine. Biomonitoring may provide a more reliable guide to the exposures than an individual has actually experienced than environmental monitoring as the individual effectively acts as the sampler. There are, however, uncertainties about the importance of interindividual differences in uptake, absorption and metabolic response that can make it difficult to relate measurements of biomarkers to pollutant intake and environmental concentrations. Biomonitoring has been widely used in workplace exposure monitoring for a wide range of substances including measurements required by health and safety regulations such as blood lead concentrations in lead exposed workers. Biomonitoring in humans has not been used as a tool in environmental regulation.

Biomarkers have not been widely used in the assessment of exposure to bioaerosols.

It is possible to use the immune response to common moulds, such as specific IgE, specific IgG or positive skin prick test as an indirect measure of exposure to these moulds. These responses vary between individuals, although group comparisons have shown that mould-exposed populations have greater levels of immune markers of mould exposure than unexposed populations. Bunger et al (2007) investigated levels of specific IgG antibodies to moulds and actinomycetes in workers as potential biomarkers of exposure, but these were not significantly different from those in control subjects. Both IgE and positive skin prick test appear to be more sensitive markers of exposure than IgG and are also more strongly related to the development of allergic symptoms (asthma and/or rhinitis). It is potentially possible to examine the immune response to a wide range of microbial species.

Other studies have measured cellular markers of inflammation (differential cell counts, inflammatory mediators, protein/enzyme markers of cellular damage) in nasal lavage fluids as markers of upper airways inflammation (eg Douwes et al, 2000; Heldal et al 2003a) or in sputum as markers of lower airways inflammation (eg Heldal et al, 2003b). These inflammatory markers are not, however, specific to bioaerosol exposure. Similarly measurable effects on respiratory health including changes in lung function parameters or airways responsiveness over a working shift, working week or longer period are not specific to bioaerosol exposure.

It is possible to measure endotoxin in blood but it is not known whether blood concentrations are linked to exposure to inhaled endotoxin. There are no readily available biomarkers of exposure to organic dust.
Overall, although biomarkers have been useful in demonstrating workplace or environmental exposure to more conventional pollutants, their application for assessing exposure to bioaerosol is likely to be limited because of variability of individual response biomarkers in human population.

### A1.7 STANDARDISATION OF METHODS

At present, the Environment Agency’s (EA) preferred method of monitoring bioaerosols around waste facilities is active impaction onto agar using Andersen or split samplers or liquid impingers. They indicate that analysis can be for viable micro-organisms based on cultivation of the sample and colony counting or counting of total micro-organisms using optical microscopy and staining (EA, 2004). A CEN (European Committee for Standardisation) working group on the “Measurement of bioaerosols in ambient air and emissions” (CEN/TC 264/WG 28) has recently been formed. At present the approach to the collection and analysis in participating member states is very different (Table A1.1).

**Table A1.1:** Summary of the approach taken to the collection and analysis of bioaerosol samples in Member States participating in CEN/TC 264/WG 28 (from minutes of first meeting)

<table>
<thead>
<tr>
<th>Country</th>
<th>Main experience</th>
<th>Sampling methods</th>
<th>Analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Composting facilities</td>
<td>Industry use Andersen samplers but some researchers favour collection on filters</td>
<td>Cultivation-based analysis</td>
</tr>
<tr>
<td>France</td>
<td>Composting facilities, cooling towers, indoor air</td>
<td>Impinger or cyclone methods for bacteria</td>
<td>Cultivation-based or indirect (PCR, FSH)</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Workplace and indoor</td>
<td>Moulds collected on filter; bacteria collected using impingers</td>
<td>Particular interest in glucans and endotoxins</td>
</tr>
<tr>
<td>Germany</td>
<td>Not stated</td>
<td>Moulds collected on filter; bacteria collected using impingers</td>
<td>Particular interest in glucans and endotoxins</td>
</tr>
</tbody>
</table>

The American Society for Testing and Materials (ASTM) method E884-82 (ASTM, 2006) is a standard developed in the US for the sampling of bacterial and fungal aerosols that is intended to be applicable to waste facilities and similar workplaces. The method recommends use of a multistage impactor (eg Andersen sampler) and all-glass liquid impingers with sampling times of 30 minutes and up to 1.5 hours, respectively. It is recommended that sampling is undertaken at least one site 300 m upwind and one site 100 m downwind of the site (three replicates upwind and five downwind).

The EA (2004) note that the predecessor to this method, ASTM E884-82 (2001), was not evaluated for suitability in England and Wales and that Andersen samplers may be inappropriate for large-diameter aerosols (eg fluffy cellulose-based particles). The sampling period may also be too long and lead to overloading of the collection media.

In the UK, the Composting Association (1999) published guidance for the monitoring of ambient air at composting facilities. This indicates that samples should be taken using two, single-stage Andersen samplers in parallel and recommends the use of selective media for viable micro-organisms. Sampling should be carried out 25 m upwind and up to 200 m downwind of the composting sites and at the nearest sensitive receptor (occupied building). Sample durations should be 20-30 minutes.
**APPENDIX 2**

**Background levels of exposure to bioaerosol**

**A2.1 ENDOTOXIN**

Table A2.1: Background concentrations of endotoxin in ambient and indoor air (Table continues overleaf)

<table>
<thead>
<tr>
<th>Location</th>
<th>Environment</th>
<th>Concentrations</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>Outdoor urban air Oldenburg, Saxony 2 East German cities</td>
<td>&lt;1.3 EU m⁻³</td>
<td>Sampling periods with higher average temperatures or reduced relative humidity were associated with higher levels of endotoxin in PM₂.₅. Endotoxin levels significantly higher during spring and summer than during winter.</td>
<td>Schulze et al (2006)</td>
</tr>
<tr>
<td>Germany</td>
<td>PM₂.₅ collected at forty outdoor monitoring sites across Munich</td>
<td>geometric mean concentration in PM₂.₅ was 1.07 EU mg⁻¹ (0.015 EU m⁻³ of sampled air)</td>
<td></td>
<td>Heinrich et al (2003).</td>
</tr>
<tr>
<td>Denmark</td>
<td>Outdoor air in towns</td>
<td>Median concentration 0.33 EU m⁻³</td>
<td></td>
<td>Madsen (2006).</td>
</tr>
<tr>
<td>US</td>
<td>Massachusetts Suburban air Urban air</td>
<td>0.39 EU m⁻³ Annual (geometric) mean</td>
<td></td>
<td>Park et al (2000).</td>
</tr>
<tr>
<td>US</td>
<td>Intake to office air in St. Louis</td>
<td>Geometric mean: 1.2 EU m⁻³ (range 0.7-2.1 EU m⁻³)</td>
<td></td>
<td>Heines et al (2000).</td>
</tr>
<tr>
<td>US</td>
<td>Outdoor air in 13 communities in Southern California</td>
<td>Geometric mean concentration of PM₁₀ of 34.6 ug m⁻³ (range, 3.0-135) and of endotoxin of 0.44 EU m⁻³ (range, 0.03-5.44). The geometric mean concentration of endotoxin in PM₁₀ was 13.6 EU mg⁻¹ (range, 0.7-96.8)</td>
<td>12 months study; highest concentrations of endotoxin in PM₁₀ found at sites downwind of Los Angeles, California, which were also the locations of highest PM₁₀. PM₁₀ and endotoxin concentrations were significantly correlated, but no correlation was found between endotoxin concentrations and other ambient pollutants (ozone, nitrogen dioxide, total acids, or PM₂.₅).</td>
<td>Mueller-Anneling et al (2004)</td>
</tr>
<tr>
<td>UK</td>
<td>Background</td>
<td>typically &lt;0.5 EU m⁻³</td>
<td>Background measured for various commercial contracts</td>
<td>IOM unpublished data</td>
</tr>
<tr>
<td>Germany</td>
<td>Industrial areas Congested streets</td>
<td>median 1.3 EU m⁻³</td>
<td></td>
<td>Madsen (2006)</td>
</tr>
<tr>
<td>Location</td>
<td>Environment</td>
<td>Concentrations</td>
<td>Comments</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Germany</td>
<td>Outdoor air in rural area</td>
<td>Winter mean: 3.6 EU m⁻³ (range &lt;1.3-20.0 EU m⁻³)</td>
<td></td>
<td>Schultze et al (2006)</td>
</tr>
<tr>
<td>Germany</td>
<td>Summer mean</td>
<td>Winter mean: 4.4 EU m⁻³ (range &lt;1.3-23.2 EU m⁻³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germany</td>
<td>Agricultural field</td>
<td>Median concentration: 2.9 EU m⁻³</td>
<td></td>
<td>Review article</td>
</tr>
<tr>
<td></td>
<td>Outdoor air downwind of livestock</td>
<td>60 ngm⁻³ (approximately 600 EU m⁻³)</td>
<td>Dust endotoxin levels not significantly associated with airborne endotoxin.</td>
<td>Hartung et al (1997)</td>
</tr>
<tr>
<td></td>
<td>buildings 50 m</td>
<td>15 ngm⁻³ (approximately 150 EU m⁻³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>15 Massachusetts homes – bedroom air</td>
<td>Maximum concentration: &lt;1 EU m⁻³</td>
<td></td>
<td>Park et al (2000)</td>
</tr>
<tr>
<td></td>
<td>median 20 EU m⁻³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poland</td>
<td>Indoor air in homes</td>
<td>typically &lt; ng m⁻³ (&lt;10 EU m⁻³)</td>
<td></td>
<td>Gorny &amp; Dutkiewcz (2002)</td>
</tr>
<tr>
<td>Canada</td>
<td>Indoor air in 20 homes</td>
<td>median 0.09 ngm⁻³ (approximately 0.1 EU m⁻³)</td>
<td></td>
<td>Miller et al (2007)</td>
</tr>
<tr>
<td></td>
<td>Living rooms</td>
<td>range 0.02-0.3 ngm⁻³ (approximately 0.02-0.3 EU m⁻³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Children’s rooms</td>
<td>mean 0.45 ngm⁻³ (approximately 4.5 EU m⁻³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>range 0.06-2.9 ngm⁻³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>Rooms of smoking students</td>
<td>12.1 pmol of LPS m⁻³ (&gt;1000 EU m⁻³)</td>
<td>Difficult to convert pmol LPS to EU – may have underestimated molecular weight of LPS</td>
<td>Larsson et al (2004)</td>
</tr>
<tr>
<td></td>
<td>Rooms of non-smoking students</td>
<td>0.1 LPS m⁻³ (about 10 EU m⁻³)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## A2.2 FUNGI

### Table A2.2: Background concentrations of fungi in ambient and indoor air (Table continues overleaf)

<table>
<thead>
<tr>
<th>Location</th>
<th>Environment</th>
<th>Concentrations</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>Urban air (outdoors)</td>
<td>mean concentrations of mesophilic fungi of 273 cfu m$^{-3}$ (range 0-7200) and mean concentrations of thermophilic fungi of 2.1 cfu m$^{-3}$ (range 0-193)</td>
<td></td>
<td>Jones and Cookson (1983)</td>
</tr>
<tr>
<td>UK</td>
<td>Urban air (outdoors)</td>
<td>concentrations of 1200 and 300 cfu m$^{-3}$ for mesophilic and thermophilic species respectively</td>
<td></td>
<td>Lacey and Crook (1988)</td>
</tr>
<tr>
<td>UK</td>
<td>Central London (outdoors)</td>
<td>total spore counts of 4425 spores m$^{-3}$ (daily mean concentration; range 39-16119)</td>
<td></td>
<td>Atkinson et al (2006)</td>
</tr>
<tr>
<td>UK</td>
<td>central Cardiff (outdoors)</td>
<td>total (viable and nonviable) fungal spores range from 100 spores m$^{-3}$ in the winter to 3500 to 4000 spores m$^{-3}$ during the summer.</td>
<td></td>
<td>Mullins (2001)</td>
</tr>
<tr>
<td>UK</td>
<td>Derby air (outdoors)</td>
<td>mean concentrations of total spores in were 10101 with a 90$^{th}$ percentile of 23790</td>
<td></td>
<td>Newsona et al (2000)</td>
</tr>
<tr>
<td>4 sites in England</td>
<td>see table below</td>
<td></td>
<td></td>
<td>DSTL</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Outdoor air in a suburban area</td>
<td>11,464 (range 5767-22,788) cfu m$^{-3}$ in winter and 4689 (range 1895-11,603) cfu m$^{-3}$ in summer</td>
<td>In summer the total fungal concentration, both indoors and outdoors of suburban homes, were significantly higher than those of urban homes. It was noted that the concentrations reported were substantially higher than those that had been reported elsewhere in the world.</td>
<td>Pei-Chih et al (2000)</td>
</tr>
<tr>
<td>US</td>
<td>outdoor concentrations of fungi in metropolitan Atlanta, Georgia in the vicinity of 50 detached houses</td>
<td>Median - 86 cfu m$^{-3}$ in the winter and 439 in the summer</td>
<td></td>
<td>(Horner et al, 2004).</td>
</tr>
<tr>
<td>Lithuania</td>
<td>Outdoor air; 20 sampling sites near busy streets</td>
<td>Lowest concentrations being measured in the winter (mean 379 cfu m$^{-3}$; range 178-522) and the highest concentrations were measured in the summer (mean 3625 cfu m$^{-3}$; range 800-6400 cfu m$^{-3}$).</td>
<td>Micromycetes of 430 species belonging to 165 genera, 19 families, 13 orders, 4 classes, and 3 phyla were isolated and identified including pathogenic species such as <em>Aspergillus niger</em>, <em>A. fumigatus</em>, <em>Cladosporium herbarum</em>, <em>Alternaria alternata</em>, and <em>Aureobasidium pullulans</em>.</td>
<td>Lugauskas et al (2003)</td>
</tr>
<tr>
<td>Location</td>
<td>Environment</td>
<td>Concentrations</td>
<td>Comments</td>
<td>Reference</td>
</tr>
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<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>US</td>
<td>2407 outdoor air samples from 1,717 buildings located across the United States</td>
<td>Mean concentrations - 930 cfum(^{-3}) (range 1-(+)8200).</td>
<td></td>
<td>Shelton et al (2002)</td>
</tr>
<tr>
<td>Poland</td>
<td>Outdoor air: City streets</td>
<td>(10^7) fungal spores m(^{-3}) of air</td>
<td>Maximum levels arose during the autumn; little difference between concentrations found in urban or rural locations.</td>
<td>Krajewski et al (2001a)</td>
</tr>
<tr>
<td>UK</td>
<td>Indoor air in 24 homes</td>
<td>Mean fungal concentrations of 1096 cfum(^{-3}) (range 28 (-)35000)</td>
<td></td>
<td>Hunter and Lea (1994)</td>
</tr>
<tr>
<td>US</td>
<td>9,619 indoor air samples from 1,717 buildings located across the US</td>
<td>Mean concentrations - 300 cfum(^{-3}) (range 1-(+)10000); Concentrations in indoor air were lower than those in outdoor air. The fungal levels were highest in the fall and summer and lowest in the winter and spring. Geographically, the highest fungal levels were found in the Southwest, Far West, and Southeast.</td>
<td>The most common culturable airborne fungi, both indoors and outdoors and in all seasons and regions, were Cladosporium, Penicillium, nonsporulating fungi, and Aspergillus. Stachybotrys chartarum was identified in the indoor air in 6% of the buildings studied and in the outdoor air of 1% of the buildings studied.</td>
<td>Shelton et al (2002)</td>
</tr>
<tr>
<td>Finland</td>
<td>Personal exposure and concentrations in the home and work environment</td>
<td>Geometric mean personal exposure concentrations were 3-12 cfum(^{-3}) for total viable fungi, 0.6-3.7 cfum(^{-3}) for Penicillium and mainly under 1 cfum(^{-3}) for other fungi with a total of 39 genera being identified in personal samples.</td>
<td>The variation in concentration of Penicillium explained 25-95% of the variations of total fungal concentration in personal exposure, home and workplace environments. Personal exposure was not necessarily related to the presence of a certain fungus in the home or work environment.</td>
<td>Toivola et al (2004)</td>
</tr>
<tr>
<td>US</td>
<td>Indoor air in 44 office buildings throughout the continental United States, as part of the Building Assessment, Survey and Evaluation (BASE) program.</td>
<td>Total fungal spore concentrations ranged from &lt; 24 to 1000 spores m(^{-3}) except for one building with natural ventilation where indoor levels were approximately 9000 spores m(^{-3}).</td>
<td>Indoor air concentrations of total spores did not vary significantly between winter and summer, between morning and afternoon monitoring periods, among climate zones or locations within a test area. Indoor-outdoor ratios of total spore concentrations typically ranged between 0.01 and 0.1 and were approximately seven times greater in winter than summer because of relatively low outdoor levels in the winter.</td>
<td>MacIntosh et al (2006)</td>
</tr>
<tr>
<td>Location</td>
<td>Environment</td>
<td>Concentrations</td>
<td>Comments</td>
<td>Reference</td>
</tr>
<tr>
<td>----------</td>
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<td>-----------</td>
</tr>
<tr>
<td>US</td>
<td>4 office buildings in Boston, Massachusetts, over a 12 month period</td>
<td>Median level of airborne fungi measured in was 21.5 cfum(^{-3}) (range 1.1-618 cfum(^{-3})).</td>
<td>Significant seasonal variation, concentrations, highest in summer and lowest in winter. Concentrations positively correlated with relative humidity and negatively related to carbon dioxide concentrations.</td>
<td>Chao et al (2002)</td>
</tr>
</tbody>
</table>
| Poland   | Homes without mould problems  
Home with mould problems  
Office air | 0-1997 cfum\(^{-3}\) (median 78 cfum\(^{-3}\))  
49-16,968 cfum\(^{-3}\) (median 239 cfum\(^{-3}\) and 504 cfum\(^{-3}\) in winter and summer respectively)  
18-1689 cfum\(^{-3}\) (median 53 cfu m\(^{-3}\) in winter and 136 cfum\(^{-3}\) in summer). | Filamentous fungi (Penicillium spp., Aspergillus spp.), and yeasts were the most commonly isolated species. | Gorny & Dutkiewicz (2002) |
| US       | six single-family homes located in the Cincinnati area | Indoor fungal and pollen counts were generally lower than outdoor concentrations. Only a small fraction of pollen appeared to penetrate from the outdoor to indoor environment whereas concentrations of airborne fungi were affected by the indoor sources and possibly a higher outdoor-to-indoor penetration of fungal spores compared to pollen grains. | | Lee et al (2006) |
| US       | 50 detached single-family homes in metropolitan Atlanta believed to be free of moisture problems or indoor fungal growth | Median winter concentrations in outdoor air, bedroom, family room and kitchen were 86, 71, 92 and 89 cfu m\(^{-3}\) respectively. The median summer concentrations were 439, 166, 189 and 166 cfu m\(^{-3}\) respectively. | In the analysis of dust for culturable fungal colonies, leaf surface fungi constituted a considerable portion (>20%) of the total colonies in at least 85% of the samples. | Horner et al (2004) |
Table A2.3: Fungal concentrations by species at 4 locations in England as reported by DSTL (Defence Science & Technology Laboratory)

<table>
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<tr>
<th>Species</th>
<th>Birmingham mean</th>
<th>Lichfield max</th>
<th>Lizard mean</th>
<th>Pershore max</th>
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### A2.3 BACTERIA

**Table A2.4:** Background concentrations of bacteria in ambient and indoor air (Table continues overleaf)

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<th>Concentrations</th>
<th>Comments</th>
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<td>UK</td>
<td>Suburban air</td>
<td>Mean bacterial concentrations in suburban air of 79 cfu m(^{-3}) (range 42-1600)</td>
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<td>Jones and Cookson (1983)</td>
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<td>UK</td>
<td>Urban air</td>
<td>500 cfu m(^{-3})</td>
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<td>Lacey and Crook (1988)</td>
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<td>Urban air</td>
<td>850 cfu m(^{-3}) (range 100-4000)</td>
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<td>Bovallius et al (1978)</td>
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<td>US</td>
<td>Mean outdoor concentration of culturable bacteria in the vicinity of 100 office buildings</td>
<td>194 cfu m(^{-3}) in the winter and 165 cfu m(^{-3}) in the summer.</td>
<td>The two dominant outdoor groups were “unknown bacteria” and Gram positive “rods”. Mesophilic bacteria (30°C) accounted for &gt;95% of culturable bacteria.</td>
<td>Tsai and Macher (2005)</td>
</tr>
<tr>
<td>US</td>
<td>Two US cities over 17 weeks</td>
<td>At least 1,800 types of bacteria were present in urban air including the consistent presence of bacterial families with pathogenic members.</td>
<td>Seasonal and meteorological influences were more important than location determining bacterial compositions.</td>
<td>Brodie et al (2007)</td>
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<tr>
<td>US</td>
<td>Indoor air: 100 large office buildings</td>
<td>Mean concentrations 116 cfum(^{-3}) (summer) 87 cfum(^{-3}) (winter); 90th percentile 175 cfum(^{-3}); 41% of samples below the 2- or 5-minute detection limits (18 or 7 cfum(^{-3}), respectively); Mesophilic bacteria (30 °C) accounted for &gt;95% of culturable bacteria which consisted primarily of unknown bacteria and Gram-positive cocci.;</td>
<td>Average concentrations were lower indoors than outdoors, except for Gram-positive cocci, which were significantly higher indoors (39 vs. 24 cfum(^{-3}))</td>
<td>Tsai and Macher (2005)</td>
</tr>
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<td>US</td>
<td>Indoor air in six family homes in Cincinnati</td>
<td>Mostly below the detection limit for culturable actinomycetes. Median indoor/outdoor ratio was 2.857.</td>
<td></td>
<td>Lee et al (2006)</td>
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<tr>
<td>US</td>
<td>Study of bioaerosol emission rates and plume characteristics of bioaerosols generated during land application of liquid Class B biosolids.</td>
<td>Neither coliphages nor coliform bacteria were detected in air downwind of spray application of liquid Class B biosolids.</td>
<td></td>
<td>Tanner et al (2005)</td>
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<td>Concentrations</td>
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<td>-------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------</td>
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<tr>
<td>Canada</td>
<td>Biosolids spreading</td>
<td>Initial results from field studies indicated that endotoxin, total coliforms,</td>
<td>LIDAR (Light Detection and Ranging) technology were used to monitor emissions and dispersion of total microorganisms, heterotrophic bacteria, fungi, and endotoxins</td>
<td>Water Environment Research Foundation (WERF) funded project (02-PUM-1), NRC (200)</td>
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<td>downwind of the source were greater than upwind concentrations; similarity</td>
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<td>between the biosolids and downwind aerosol population; no similarity</td>
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<td>between biosolids and upwind aerosol population.</td>
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<td>Germany</td>
<td>Outdoor air about 100 m from pig</td>
<td>17800 cfum⁻³ in winter and 930 cfm⁻³ in summer.</td>
<td>Airborne bacteria</td>
<td>Platz et al (1995)</td>
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<td>Poland</td>
<td>City streets</td>
<td>Concentrations of Actinomycetes were below detection limit (&lt;10⁶ cfu m⁻³)</td>
<td>Krajewski et al (2001a)</td>
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<td>Poland</td>
<td>Indoor air: City flats</td>
<td>&lt;100-1000 cfum⁻³</td>
<td>Mesophilic bacteria</td>
<td>Krajewski et al (2001a)</td>
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<td>Without mould problems</td>
<td>endospore-forming bacilli (<em>Bacillus</em> spp.). Gram-negative bacteria</td>
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<td>With mould problems</td>
<td>(<em>Pseudomonadaceae, Aeromonas</em> spp.) were the most commonly isolated species.</td>
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<td>Bioaerosol emissions associated</td>
<td>Effects on airborne bacterial concentrations at distances of up to 450-480m</td>
<td>Sciafe et al (2007) (review)</td>
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### A2.4 BETA (1→3) GLUCAN

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<td>Indoor air in 20 homes</td>
<td>median: 1.30 (range 0.5-2.8)</td>
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<td>Beijer et al (2002)</td>
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<td>high exposure – 17 homes</td>
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</table>
### A2.5 BACKGROUND BIOAEROSOL CONCENTRATIONS REPORTED IN STUDIES OF THE WASTE INDUSTRY

Table A2.6: Summary of background bioaerosol levels cited in studies of the waste industry. **MB**: Mesophilic bacteria, **TB**: Thermophilic bacteria, **GNB**: Gram negative bacteria, **MA**: Mesophilic actinomycetes, **TA**: Thermophilic actinomycetes, **TF**: Thermophilic fungi, **XF**: Xerophilic fungi, **LOD**: Limit of detection, **N.D.**: Not determined.

<table>
<thead>
<tr>
<th>Source, activity, job type or environment</th>
<th>Total Bacteria (cfu m(^{-3}))</th>
<th>Bacteria (cfu m(^{-3}))</th>
<th>Aspergillus fumigatus (cfu m(^{-3}))</th>
<th>Actinomyces (cfu m(^{-3}))</th>
<th>Fungal spores (cfu m(^{-3}))</th>
<th>Endotoxin (EU m(^{-3}) except where stated)</th>
<th>(\beta-(1\rightarrow3))-glucans (ng m(^{-3}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference levels</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>524</td>
<td>0.63</td>
<td></td>
<td>Neumann et al (2005)</td>
</tr>
<tr>
<td>Reference areas</td>
<td>&lt;10(^{-4})</td>
<td>&lt;10(^{-4})</td>
<td>&lt;10(^{-4})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bunger et al (2000)</td>
</tr>
<tr>
<td>Background: January February March</td>
<td>2400</td>
<td>7000</td>
<td>2500</td>
<td>7000</td>
<td>12000</td>
<td></td>
<td></td>
<td>Taha et al (2007)</td>
</tr>
<tr>
<td>Upwind (composting) Upwind (composting)</td>
<td>8.4x10(^{-3})</td>
<td>N.D.</td>
<td>1.9x10(^{-3})</td>
<td></td>
<td>1.25x10(^{-3})</td>
<td></td>
<td></td>
<td>Herr et al (2003a)</td>
</tr>
<tr>
<td>75 m upwind of composting</td>
<td>433</td>
<td>0</td>
<td>100</td>
<td></td>
<td>0.16 ng m(^{-3})</td>
<td></td>
<td></td>
<td>Studies reviewed by Prasad et al (2004)</td>
</tr>
<tr>
<td>Upwind of a recycling plant (range 3 sites): Summer Winter</td>
<td>250-5820</td>
<td>150-1220</td>
<td>GNB: 120 GNB: ND</td>
<td>120-3960</td>
<td>10-7870</td>
<td>0.14 ng m(^{-3}) (0.01-0.41)</td>
<td>0.13 ng m(^{-3}) (0.01-0.78)</td>
<td>Lavoie and Guertin (2001)</td>
</tr>
<tr>
<td>Off-site samples (composting)</td>
<td>2.08x10(^{-3}) (0.16-17.6 x10(^{-3}))</td>
<td>0 (0-1.19 x10(^{-3}))</td>
<td>17 (0.2-1.8)</td>
<td>1.5 ng m(^{-3}) (0.8-2.1)</td>
<td></td>
<td></td>
<td></td>
<td>Hryhorczuk et al (2001)</td>
</tr>
<tr>
<td>Background</td>
<td>110</td>
<td></td>
<td>17</td>
<td>0.8</td>
<td>0.5 ng m(^{-3})</td>
<td></td>
<td></td>
<td>Kiviranta et al (1999)</td>
</tr>
<tr>
<td>Background (City Streets)</td>
<td>MB: 1.1 x10(^{+3}) (0.1-2.3)</td>
<td>(0.13-6.7)</td>
<td>17 (12-22)</td>
<td>0.5 ng m(^{-3})</td>
<td></td>
<td></td>
<td></td>
<td>Krajewski et al (2002)</td>
</tr>
<tr>
<td>Background (Dwellings)</td>
<td>MB: 1.3 x10(^{+3}) (0.2-1.8)</td>
<td>(0.02 x10(^{3}))</td>
<td>1.5 ng m(^{-3}) (0.8-2.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2002)</td>
</tr>
</tbody>
</table>

**Footnotes:**
- **MB**: Mesophilic bacteria
- **TB**: Thermophilic bacteria
- **GNB**: Gram negative bacteria
- **MA**: Mesophilic actinomycetes
- **TA**: Thermophilic actinomycetes
- **TF**: Thermophilic fungi
- **XF**: Xerophilic fungi
- **LOD**: Limit of detection
- **N.D.**: Not determined
<table>
<thead>
<tr>
<th>Source, activity, job type or environment</th>
<th>Total Bacteria (cfu m(^{-3}))</th>
<th>Bacteria (cfu m(^{-3}))</th>
<th>Aspergillus fumigatus (cfu m(^{-3}))</th>
<th>Actinomycetes (cfu m(^{-3}))</th>
<th>Fungal spores (cfu m(^{-3}))</th>
<th>Endotoxin (EU m(^{-3}) except where stated)</th>
<th>(\beta-(1\rightarrow3))-glucans (ng m(^{-3}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background (urban)</td>
<td>(5.9 \times 10^7) ((4.8-16.1 \times 10^7))</td>
<td></td>
<td></td>
<td></td>
<td>(6.0 \times 10^7) ((4.8-11.7 \times 10^7))</td>
<td></td>
<td></td>
<td>Lavoie et al (2006)</td>
</tr>
<tr>
<td>Background (rural)</td>
<td>(5.9 \times 10^7) ((5.8-6.0 \times 10^7))</td>
<td></td>
<td></td>
<td></td>
<td>(5.85 \times 10^7) ((3.8-6.0 \times 10^7))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outside air in summer</td>
<td></td>
<td>GNB: 10</td>
<td>TA: 10</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
<td>From studies reviewed in Forcier (2002)</td>
</tr>
<tr>
<td>Ambient, UK Suburban urban/industrial in homes</td>
<td>79 ((42-1600)) 500</td>
<td></td>
<td></td>
<td></td>
<td>273 ((0-7200)) 1200 1096 ((28-35000))</td>
<td></td>
<td></td>
<td>From studies reviewed in Swan et al (2003)</td>
</tr>
<tr>
<td>Ambient, France Outdoor ambient, Paris</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2999-9841 92 ((3-675))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient, Netherlands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>941 0-15643</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient, Austria (rural)</td>
<td>327</td>
<td></td>
<td></td>
<td></td>
<td>185</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient, Scandinavia Urban Rural</td>
<td>850 ((100-4000)) 99 ((2-3400))</td>
<td></td>
<td></td>
<td></td>
<td>750</td>
<td></td>
<td>930 ((0-\rightarrow8200)) 700 600</td>
<td></td>
</tr>
<tr>
<td>Ambient, Finland</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>750</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient, US Urban</td>
<td>1500</td>
<td></td>
<td></td>
<td></td>
<td>930 ((0-\rightarrow8200)) 700 600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambient, US Rural</td>
<td>2000</td>
<td></td>
<td></td>
<td></td>
<td>930 ((0-\rightarrow8200)) 700 600</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX 3

Emissions of Bioaerosols from Waste & Processing Activities and associated exposures

A3.1 EMISSION CHARACTERISTICS & INFLUENCING FACTORS

Bioaerosols become airborne by processes that include forcible release, mechanical disruption and nebulisation. Bioaerosol emissions from waste are dependent on a wide range of factors that consequently affect the potential for exposure. Principally, these are i) waste composition, ii) conditions of storage, and iii) treatment processes. Associated with these factors are more specific attributes including: how the waste was generated and its physical form; the storage container type; the profile of microbiological species present; and physical conditions including temperature and pH.

Many of these attributes influence the microbial population of biowaste and how it changes through time as the waste progressively decomposes either during storage or during active treatment, for example, in composting. As a result, the profile of emissions may also vary as a function of the age of the waste, analogous to that observed with VOC emissions from landfill waste.

A3.2 WASTE PREPARATION STORAGE & COLLECTION

Storage of domestic waste prior to collection

The storage of biological wastes from domestic sources, prior to collection, is associated with the development of thermophilic and thermotolerant moulds, especially of mucoraceous species and aspergilli, including the human pathogen A. fumigatus. Several studies have investigated the factors that may affect bioaerosol emission from stored waste. In two series of experiments Reiss (1995) demonstrated that the following procedures can reduce the number of spores in the air in the bio-containers above the biological wastes: (1) wrapping the wastes in portions in newsprint reduces the number of colony-forming units by about 50-70%; (2) cleaning of the container after each emptying with diluted vinegar reduces the number of cfu by up to 80%; and (3) placing the container at shady sites so that the temperature of the air inside the bio-containers is approximately 5-8 °C lower than at sunny places also decreases the number of cfu in the air above biological wastes.

Heldal et al (2001) investigated the influence of different waste storage systems on the emission of bioaerosols and gases from compostable household waste (Table 5.1). Batches of waste were stored for 14 days in different storage systems: ventilated containers (compostainers) with or without added structure material and closed containers with or without an added preservative. Hydrogen sulphide and mercaptans developed in the closed container, and the concentration of ammonia increased continuously in both systems to 140 ppm. The microbial content for the incubated waste was higher for closed containers than for compostainers, and waste in closed containers generated a liquid rich in endotoxin and bacteria. The aerosols emitted from the waste consisted mainly of fungal spores, especially Aspergillus fumigatus, and no significant differences were observed between the systems although there was a high potential for endotoxin emissions from waste stored in closed containers. The use of a preservative prevented microbial growth and reduced the emission of bioaerosols and gases substantially.
Table A3.1: Reported bacterial, fungal and endotoxin contents of domestic waste (Heldal et al., 2001)

<table>
<thead>
<tr>
<th>Source</th>
<th>Total Bacteria</th>
<th>Bacteria</th>
<th>Gram-negative bacteria</th>
<th>Aspergillus fumigatus</th>
<th>Actinomycetes</th>
<th>Fungal spores</th>
<th>Endotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh waste</td>
<td>3x10⁶ counts g⁻¹</td>
<td>0.6x10⁶ cfu g⁻¹ (37ºC); 2x10⁵ cfu g⁻¹ (25ºC)</td>
<td>0.7x10⁶ cfu g⁻¹</td>
<td>&lt;0.2x10⁷ cfu g⁻¹</td>
<td>0.01x10⁶ cfu g⁻¹ (55ºC); 0.001x10⁶ cfu g⁻¹ (25ºC)</td>
<td>0.01x10⁶ cfu g⁻¹</td>
<td>0.01x10⁶ EU g⁻¹</td>
</tr>
<tr>
<td>Waste + preservative</td>
<td>920 x10⁶ counts g⁻¹</td>
<td>0.2 x10⁶ cfu g⁻¹ (37ºC); 0.1 x10⁵ cfu g⁻¹ (25ºC)</td>
<td>0.1 x10⁶ cfu g⁻¹</td>
<td>0.2 x10⁶ cfu g⁻¹</td>
<td>&lt;0.0002 x10⁶ cfu g⁻¹ (55 &amp; 25ºC)</td>
<td>0.001 x10⁶ cfu g⁻¹</td>
<td>0.01 x10⁶ EU g⁻¹</td>
</tr>
<tr>
<td>Compostainer: Waste only</td>
<td>102x10⁶ cfu g⁻¹</td>
<td>7100x10⁶ cfu g⁻¹ (37ºC); 2600 x10⁶ cfu g⁻¹ (25ºC)</td>
<td>0.2 x10⁶ cfu g⁻¹</td>
<td>&lt;1 x10⁶ cfu g⁻¹ (55ºC); 0.1 x10⁵ cfu g⁻¹ (25ºC)</td>
<td>1 x10⁶ cfu g⁻¹</td>
<td>3 x10⁶ cfu g⁻¹</td>
<td>3 x10⁶ EU g⁻¹</td>
</tr>
<tr>
<td>Waste + straw</td>
<td>148 x10⁶ cfu g⁻¹</td>
<td>9600x10⁶ cfu g⁻¹ (25ºC); 4100 x10⁶ cfu g⁻¹ (25ºC)</td>
<td>0.1 x10⁶ cfu g⁻¹</td>
<td>0.2 x10⁶ cfu g⁻¹</td>
<td>&lt;0.001 x10⁶ cfu g⁻¹ (55ºC); 0.1 x10⁵ cfu g⁻¹ (25ºC)</td>
<td>19 x10⁶ cfu g⁻¹</td>
<td>4 x10⁶ EU g⁻¹</td>
</tr>
<tr>
<td>Closed container: Waste only</td>
<td>130 x10⁶ counts g⁻¹</td>
<td>12000 x10⁶ cfu g⁻¹ (37ºC); 146000 x10⁶ cfu g⁻¹ (25ºC)</td>
<td>13000 x10⁶ cfu g⁻¹ (37ºC); 300 x10⁶ cfu g⁻¹ (25ºC)</td>
<td>1 x10⁶ cfu g⁻¹</td>
<td>&lt;1 x10⁶ cfu g⁻¹ (55ºC); 0.4 x10⁵ cfu g⁻¹ (25ºC)</td>
<td>10 x10⁶ cfu g⁻¹</td>
<td>4 x10⁶ EU g⁻¹</td>
</tr>
<tr>
<td>Percolate (microbial content per ml)</td>
<td>388 x10⁶ cfu g⁻¹</td>
<td>146000 x10⁶ cfu g⁻¹ (25ºC); 37900 x10⁶ cfu g⁻¹ (37ºC); 25500 x10⁶ cfu g⁻¹ (25ºC)</td>
<td>0.01 x10⁶ cfu g⁻¹</td>
<td>0.01 x10⁶ cfu g⁻¹</td>
<td>&lt;0.0001 x10⁶ cfu g⁻¹ (55 &amp; 25ºC)</td>
<td>4 x10⁶ cfu g⁻¹</td>
<td>4 x10⁶ EU g⁻¹</td>
</tr>
</tbody>
</table>
Wouters *et al* (2000) evaluated the association between indoor storage of organic waste and levels of microbial agents in house dust. The levels of bacterial endotoxins, mould, beta (1→3)-glucans, and fungal extracellular polysaccharides (EPS) of *Aspergillus* and *Penicillium* species were determined in house dust samples collected in 99 homes in the Netherlands. In homes in which separated organic waste was stored indoors for 1 week or more, the levels of endotoxin, EPS, and glucan were significantly higher on both living room and kitchen floors than the levels in homes in which only nonorganic residual waste was stored indoors (3.2-, 7.6-, and 4.6-fold, respectively). Significantly increased levels of endotoxin and EPS were observed, 2.6- and 2.1-fold respectively, when separated organic waste was stored indoors for 1 week or less, whereas storage of nonseparated waste indoors had no significant effect on microbial levels. The presence of textile floor covering was another significant determinant of microbial levels.

*Emissions during waste collection*

In a study published in German, Martens *et al* (1999) investigated the relationship between the types of waste being collected and the nature of bioaerosol emissions. The lowest emissions of airborne microorganisms were found during the collection of the paper waste fraction with higher emissions arising during the collection of unseparated household wastes, source separated bio-wastes and the remaining residual waste fraction. No significant differences were found in emissions arising during collection of these three waste types, although there was a tendency to higher values for the thermotolerant mould *A. fumigatus* during the collection of bio- and residual wastes which may have been related to longer collection intervals (bio- and residual wastes were partially collected every two weeks, unseparated wastes every week).

**A3.3 WASTE PROCESSING**

**A3.3.1 Overview**

The waste management process expected to have the greatest potential to generate bioaerosols is composting. Landfill activities may also be an important source of bioaerosols. Other waste management processes (including recycling, incineration, and anaerobic digestion) have the potential to produce bioaerosol emissions, but most studies have focussed principally on the more ‘traditional’ pollutants including metals, dioxins, NO\textsubscript{x}, SO\textsubscript{x}, acid gases, and VOCs (NSCA, 2002; HRB, 2003; Defra, 2004). The Defra review of the environmental and health effects of waste management considered composting, mechanical & biological treatment (MBT), anaerobic digestion, pyrolysis, incineration, and landfill. The only emissions considered that are relevant to bioaerosols were particulate matter and total VOCs. The report acknowledged that composting generated the highest levels of particulate matter emission, although no further speciation was conducted. The highest emissions of VOCs were reported from landfill, composting and MBT. Although emissions of microorganisms were thought to be significant, they were not considered because of the lack of exposure-response functions and limited availability of emissions data.

**A3.3.2 Composting**

In their review for HSE, Swan *et al* (2003) describe the four main approaches that have been used in commercial composting:

- Windrow in which feedstock is laid out in long rows and episodically turned to ensure that the material remains aerated;
- Aerated static pile in which air is blown or sucked through the composting materials;
- In-vessel systems including small scale containers for decentralized use, tunnels, agitated bays, rotating drums, silos or tower systems and enclosed halls; and
- Vermicomposting in which selected species of earthworm are used to help compost the wastes.
The composting process can be divided into three key stages (Gilbert et al., 2001):

- High rate composting when micro-organisms consume forms of carbon that can be readily broken down and a high rate of biological activity is associated with high oxygen demand and heat generation;
- Stabilisation when micro-organisms break down fairly readily forms of carbon such as cellulose, biological activity starts to decline and oxygen demand and temperature reduce; and
- Maturation when the compost is recolonised by soil microbes and lower rates of biological activity are associated with a low to medium oxygen demand and temperatures of less than 50°C.

A number of studies have investigated the microbial processes involved in composting and the change in the microbial communities present as the composting process proceeds. During the initial phase of high rate composting, mesophilic microbial species initially dominate but are succeeded by thermophilic species such as actinomycetes as the temperature increases. Thermotolerant fungal species such as Aspergillus fumigatus may also increase during this phases. During stabilisation thermophilic species dominate and pathogenic species should be largely destroyed (Swan et al., 2003). During the maturation stage the bacterial diversity increases.

Ryckeboer et al. (2003) monitored the taxonomic and functional sub-populations of micro-organisms during the composting of vegetable, fruit and garden waste in a composting bin system. All counts decreased during the thermophilic (high rate) phase of the composting, but increased again when the temperature declined (stabilisation). Total microbial activity, measured with an enzyme activity assay, decreased during the thermophilic phase, increased substantially thereafter, and decreased again during maturation. Bacteria dominated during the thermophilic phase while fungi, streptomycetes and yeasts were below the detection limit. Different bacterial populations were found in the thermophilic and mesophilic phases. In fresh wastes and during the peak-heating phase, all bacterial isolates were bacilli. During the cooling and maturation phase the bacterial diversity increased to include other Gram-positive and Gram-negative bacteria. Among the fungi, Aspergillus spp. and Mucor spp. were predominant after the thermophilic phase.

Hassan et al. (2001) investigated the prevailing physico-chemical conditions and microbial community during the composting of municipal solid waste in a semi-industrial pilot plant employing moderate aeration during the composting process. Auto-sterilization induced by relatively high temperatures (60-55°C) caused a significant change in bacterial communities; Escherichia coli and faecal Streptococci populations decreased, from 2 x 10^7 to 3.1 x 10^3 and 10^8 to 1.5 x 10^6 cells/g waste dry weight (WDW) respectively; yeasts and filamentous fungi decreased from 4.5 x 10^6 to 2.6 x 10^5 cells/g WDW and mesophilic bacteria were reduced from 5.8 x 10^9 to 1.8 x 10^7 bacteria/g WDW. Conversely, the number of bacterial spores increased at the beginning of the composting process, but after the third week their number decreased notably. Salmonella disappeared completely from compost by the 25th day as soon as the temperature reached 60 °C, and the bacterial population increased gradually during the cooling phase. While Staphylococci seemed to be the dominant bacteria during the mesophilic phase and at the beginning of the thermophilic phase, bacilli predominated during the remainder of the composting cycle. Compost sonication for about three minutes induced the inactivation of delicate bacteria, in particular gram-negatives. In contrast, gram-positive bacteria, especially micrococcus, spores of bacilli, and fungal propagules survived and reached high concentrations in the compost. Other studies have established that microbial biomass carbon and nitrogen dynamics during the composting of urban solid waste are consistent with a shift in the composition of the microbial populations from one dominated by bacteria and actinomycetes to one dominated by fungi (Ben Ayed et al., 2007).

Swan et al. (2003) provide a summary of the temperatures and times required to kill a range of human, animal and plant pathogens. The elimination temperatures for some pathogens are 100°C or higher (eg the causal agents for tetanus, anthrax and scapie). Most other common pathogens such as Salmonella species are eliminated at temperatures of 55 to 60 °C. Clearly
emissions at lower temperatures could include these species if there are sources of infection within the composting material such as waste food.

In a study published in German, Knop et al (1996a) investigated the effectiveness of composting for the removal of pathogens using *Salmonella* as an indicator species. *Salmonella* was detected in 36% of the investigated fresh compost samples. Every time *Salmonella* was detected in input material, the same serotypes were to be found in seepage water and survived there until the end of intensive rot. In laboratory experiments the maximum survival time of *Salmonella enteritidis* in seepage water at +5 °C was 42 days. Seepage water was observed to be a reservoir for the survival of *Salmonellae* and is likely to be a significant source of contamination.

A study undertaken by Bohnel and Lube (2000) highlighted the potential health risks associated with pathogenic anaerobic spore formers, such as *Clostridium botulinum* that may be present in compost. About 50% of the commercially available composts sampled contained *C. botulinum*. They suggested that the separate storage and collection of household biowaste may be one factor leading to the contamination of compost end-products.

Taha *et al* (2005) investigated the fugitive release of bioaerosols from static compost piles at a green waste composting facility in South East England. Wind tunnel experiments conducted on the surface of static windrows generated specific bioaerosol emission rates at ground level of between 13 and 22 x 10^3 cfum^{-2}s^{-1} for mesophilic actinomycetes and between 8 and 11 x 10^3 cfum^{-2}s^{-1} for *Aspergillus fumigatus*.

In a recent study undertaken for the EA by Crook *et al* (2006, draft), a range of microbial species was found in bioaerosol generated from composts (Table A3.2). The microbial population in samples changed during the composting process with most of the 4 sites showing a gradual increase in the numbers of thermophilic species followed by a subsequent decline during compost maturation. The dustiness of the compost (potential to emit dust) also tended to increase with time and was closely related to compost moisture content.

### Table A3.2: Predominant micro-organisms isolated in bioaerosol samples collected at 4 composting facilities in England

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhodococcus rhodochrous</em></td>
<td><em>Aspergillus fumigatus</em></td>
</tr>
<tr>
<td><em>Serratia rubidaea</em></td>
<td><em>Penicillium</em> spp.</td>
</tr>
<tr>
<td><em>Pseudoxanthomonas</em> sp.</td>
<td><em>Cladosporium cladosporioides</em></td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td><em>Basidiomycete</em> yeast sp.</td>
</tr>
<tr>
<td><em>Acinetobacter</em> sp.</td>
<td><em>Absidia corymbifera</em></td>
</tr>
<tr>
<td><em>Staphylococcus</em> sp.</td>
<td><em>Emericella nidulans</em></td>
</tr>
<tr>
<td><em>Streptomyces</em> sp.</td>
<td><em>Galactomyces geotrichum</em></td>
</tr>
<tr>
<td><em>Corynebacterium callunae</em></td>
<td><em>Paeciomyces</em> sp.</td>
</tr>
<tr>
<td><em>Arthrobacter</em> sp.</td>
<td><em>Talaromyces</em> sp.</td>
</tr>
<tr>
<td><em>Norcardiopsis</em> sp.</td>
<td><em>Thermobifida</em> fusca</td>
</tr>
</tbody>
</table>

Fischer *et al* (1999a and b) investigated the production of microbial volatile organic compounds (MVOC) by fungi derived from biowaste using synthetic agar (YES), compost-extract media (CEA VAR 1-3) and compost and the occurrence of VOC/MVOC in relation to the presence of airborne fungi in composting facilities. Thirteen airborne fungal species frequently isolated in composting plants were screened for microbial volatile organic compounds (MVOC). Various hydrocarbons of different chemical groups were identified by GC-MS including a large number of terpenes, compounds that are likely to influence compost odour perception (Muller *et al*, 2004a). Some compounds such as 3-methyl-1-butanol and 1-octen-3-ol were produced by a number of species, whereas some volatiles were specific for single species. In sampling at composting sites, a number of non-specific MVOC were found, some of which appeared to be correlated with a certain species composition or with microbial activity. The correlations between single volatiles and certain fungi found *in situ* did not,
however, match with the species-specific volatiles obtained from pure cultures. Muller et al (2004a) investigated the impact of process engineering on the dispersal of odorous compounds at three different waste composting facilities. MVOC emission depended on the degree of biodegradation with terpenes like alpha-pinene, camphene and camphor forming an important component of the emissions.

A3.3.3 Other waste management processes

There has been little investigation of bioaerosol emissions from landfill with most studies being focussed on pollutants such as metals, dioxins, NOx, SOx, acid gases, VOCs and particulates (NSCA, 2002; HRB, 2003; Defra, 2004). This is also reflected in the Health Protection Agency’s Position Statement on Municipal Solid Waste Incineration (HPA, 2005).

An experimental study aimed at characterising the microbiology of bioaerosols and leachate resulting from a sanitary landfill for solid urban waste situated near Rome was undertaken by Borrello et al (1999). Bioaerosol sampling was performed by using the active sampling method referred to as surface air system and the micro-organisms (bacteria and fungi) believed to be of relevance on bioaerosol and leachate with a view to hygienic risks, were investigated. However, the findings are not described in the PubMed abstract of the Italian article.

A3.4 MEASURES USED TO CONTROL BIOAEROSOL EMISSIONS FROM COMPOSTING

The Composting Association (2004) factsheet on bioaerosols suggests the following measures may be used to reduce bioaerosol emissions:

- Increased moisture control of feedstocks;
- Isolation of the screening operation from the composting operation;
- Adjustment of the airflow to reduce drying prior to screening operations;
- Use of both dust control and collection systems in dry climates;
- Use of sweepers and water vehicles to control dust in roadways;
- Placement of a dust hood and a bag-house dust collection system over the screen;
- Placement of water mist system over the screen conveyers to dampen the dust; and
- Use of advanced process technologies such as “in-vessel” systems.

In a study conducted for the Environment Agency, Wheeler et al (2001) recommended two measures to minimise bioaerosol emissions from open wind row composting operations:

- Ensure windrows are kept in an aerobic condition; and
- Only accept feedstocks that are not in an advanced stage of decomposition.

In the summer, anaerobic decomposition of containerised green waste during storage over the weekend prior to a Monday morning delivery poses a significant problem for some sites. Wheeler et al note the potential of in-vessel composting systems to capture and control odours, provided that buildings remain closed but fugitive emissions can cause significant nuisance. These can be minimised by the use of negative air pressure, rapidly closing vehicle entrances and self closing personnel doors. Biofilters were identified as a cost-effective method for minimising odour emissions. Another approach to managing odour emissions is to spray odour-absorbing compounds around the perimeter of the site but the effectiveness of this measure has yet to be fully assessed (Wheeler et al, 2001).

In a study of seven commercial composting plants with different operating conditions and biofilter designs, Sanchez-Monederro et al (2003) evaluated the effectiveness of biofiltration as a method for the control of releases of airborne micro-organisms. In all plants, the biofilters were originally designed for odour control. They found that biofiltration achieved an average
reduction of more than 90% and 39% in the concentrations of *Aspergillus fumigatus* and mesophilic bacteria, respectively. In all the plants, the airborne *A. fumigatus* concentration after the biofilter was lower than 1.2 x 10^3 cfum^-3 independent of the inlet concentration, whereas the mesophilic bacteria concentration was dependent on the inlet concentration. The fungus, whose spores had a maximum of diameter size distribution between 2.1 and 3.3 micrometres, appeared to be more effectively captured in the biofilter than the bacteria, which had diameters mainly between 1.1 and 2.1 micrometres.

Schelegelmich *et al* (2005) investigated the reduction of airborne microbes in the waste gas of biowaste composting processes using bioscrubber/biofilter combinations ranging from laboratory-scale to technical-scale. Although these biological systems were primarily designed for odour control, they also reduced bioaerosol emissions, although a reduction to background levels was not achieved. The bioscrubber, if equipped with a droplet separator, played a major role in emissions reduction, whereas the biofilter acted as a source for microbial emissions originating from the filter material.

Several studies have investigated how process conditions can reduce the potential harmfulness of bioaerosol emissions. Bagge *et al* (2005) undertook a study in full-scale, commercial biogas plants (BGP), processing low-risk animal waste with a separate pre-pasteurisation at 70°C for 60 minutes as required by EEC regulation 1774/2002. They sampled four BGPs on six occasions during 1 year. Samples were analysed for indicator bacteria (*Escherichia coli, Enterococcus* spp. and coliforms), spore-forming bacteria (*Clostridium* spp. and *Bacillus* spp.) and bacterial pathogens (*salmonella, listeria, campylobacter* and VTEC O157). *Salmonella* was the most frequently isolated pathogen before pasteurisation In general, the treatment adequately reduced both indicator and pathogenic bacteria but spore-forming bacteria were not reduced. Recontamination and regrowth of bacteria in biowaste was frequently noted after pasteurisation and digestion.

Suarez-Estrella *et al* (2003) investigated the effect of high temperatures generated during composting process, on the phytopathogen fungus *Fusarium oxysporum* f.sp. *melonis*. Vegetable residues infected with *F. oxysporum* f.sp. *melonis* were included in compost windrows subjected to different treatments. After 2-3 days of composting, there was complete elimination of fungi. The results of experiments at temperatures of 45, 55 and 65 °C confirmed the role of temperature in eliminating fungal persistence. Treatment at 65 °C was especially effective, whereas 45 °C eliminated fungal persistence only after 10 days.

Vinneras *et al* (2006) evaluated two alternative methods for removing the infection risk associated with organic fertilisers derived from faeces and manure: thermophilic composting and ammonia-based treatment. Thermal composting of faecal matter and food waste resulted in a treatment temperature of over 65°C in a 90L reactor. By using insulation and turning the compost three times during the high temperature period, it was possible reduce pathogens by a factor of 10^5. Small scale composting of the same material indicated less efficient reduction of faecal bacteria at temperatures around 50 °C. The use of ammonia to increase the pH to above 9 within an hour was effective in reducing indicator organisms for bacteria (*Salmonella* spp. and faecal coliforms, *Enterococcus* spp.). In an earlier study, Vinneras *et al* (2003) showed that the best mixture for dry thermal composting was a mix of faeces, food waste and amendment. The urine was collected separately by use of urine-diverting toilets. The results of a pilot-scale experiment indicated that composting could be effective for disinfection of faecal matter, if the reactor is sufficiently well insulated for a high temperature to develop throughout the material.
APPENDIX 4

Exposure to bioaerosols from waste management activities

A4.1 WASTE COLLECTION

Table A4.1.1: Exposure concentrations associated with waste collection

Key: MB: Mesophilic bacteria, TB: Thermophilic bacteria, TF: Thermophilic fungi,
XF: Xerophilic fungi, Inhl: Inhalable, resp: respirable

<table>
<thead>
<tr>
<th>Source, activity, job type or environment</th>
<th>Study location</th>
<th>Dust (mg m$^{-3}$)</th>
<th>Total Bacteria (cfu m$^{-3}$)</th>
<th>Bacteria (cfu m$^{-3}$)</th>
<th>Aspergillus fumigatus (cfu m$^{-3}$)</th>
<th>Fungal spores (cfu m$^{-3}$) except where stated</th>
<th>Endotoxin (EU m$^{-3}$)</th>
<th>Beta(1→3) glucans (ng m$^{-3}$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biowaste collection</td>
<td>Germany</td>
<td>10$^{-7}$</td>
<td></td>
<td></td>
<td>10$^{-7}$</td>
<td></td>
<td></td>
<td></td>
<td>Bunger et al (2000)</td>
</tr>
<tr>
<td>Household organic waste collection</td>
<td>Norway</td>
<td>0.37 (0.10-2.10)</td>
<td>0.80 x10$^{5}$ counts m$^{-3}$ (0.06-3.80 x10$^{6}$)</td>
<td>0.2 x10$^{6}$ spore m$^{-3}$ (0-0.2 x10$^{6}$)</td>
<td>13 (7-180)</td>
<td>52 (5-220)</td>
<td>Heldal et al (2003b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source segregated and mixed household waste collection</td>
<td>Norway</td>
<td>0.2</td>
<td>0.4 x10$^{5}$ (5$^{*}$ bacteria, 7% of count)</td>
<td>0.1 x10$^{4}$</td>
<td>1.8</td>
<td>Heldal &amp; Eduard (2004)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Household Waste collection: Driver</td>
<td>Poland</td>
<td>6.3 (1.1-16)</td>
<td>MB: 267 x10$^{3}$ (22-750)  TB: 1.7 x10$^{3}$ (0.3-3.3) MB: 59 x10$^{3}$ (3.8-190) TB: 1.4 x10$^{3}$ (0.13-6.3)</td>
<td>30 (6.2-61)</td>
<td>36 ng m$^{-3}$ (0.9-101)</td>
<td></td>
<td></td>
<td></td>
<td>Krajewski et al (2002)</td>
</tr>
<tr>
<td>Loader</td>
<td></td>
<td>7.7 (0.6-24)</td>
<td></td>
<td></td>
<td>63 (6.8-132)</td>
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</tbody>
</table>
### Table A4.2

**Table A4.2**: Exposure concentrations associated with working in waste transfer stations in the Netherlands (van Tongeren *et al*, 1997)

| Activity/ job type | Dust  
|:------------------|:--------|:----------|:-----------|:----------|
|                   | (mg m⁻³) | (cfu m⁻³) | (cfu m⁻³) | (EU m⁻³) |
| Waste Transfer (Remote operation; Plant A) Indoor waste storage pit | 1.1 (0.3-3.4) | 13.2 x10⁵ (0.4-1018.2 x10³) | 16.2 x10⁵ (0.2-1487 x10³) | 5.1 (2.3-13.7) |
| Waste Transfer (Manual operation; Plant B) Enclosed outside area | 1.5 (0.3-7.9) | 11.2 x10⁴ (0.3-795.1 x10³) | 39.8 x10⁴ (0.8-826.9 x10³) | 4.5 (1.6-13.0) |
### Table A4.3: On-site exposure concentrations associated with composting

<table>
<thead>
<tr>
<th>Activity, job type or environment</th>
<th>Study location</th>
<th>Dust (mg m(^{-3}))</th>
<th>Total Bacteria (cfu m(^{-3}))</th>
<th>Bacteria (cfu m(^{-3}))</th>
<th>Aspergillus fumigatus (cfu m(^{-3}))</th>
<th>Actinomycetes (cfu m(^{-3}))</th>
<th>Fungal spores (cfu m(^{-3}))</th>
<th>Endotoxin (EU m(^{-3}) except where stated)</th>
<th>Beta(1→3) glucans (ng m(^{-3}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant area</td>
<td>Germany</td>
<td>10(^{5})</td>
<td>10(^{6})</td>
<td>10(^{5})</td>
<td>1.99 ng m(^{-3})</td>
<td></td>
<td></td>
<td>0.79 (0.12-14.45)</td>
<td></td>
<td>Bunger et al (2000)</td>
</tr>
<tr>
<td>On-site Samples</td>
<td>US</td>
<td>4.5 x10(^{-3})</td>
<td>GNB: 2.05 x10(^{-3})</td>
<td>84 (0-1520)</td>
<td>1.99 ng m(^{-3})</td>
<td></td>
<td></td>
<td>0.79 (0.12-14.45)</td>
<td></td>
<td>Hryhoczuk et al (2001)</td>
</tr>
<tr>
<td>Composting Plant</td>
<td>Netherlands</td>
<td>4.5 (0.7-55.1)</td>
<td>GNB: 1.6 x10(^{-3})</td>
<td>40.0 x10(^{-2})</td>
<td>5.1 ng m(^{-3})</td>
<td></td>
<td></td>
<td></td>
<td>van Tongeren et al (1997)</td>
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<tr>
<td>Composting plant:</td>
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<td></td>
<td>Taha et al (2007)</td>
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<tr>
<td>Window turning(^{a})</td>
<td></td>
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<td>Range across 4 locations</td>
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<tr>
<td>Shredding(^{b})</td>
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<td>Range across 2 locations</td>
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<tr>
<td>(mean range across 2 sites):</td>
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<td>Turning</td>
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<tr>
<td>Shredding(^{b})</td>
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<tr>
<td>Compost workers at 4 sites</td>
<td>UK</td>
<td>0.39-2.88</td>
<td>1000-3500</td>
<td>TA6500-114000</td>
<td>TF-2600-16700</td>
<td>MF2600-73900</td>
<td>3.3-3.6</td>
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<td>Crook et al (Draft, 2006)</td>
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<tr>
<td>Composting Centre</td>
<td>Various</td>
<td>GNB: 10(^{5})</td>
<td>TA: 10(^{4})</td>
<td>10(^{5})</td>
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<td>Studies reviewed by Forzier (2002)</td>
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<td>Near rotating sieve</td>
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<tr>
<td>Biofilter exhaust</td>
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<tr>
<td>Production workers</td>
<td>Netherlands</td>
<td>1.3 (&lt;0.3-5.3)</td>
<td></td>
<td>527 (220-1712)</td>
<td>3620 (&lt;150-13180)</td>
<td></td>
<td></td>
<td></td>
<td>Douwes et al (2000)</td>
<td></td>
</tr>
<tr>
<td>Activity, job type or environment</td>
<td>Study location</td>
<td>Dust (mg m(^{-3}))</td>
<td>Total Bacteria (cfu m(^{-3}))</td>
<td>Bacteria (cfu m(^{-3}))</td>
<td>Aspergillus fumigatus (cfu m(^{-3}))</td>
<td>Actinomycetes (cfu m(^{-3}))</td>
<td>Fungal spores (cfu m(^{-3}))</td>
<td>Endotoxin (EU m(^{3}) except where stated)</td>
<td>Beta(1→3) glucans (ng m(^{-3}))</td>
<td>Reference</td>
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</tr>
<tr>
<td>Composting Plant site worker</td>
<td>Poland</td>
<td>4.6 (0.8-10)</td>
<td>MB: 919 x10(^{7}) (26-6278)</td>
<td>TB: 64 x10(^{5}) (4.4-390)</td>
<td>19 (1.6-56)</td>
<td>76 ng m(^{-3}) (10-324)</td>
<td>Krajewski et al (2002)</td>
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<tr>
<td>Composting Plant machine operator</td>
<td>Poland</td>
<td>4.9 (2.3-10)</td>
<td>MB: 323 x10(^{7}) (19-540)</td>
<td>TB: 257 x10(^{5}) (9.8-890)</td>
<td>27 (5.8-69)</td>
<td>61 ng m(^{-3}) (9.1-114)</td>
<td>Krajewski et al (2002)</td>
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<td>Technical personnel</td>
<td>Netherlands</td>
<td>1.5 (0.7-7.3)</td>
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<td></td>
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<td>373 (141-3544)</td>
<td>4850 (1.03-53.23)</td>
<td>Douwes et al (2000)</td>
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<tr>
<td>Supervisors</td>
<td>Netherlands</td>
<td>1.8 (0.5-22.8)</td>
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<td></td>
<td></td>
<td></td>
<td>418 (107-1678)</td>
<td>4280 (1400-10380)</td>
<td>Douwes et al (2000)</td>
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<tr>
<td>Bulldozer operator</td>
<td>Netherlands</td>
<td>0.5 (&lt;0.3-12.2)</td>
<td></td>
<td></td>
<td></td>
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<td>75 (&lt;6-357)</td>
<td>0.54 (&lt;0.15-4.83)</td>
<td>Douwes et al (2000)</td>
<td></td>
</tr>
<tr>
<td>Bulldozer operator (reloading</td>
<td>Poland</td>
<td>2.5 (1.9-3.2)</td>
<td>MB: 78 x10(^{7}) (31-170)</td>
<td>TB: 29 x10(^{5}) (6.1-59)</td>
<td>16 (11-26)</td>
<td>14 ng m(^{-3}) (9.2-20)</td>
<td>Krajewski et al (2002)</td>
<td></td>
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<tr>
<td>Control room</td>
<td>Finland</td>
<td>0.6 (&lt;0.3-3.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>133 (10-366)</td>
<td>650 (&lt;150-16210)</td>
<td>Douwes et al (2000)</td>
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<tr>
<td>Composting Site Process Hall</td>
<td>Netherlands</td>
<td>0.4 (&lt;0.3-2.2)</td>
<td></td>
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<td>74 (8-2016)</td>
<td>570 (&lt;150-12300)</td>
<td>Douwes et al (2000)</td>
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<td>Composting Site Workshop</td>
<td>Netherlands</td>
<td>0.4 (&lt;0.3-0.8)</td>
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<td>101 (30-231)</td>
<td>364 (&lt;150-1930)</td>
<td>Douwes et al (2000)</td>
<td></td>
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<tr>
<td>Composting site Canteen &amp; offices</td>
<td>Netherlands</td>
<td>0.4 (&lt;0.3-0.8)</td>
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<td>100 (30-231)</td>
<td>364 (&lt;150-1930)</td>
<td>Douwes et al (2000)</td>
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<tr>
<td>Plant area after work shift</td>
<td>Germany</td>
<td>10(^{7})</td>
<td>10(^{7})</td>
<td>10(^{7})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bunger et al (2000)</td>
<td></td>
</tr>
<tr>
<td>Drum composting plant treating</td>
<td>Finland</td>
<td>0.6-0.7 mgm(^{-3})</td>
<td>GM total microbial concentration 21.8 million pcs/m(^{3})</td>
<td>13.9 million pcs/m(^{3})</td>
<td>1.4 million pcs/m(^{3})</td>
<td></td>
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<td>Tolvanen et al (2005)</td>
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<td>Catering waste receiving hall</td>
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<td>Drum composting hall</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Source, activity, job type or environment</th>
<th>Location</th>
<th>Total Bacteria (cfu m⁻³ except where stated)</th>
<th>Gram-negative bacteria (cfu m⁻³)</th>
<th>Aspergillus fumigatus (cfu m⁻³ except where stated)</th>
<th>Actinomycetes (cfu m⁻³ except where stated)</th>
<th>Fungal spores (cfu m⁻³ except where stated)</th>
<th>Endotoxin (EU m⁻³ except where stated)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upwind (composting) Germany</td>
<td>8.4-18x10⁴</td>
<td>N.D.</td>
<td>1.9-3.6x10⁷</td>
<td></td>
<td></td>
<td>0.16 ng m⁻³</td>
<td>Herr et al (2003a)</td>
<td></td>
</tr>
<tr>
<td>Upwind of composting (75m) Various</td>
<td>433</td>
<td>0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>Studies reviewed by Prasad et al (2004)</td>
<td></td>
</tr>
<tr>
<td>Downwind of composting Germany 200m NW 250m WNW 300m N 320m NW 550m N</td>
<td></td>
<td>2.2-51x10⁴ 3.9-17x10⁴ 0.44-8.3x10⁴ 0.68-5.9x10⁴ 0.083-0.43x10⁴</td>
<td>2.3-55x10⁴ 1.9-11x10⁶ 0.28-6.0x10⁴ 0.13-5.0x10⁴ &lt;5-990</td>
<td>7.7-130x10⁴ 13-46x10² 4.3-17x10³ 3.9-19x10² 2.3-4.1x10³</td>
<td></td>
<td></td>
<td>Herr et al (2003a)</td>
<td></td>
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<tr>
<td>Downwind of composting (150m) Various</td>
<td>2830</td>
<td>200</td>
<td>390</td>
<td></td>
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<td>0.24 ng m⁻³</td>
<td>Studies reviewed by Prasad et al (2004)</td>
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</tr>
<tr>
<td>Upwind of recycling plants (3 sites): Summer Winter Canada</td>
<td>250-5820 150-1220</td>
<td>120 N.D.</td>
<td>120-3960 10-7870</td>
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<td>Lavoie and Guertin (2001)</td>
<td></td>
</tr>
<tr>
<td>Downwind of recycling plants (3 sites): Summer Winter Canada</td>
<td>520-5650 N.D.</td>
<td>250 N.D.</td>
<td>730-3095 N.D.</td>
<td></td>
<td></td>
<td></td>
<td>Lavoie and Guertin (2001)</td>
<td></td>
</tr>
<tr>
<td>3 composting plants. 80 m from site 150 m from site UK</td>
<td>&lt; 100</td>
<td>&lt; 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wheeler et al (2001)</td>
<td></td>
</tr>
</tbody>
</table>
Table A4.5. Bioaerosol concentrations measured in areas surrounding 4 composting facilities investigated by Crook et al (2006). LOD: limit of detection

<table>
<thead>
<tr>
<th>Activity</th>
<th>Distance from site</th>
<th>Dust ( (\text{mg m}^{-3}) )</th>
<th>Total Microbes ( (\text{microbes x10}^3 \text{ m}^{-3}) )</th>
<th>Aspergillus fumigatus ( (\text{cfu m}^{-3}) )</th>
<th>Thermophilic Actinomycetes ( (\text{cfu m}^{-3}) )</th>
<th>Mesophilic fungi ( (\text{cfum}^{-3}) )</th>
<th>Thermophilic fungi ( (\text{cfu m}^{-3}) )</th>
<th>Endotoxin ( (\text{EU m}^{-3}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No operation</td>
<td>50 m upwind</td>
<td>0.0067</td>
<td>5.6-4600</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>230</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td>50 m downwind</td>
<td>0.2</td>
<td>5.5 -9200</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>76</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td>120 - 150 m downwind</td>
<td>0.1</td>
<td>15-15000</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>320</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Shredding green waste</td>
<td>50 m upwind</td>
<td>0.032-0.53</td>
<td>9-4300</td>
<td>24</td>
<td>&lt;LOD</td>
<td>96</td>
<td>24</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td>50 m downwind</td>
<td>0.036-0.055</td>
<td>≤5400</td>
<td>45</td>
<td>&lt;LOD</td>
<td>160</td>
<td>45</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td>120 - 150 m downwind</td>
<td>0.028</td>
<td>23-5400</td>
<td>2100</td>
<td>&lt;LOD</td>
<td>1800</td>
<td>2100</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Turning</td>
<td>50 m upwind</td>
<td>0.035-0.096</td>
<td>5.6</td>
<td>19</td>
<td>1300</td>
<td>1700</td>
<td>9500</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td>50 m downwind</td>
<td>0.049-0.057</td>
<td>5.5</td>
<td>9000</td>
<td>160</td>
<td>160</td>
<td>540</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td></td>
<td>120 - 150 m downwind</td>
<td>0.019-0.040</td>
<td>15</td>
<td>740</td>
<td>308</td>
<td>230</td>
<td>56</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>
A4.4 MATERIALS RECYCLING

Table A4.6: Exposure concentrations associated with materials recovery. Key: **MB**: Mesophilic bacteria, **TB**: Thermophilic bacteria, **GNB**: gram negative bacteria; **MA**: Mesophilic actinomycetes, **TA**: Thermophilic actinomycetes, **TF**: Thermophilic fungi, **XF**: Xerophilic fungi, **LOD**: Limit of detection.

<table>
<thead>
<tr>
<th>Activity, job type or environment</th>
<th>Plant location</th>
<th>Dust (mg m(^{-3}))</th>
<th>Bacteria (cfu m(^{-3}))</th>
<th>Actinomycetes (cfu m(^{-3}))</th>
<th>Fungal spores (cfu m(^{-3}))</th>
<th>Endotoxin (EU m(^{-3}) except where stated)</th>
<th>Beta (1→3) glucan (ngm(^{-3}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-treatment &amp; crushing</strong></td>
<td>Finland</td>
<td>0.4 (&lt;0.01-0.7)</td>
<td>MB: 55290 (7370-236960)</td>
<td>MA: 610 (90-4150)</td>
<td>MF: 96620 (30460-226240)</td>
<td>210.5 (50-980)</td>
<td>Tolvanen and Hänninen (2006)</td>
<td></td>
</tr>
<tr>
<td><strong>Bioreactor Hall (impactor collection)</strong></td>
<td>Finland</td>
<td>0.1 (&lt;0.01-0.5)</td>
<td>MB: 2620 (710-6500)</td>
<td>MA: 260 (0-710)</td>
<td>MF: 440 (285-850)</td>
<td>194.3 (4.2-1100)</td>
<td>Tolvanen and Hänninen (2006)</td>
<td></td>
</tr>
<tr>
<td><strong>Drying Hall (impactor collection results)</strong></td>
<td>Finland</td>
<td>0.4 (&lt;0.01-1.5)</td>
<td>MB: 120 (35-490)</td>
<td>MA: 20 (0-20)</td>
<td>MF: 20 (0-35)</td>
<td>16.2 (8.4-31)</td>
<td>Tolvanen and Hänninen (2006)</td>
<td></td>
</tr>
<tr>
<td><strong>Worker Personal Exposure</strong></td>
<td></td>
<td>0.42-0.58</td>
<td></td>
<td></td>
<td></td>
<td>21.4-38.7</td>
<td>Mahar et al (1999)</td>
<td></td>
</tr>
<tr>
<td><strong>Area Exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20.7-38.4 22.5-34.5</td>
<td>Mahar (2002)</td>
<td></td>
</tr>
<tr>
<td><strong>RDF Plant</strong></td>
<td></td>
<td>0.42-0.58</td>
<td></td>
<td></td>
<td></td>
<td>21.4-38.7</td>
<td>Mahar (2002)</td>
<td></td>
</tr>
<tr>
<td>Activity, job type or environment</td>
<td>Plant location</td>
<td>Dust (mg m(^{-3}))</td>
<td>Bacteria (cfu m(^{-3}))</td>
<td>Actinomycetes (cfu m(^{-3}))</td>
<td>Fungal spores (cfu m(^{-3}))</td>
<td>Endotoxin (EU m(^{-3}) except where stated)</td>
<td>Beta (1→3) glucan (ngm(^{-3}))</td>
<td>Reference</td>
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</tr>
<tr>
<td>Dry waste (plastic &amp; paper)</td>
<td>Finland</td>
<td>0.9 (LOD-1.3)</td>
<td>MB: 14200 (2600-38400); 1400 when process was off TB: 15000 (3500-98900); 530 when process was off</td>
<td>MA: 1100; 70 when process off TA: 590 (90-3600)</td>
<td>MF: 55200 (5400-202000); 3000 when process was off TF: 6500 (330-21800); 450 when process was off</td>
<td>440 ng m(^{-3}) (4.7-1000)</td>
<td>Tolvanen (2001)</td>
<td></td>
</tr>
<tr>
<td>MRF waste delivery area</td>
<td>Germany</td>
<td>Short term peaks &gt; 6 mgm-3, full shift &lt;&lt; 6 mgm-3</td>
<td>Total microbes ≤6.9 x 10^5 cfum(^{-3})</td>
<td>Endotoxin concentrations &gt; 200 EU m(^{-3}) irrespective of the measurement place, except near after-crusher where the average concentration was 60 EU m(^{-3}).</td>
<td>6.6 x 10^6 cfum(^{-3})</td>
<td></td>
<td>Knop et al (1996b)</td>
<td></td>
</tr>
<tr>
<td>Dry waste treatment plant:</td>
<td>Finland</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tolvanen (2004)</td>
</tr>
<tr>
<td>In processing hall</td>
<td></td>
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<tr>
<td>Near a conveyor belt</td>
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<tr>
<td>Near a jigger</td>
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<tr>
<td>Near a bailer</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Near the after crusher</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Waste sorting:</td>
<td>Canada</td>
<td>9.6 x 10^3 – 13 x10^3</td>
<td>1840-6110</td>
<td></td>
<td></td>
<td></td>
<td>60</td>
<td>Lavoie &amp; Guertin (2001)</td>
</tr>
<tr>
<td>Activity, job type or environment</td>
<td>Plant location</td>
<td>Dust (mg m$^{-3}$)</td>
<td>Bacteria (cfu m$^{-3}$)</td>
<td>Actinomycetes (cfu m$^{-3}$)</td>
<td>Fungal spores (cfu m$^{-3}$)</td>
<td>Endotoxin (EU m$^{-3}$ except where stated)</td>
<td>Beta (1→3) glucan (ngm$^{-3}$)</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------</td>
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<td>-----------</td>
</tr>
<tr>
<td>Personal exposure measurements for MRF workers</td>
<td>England and Wales</td>
<td>Median (Range)</td>
<td>2.21 (0-8.95)</td>
<td>2.85 (0-18.59)</td>
<td>8.21 (2.01-62.61)</td>
<td>4.09 (0-45.02)</td>
<td>4.11 (1.29-40.15)</td>
<td>4.17 (0.31-27.25)</td>
</tr>
</tbody>
</table>
### A4.5 REFUSE-DERIVED FUEL

**Table A4.7:** Exposure concentrations associated with refuse-derived fuel from municipal waste in the US. Key: **MB:** Mesophilic bacteria, **TB:** Thermophilic bacteria, **MA:** Mesophilic actinomycetes, **TA:** Thermophilic actinomycetes, **TF:** Thermophilic fungi

<table>
<thead>
<tr>
<th>Activity, job type or environment</th>
<th>Dust (mg m⁻³)</th>
<th>Total microbes viable and nonviable</th>
<th>Bacteria (cfu m⁻³)</th>
<th>Actino-mycetes (cfu m⁻³)</th>
<th>Fungal spores (cfu m⁻³)</th>
<th>Endotoxin (EU m⁻³ except where stated)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worker Personal Exposure (range across 2 plants): 1995-2000</td>
<td>0.42-0.58</td>
<td>6.8 x 10⁵ organisms m⁻³ (determined using fluorescence microscopy)</td>
<td>14200 (2600-38400); 1400 when process was off</td>
<td>15000 (3500-98900); 530 when process was off</td>
<td>55200 (5400-202000); 30000 when process was off</td>
<td><strong>29.0 (2.9 ng m⁻³)</strong></td>
<td>Mahar (2002)</td>
</tr>
<tr>
<td>Area Exposure (range across 2 plants): 1995-2000</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mahar (2002)</td>
</tr>
<tr>
<td>RDF Plant (range across 2 plants) Mean personal exposure (35 workers)</td>
<td>0.9 (LOD-1.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mahar et al (1999)</td>
</tr>
<tr>
<td>Dry waste (plastic &amp; paper) unloading &amp; pre-crushing (impactor collection results)</td>
<td>4.8 x 10⁶ particles m⁻³</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tolvanen (2001)</td>
</tr>
<tr>
<td><strong>Endotoxin (EU m⁻³ except where stated)</strong></td>
<td><strong>20.7-38.4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mahar (2002)</td>
</tr>
<tr>
<td><strong>Endotoxin (EU m⁻³ except where stated)</strong></td>
<td><strong>25.1-60.6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mahar (2002)</td>
</tr>
<tr>
<td><strong>Endotoxin (EU m⁻³ except where stated)</strong></td>
<td><strong>440 ng m⁻³</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Tolvanen (2001)</td>
</tr>
<tr>
<td><strong>Endotoxin (EU m⁻³ except where stated)</strong></td>
<td><strong>1000 ng m⁻³</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mahar et al (1999)</td>
</tr>
<tr>
<td><strong>Endotoxin (EU m⁻³ except where stated)</strong></td>
<td><strong>4.7 and 33 ng m⁻³</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mahar (2002)</td>
</tr>
</tbody>
</table>
### A4.6 MECHANICAL BIOLOGICAL TREATMENT

Table A4.8: Exposure concentrations associated with MBT in Finland (Tolvanen and Hanninen, 2006) Key: **MB**: Mesophilic bacteria, **TB**: Thermophilic bacteria, **MA**: Mesophilic actinomycetes, **TA**: Thermophilic actinomycetes, **MF**: Thermophilic fungi, **XF**: Xerophilic fungi,

<table>
<thead>
<tr>
<th>Activity, job type or environment</th>
<th>Dust (mg m$^{-3}$)</th>
<th>Bacteria (cfu m$^{-3}$)</th>
<th>Actino-mycetes (cfu m$^{-3}$)</th>
<th>Fungal spores (cfu m$^{-3}$)</th>
<th>Endotoxin (EU m$^{-3}$ except where stated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment &amp; crushing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(impactor collection)</td>
<td>0.4 (&lt;0.01-0.7)</td>
<td>MB: 55290 (7370-236960)</td>
<td>MA: 610 (90-4150)</td>
<td>MF: 96620 (30460-226240)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TB: 12450 (1010-552110)</td>
<td>TA: 220 (35-1200)</td>
<td>TF: 3070 (180-155900)</td>
<td></td>
</tr>
<tr>
<td>Bioreactor Hall</td>
<td>0.1 (&lt;0.01-0.5)</td>
<td>MB: 2620 (710-6500)</td>
<td>MA: 260 (0-710)</td>
<td>MF: 440 (265-850)</td>
<td></td>
</tr>
<tr>
<td>(impactor collection)</td>
<td></td>
<td>TB: 80 (20-250)</td>
<td>TA: 30 (0-35)</td>
<td>TF: 35 (0-35)</td>
<td></td>
</tr>
<tr>
<td>Drying Hall</td>
<td>0.4 (&lt;0.01-1.5)</td>
<td>MB: 120 (25-490)</td>
<td>MA: 20 (0-20)</td>
<td>MF: 20 (0-35)</td>
<td></td>
</tr>
<tr>
<td>(impactor collection results)</td>
<td></td>
<td>TB: 30 (0-70)</td>
<td>TA: No growth</td>
<td>TF: 20 (0-20)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>210.5 (50-980)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>194.3 (4.2-1100)</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>16.2 (5.4-31)</td>
</tr>
</tbody>
</table>
### A4.7 WASTE INCINERATION

**Table A4.9**: Exposure concentrations associated with incineration

Key: **MB**: Mesophilic bacteria, **TB**: Thermophilic bacteria, **MA**: Mesophilic actinomycetes, **TA**: Thermophilic actinomycetes, **TF**: Thermophilic fungi,

<table>
<thead>
<tr>
<th>Activity/ job type/ location</th>
<th>Dust (mg m(^{-3}))</th>
<th>Bacteria (cfu m(^{-3}))</th>
<th>Actinomycetes (cfu m(^{-3}))</th>
<th>Fungal spores (cfu m(^{-3}))</th>
<th>Endotoxin (EU m(^{-3}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combustion area (office level)</td>
<td>0.2 (&lt;0.01-1.0)</td>
<td>MB: 335 (90-1310)</td>
<td>TA: 50 (10-320)</td>
<td>MF: 1725 (405-10105)</td>
<td>15.7 (1.7-46)</td>
<td>Tolvanen and Hänninen (2005)</td>
</tr>
<tr>
<td>(impactor collection results)</td>
<td>TB: 50 (0-95)</td>
<td>MA: 25 (0-70)</td>
<td>TF: 50 (15-1925)</td>
<td></td>
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</tr>
<tr>
<td>Combustion area (slag pool level)</td>
<td>0.3 (&lt;0.01-0.5)</td>
<td>MB: 1245 (175-27370)</td>
<td>TA: 23 (0-23)</td>
<td>MF: 1380 (160-4100)</td>
<td>223.4 (1.8-1300)</td>
<td>Tolvanen and Hänninen (2005)</td>
</tr>
<tr>
<td></td>
<td>TB: 65 (0-175)</td>
<td>MA: 70 (0-580)</td>
<td>TF: 75 (0-140)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TB: 2670 (880-4150)</td>
<td>MA: 2170 (280-25070)</td>
<td>TF: 5235 (390-293990)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Crane room</td>
<td>0.4 (0-1.3)</td>
<td>MB: 470 (42-3270)</td>
<td>TA: 130 (0-900)</td>
<td>MF: 1945 (230-20350)</td>
<td>30.3 (2.4-120)</td>
<td>Tolvanen and Hänninen (2005)</td>
</tr>
<tr>
<td></td>
<td>TB: 135 (0-350)</td>
<td>MA: 120 (20-810)</td>
<td>TF: 195 (10-2665)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domestic waste incineration</td>
<td>10(^{7})</td>
<td>10(^{7})</td>
<td>10(^{7})</td>
<td>From studies reviewed in Swan et al (2003)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
There is very little information about bioaerosol exposure in relation to landfill operations. Krajewski et al. (2002) reported that the mean exposure concentrations for landfill machine operator and a landfill site worker to dust were 0.9 and 0.3 mg m$^{-3}$ respectively, concentrations of fungi were 25 and 3.1 cfu m$^{-3}$ respectively and endotoxin concentrations were about 400 ng m$^{-3}$ in both jobs. Mean concentrations of mesophilic bacteria were about 4 x 10$^3$ cfu m$^{-3}$ for both jobs, but the mean exposure to thermophilic bacteria was much higher for the machine operator (25 x 10$^3$ compared with 3.1 x 10$^3$ cfu m$^{-3}$).

In a study of 5 UK landfill sites (Table A4.9), Swan et al. (2004) reported widely variable onsite (Table A4.10) and workplace (Table A4.11) exposure concentrations.

### Table A4.10: Characteristics of landfill sites investigated by Swan et al. (2004)

<table>
<thead>
<tr>
<th>Site</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Accepts about 2000 tonnes/day of waste, predominantly household with some industrial and commercial spoils; daily cover with soil</td>
</tr>
<tr>
<td>B</td>
<td>Accepts about 1200 tonnes/day of waste, predominantly domestic and trade with small quantities of hazardous waste eg industrial sludges, contaminated soil</td>
</tr>
<tr>
<td>C</td>
<td>Accepts about 9000-13000 tonnes/week; approximately equivalent quantities of household and inert waste, also accepts mildly contaminated soil; daily cover with soil</td>
</tr>
<tr>
<td>D</td>
<td>Accepts household and industrial waste from light industry, no hazardous waste; daily cover soil, felt material and offcuts from local car industry, sometimes green material</td>
</tr>
<tr>
<td>E</td>
<td>Smallest site investigated; accepts 6-7000 tonnes/week; building waste (&gt;50%) and household waste; daily cover low grade green waste/compost from own facility in summer; soil in winter</td>
</tr>
</tbody>
</table>

### Table A4.11: Concentrations of bioaerosol measured on landfill sites upwind and downwind of active operations

<table>
<thead>
<tr>
<th>Site</th>
<th>Dust mg m$^{-3}$</th>
<th>Total bacteria cfu m$^{-3}$</th>
<th>Coliforms</th>
<th>Thermophilic bacteria</th>
<th>Fungi</th>
<th>Endotoxin EU m$^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upwind</td>
<td>Downwind</td>
<td>Upwind</td>
<td>Downwind</td>
<td>Upwind</td>
<td>Downwind</td>
</tr>
<tr>
<td>A</td>
<td>0.15-1.06</td>
<td>0.02-1.29</td>
<td>418-509</td>
<td>2432-7808</td>
<td>0</td>
<td>0.277</td>
</tr>
<tr>
<td>B</td>
<td>0.0-0.17</td>
<td>0.02-1.18</td>
<td>0-4304</td>
<td>1163-78903</td>
<td>0.231</td>
<td>192-6998</td>
</tr>
<tr>
<td>C</td>
<td>0.01-3.37</td>
<td>0.7-5.07</td>
<td>0-8537</td>
<td>2439-439883</td>
<td>0.58</td>
<td>0-10704</td>
</tr>
<tr>
<td>D</td>
<td>0.1-0.5</td>
<td>0.02-3.77</td>
<td>1-21311</td>
<td>167-16612</td>
<td>0</td>
<td>0-822</td>
</tr>
<tr>
<td>E</td>
<td>0.07-1.78</td>
<td>0.23-1.73</td>
<td>424-70580</td>
<td>1303-71023</td>
<td>0-1416</td>
<td>10-1885</td>
</tr>
</tbody>
</table>
**Table A4.12:** Personal exposure concentrations for workers on 5 landfill sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Dust mg m⁻³</th>
<th>Total bacteria cfu m⁻³</th>
<th>Colliforms</th>
<th>Thermophilic bacteria</th>
<th>Fungi</th>
<th>Endotoxin EU m⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0-1.46</td>
<td>412-23134</td>
<td>0-208</td>
<td>0-373</td>
<td>0-3113</td>
<td>&lt;0.5-168</td>
</tr>
<tr>
<td>B</td>
<td>0.22-8.67</td>
<td>7903-825000</td>
<td>1522-27778</td>
<td>0-2257</td>
<td>540-262153</td>
<td>&lt;0.5-65</td>
</tr>
<tr>
<td>C</td>
<td>0.25-4.08</td>
<td>2475-382653</td>
<td>0-9091</td>
<td>0-20833</td>
<td>0-32232</td>
<td>1.4-80.27</td>
</tr>
<tr>
<td>D</td>
<td>0.07-1.81</td>
<td>9-92227</td>
<td>0-232</td>
<td>0-217</td>
<td>8-1334</td>
<td>&lt;5-797</td>
</tr>
<tr>
<td>E</td>
<td>0.05-2.57</td>
<td>5169-72176</td>
<td>194-2627</td>
<td>840-14017</td>
<td>339-10982</td>
<td>6.1-64.6</td>
</tr>
</tbody>
</table>
APPENDIX 5

Review of published exposure response information

A5.1 ORGANIC DUST

Table A5.1: Exposure-response information for exposure to organic dusts in the waste industry; NAL – nasal lavage fluid; GM – geometric mean; GSD – geometric standard deviation; AM – arithmetic mean; OR – odds ratio; MRF – Materials Recycling Facility; FEV₁ – forced expiratory volume in 1 second (a measure of lung function); NOEL – no observed effect level; LOEL – lowest observed effect level

<table>
<thead>
<tr>
<th>Exposed population</th>
<th>Control population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Threshold</th>
<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>47 Dutch waste collectors, 22% employed for &lt;6 months</td>
<td>15 office workers from same facilities employed for &gt;6 months</td>
<td>GM: 0.58 GSD: 2.6 Range: &lt;0.2-9.1 mgm⁻³ inhalable dust</td>
<td>Changes in NAL parameters, significant increase in IL8 (an inflammatory mediator)</td>
<td>Not determined, lowest exposure &lt;0.2 mgm⁻³</td>
<td>Not demonstrated, no significant difference between workers employed for &lt; or &gt; 6 months; higher prevalence of chronic respiratory symptoms in controls (smaller proportion of controls were current smokers)</td>
<td>Waste collectors showed significantly more cough, phlegm and itchy nose than controls; increase in inflammation in waste collection workers during week; no evidence of habituation; stronger relationships between NAL parameters and dust than with endotoxin – authors suggest potential importance of unmeasured components such as peptidoglycans</td>
<td>Wouters et al (2002)</td>
</tr>
<tr>
<td>25 Norwegian waste collection workers involved in collecting food and garden waste</td>
<td>-</td>
<td>AM 0.55 mgm⁻³, GM 0.37 mgm⁻³ Range 0.1-2.1 mgm⁻³ inhalable dust</td>
<td>Significant decline in FEV₁ over working week; no relationship between exposure and symptoms</td>
<td>Not demonstrated</td>
<td>Relationship between measured respiratory parameters and dust not specifically discussed</td>
<td>Heldal et al (2003a)</td>
<td></td>
</tr>
<tr>
<td>31 Norwegian waste collection workers involved in collecting food and garden waste</td>
<td>-</td>
<td>Median 0.35 mgm⁻³ Range 0.1-2.1 mgm⁻³ inhalable dust</td>
<td>Evidence of mild upper airways inflammation in NAL but no relationship with respiratory symptoms</td>
<td>Organic dust not specifically linked to markers of inflammation</td>
<td>Not demonstrated</td>
<td>Not possible to relate NAL parameters to symptoms; authors identify endotoxin and beta (1-&gt;3) glucan as important components of organic dust</td>
<td>Heldal et al (2003b)</td>
</tr>
<tr>
<td>Exposed population</td>
<td>Control population</td>
<td>Exposure</td>
<td>Health effects</td>
<td>Threshold</td>
<td>Exposure-response</td>
<td>Comments</td>
<td>Study</td>
</tr>
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</tr>
<tr>
<td>Questionnaire study of 22 Norwegian waste collection workers</td>
<td></td>
<td>AM 0.22 mgm⁻³, GM 0.17 mgm⁻³, Range 0.04-0.91 mgm⁻³ inhalable dust</td>
<td>Nonsignificant association with nasal irritation, eye irritation</td>
<td>Eye irritation reported at 0.2 mgm⁻³</td>
<td>Not demonstrated</td>
<td>Exposure monitoring may have influenced reporting of symptoms; correlation between dust and endotoxin concentrations; dust does not appear to have been a particularly relevant marker of bioaerosol exposure</td>
<td>Heldal &amp; Eduard (2004)</td>
</tr>
<tr>
<td>Study of about 175 workers in 9 MRFs in England and Wales (159 participated)</td>
<td></td>
<td>Plant means ranged from 2.1-20.3 mgm⁻³; highest measurement 63 mgm⁻³</td>
<td>Significant increase in irritated nose/sneezing; Nonsignificant increase in cough with phlegm, dry cough, chest tightness and nausea</td>
<td>5 mgm⁻³</td>
<td>OR for &gt; 5 mgm⁻³ for nasal irritation was 2.6</td>
<td>Significant increase in irritated nose/sneezing; Nonsignificant increase in cough with phlegm, dry cough, chest tightness and nausea; 25% of workers had been employed for &lt;6 months in MRF and only 30% with &gt;2 years employment; tendency for self-reported respiratory and gastrointestinal symptoms to increase with length of employment; inhalable dust exposures relatively high compared with other studies and WEL</td>
<td>Gladding et al (2003)</td>
</tr>
<tr>
<td>Dutch compost workers 14 in 1st survey, 15 in 2nd survey</td>
<td>University staff/students</td>
<td>GM 1st survey (2nd survey) Bulldozer – 0.5 (0.3) Tech personnel – 1.5 (1.2) Supervisors – 1.8 (0.7) Production workers 1.3 (1.1) mgm⁻³</td>
<td>1st survey – NAL parameters suggested higher level of chronic upper airways inflammation in compost workers; no significant difference between compost workers and controls in 2nd study</td>
<td>NOEL – 2nd survey 0.3-1.1 mgm⁻³; LOEL – 1st survey 0.5-1.8 mgm⁻³</td>
<td>Higher prevalence of nasal symptoms, cough and cough with phlegm during 1st survey than 2nd, low incidence of shortness of breath, wheezing and chest tightness in both surveys; in 1st survey pre-shift inflammatory markers greater at &gt;461 EU m⁻³ (median exposure); but no correlation with cross shift changes</td>
<td>Two surveys carried out at an interval of one year during which time industrial hygiene at the plant was much improved; results may suggest potential recovery from chronic inflammation of the upper airways, but sample size extremely small; endotoxin and beta (1-3) glucan moderately correlated; limited evidence that exposure to &gt; 461 EU m⁻³ associated with development of chronic inflammation, although little evidence for acute effects at this level of exposure; reduction in dust exposure between two surveys was relatively small suggesting that endotoxin was main driver of observed effects</td>
<td>Douwes et al (2000)</td>
</tr>
<tr>
<td>Canadian study of 226 employees at 36 randomly selected liquor stores with bottle recycling and in-house glass breaking</td>
<td></td>
<td>GM 0.18 mgm⁻³ for inhalable dust; 3.6 EU m⁻³ for endotoxin (270 personal samples); 1064 cfu gm⁻¹ for viable fungi (648 area samples).</td>
<td>Employees reported more work related chronic chest tightness and chronic nasal symptoms than unexposed controls.</td>
<td>Inhalable particulate matter levels &gt;0.2 mgm⁻³ were associated with acute upper airway irritation.</td>
<td>Somatic symptoms were associated with measures of psychosocial job strain. Acute chest symptoms were associated with breaking visibly mouldy bottles, but not with measured fungal counts.</td>
<td>Somatic symptoms were associated with measures of psychosocial job strain. Acute chest symptoms were associated with breaking visibly mouldy bottles, but not with measured fungal counts.</td>
<td>Kennedy et al (2004)</td>
</tr>
</tbody>
</table>
Table A5.2: Exposure-response information for exposure to organic dusts in other industries; NAL – nasal lavage fluid; GM – geometric mean; GSD – geometric standard deviation; AM – arithmetic mean; OR – odds ratio; FEV$_1$ – forced expiratory volume in 1 second (a measure of lung function); ODTS - Organic Dust Toxic Syndrome

<table>
<thead>
<tr>
<th>Study population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Threshold</th>
<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross sectional study of 1032 UK workers exposed to organic dusts in 9 industries</td>
<td>Organic dust</td>
<td>Dose-response relationship between organic dust and upper and lower respiratory symptoms and an association with OTDS</td>
<td></td>
<td>Prevalence of symptoms ranged from &lt; 5% at concentrations of 0.1 mgm$^{-3}$ to &gt;30% at 30 mgm$^{-3}$; 10 mgm$^{-3}$ was associated with a symptom prevalence of about 25%.</td>
<td>Strong correlation between endotoxin exposure and symptoms</td>
<td>Simpson et al (1998)</td>
</tr>
<tr>
<td>Studies in cotton workers – no increased risk</td>
<td>Cotton dust</td>
<td>Byssinosis, an obstructive respiratory disease characterised by coughing, wheezing and chest tightness</td>
<td>greater than: 1) 0.4-1.2 mgm$^{-3}$ dust 2) 0.3-15 mgm$^{-3}$ dust</td>
<td>1) FEV1% and FVC% were significantly associated with the cumulative exposure to respirable endotoxin.</td>
<td>Workers typically habituate to cotton dust exposure during the working week and their respiratory symptoms are worst on Monday morning following a weekend away from work</td>
<td>1) Sigsgaard et al (1992) 2) Cinkotai et al (1977)</td>
</tr>
<tr>
<td>Studies in cotton workers – increased risk</td>
<td>Cotton dust 0.3-2.0 mgm$^{-3}$</td>
<td>Byssinosis</td>
<td></td>
<td>Dust and bacterial concentrations strongly correlated</td>
<td>Haglind et al (1981)</td>
<td></td>
</tr>
<tr>
<td>Studies in cotton workers – no increased risk</td>
<td>Cotton dust 0.5-6.9 mgm$^{-3}$</td>
<td>Reduced lung function</td>
<td>greater than: 1) 0.2-2.5 mgm$^{-3}$ dust 2) 0.12-0.55 mgm$^{-3}$ dust</td>
<td>Dust was strongly correlated with endotoxin and Rylander (1984)</td>
<td>Kennedy et al (1987)</td>
<td>Castellan et al (1987)</td>
</tr>
<tr>
<td>Studies in cotton workers – increased risk</td>
<td>Cotton dust 0.5-6.9 mgm$^{-3}$</td>
<td>Respiratory symptoms</td>
<td>&gt;0.2-2.5 mgm$^{-3}$</td>
<td></td>
<td>Haglind et al (1984)</td>
<td></td>
</tr>
<tr>
<td>Pig workers</td>
<td>0.5-23 mgm$^{-3}$</td>
<td>No association between exposure to dust and respiratory symptoms or lung function.</td>
<td></td>
<td>No association between exposure to dust and respiratory symptoms or lung function.</td>
<td>Heederik et al (1991)</td>
<td></td>
</tr>
<tr>
<td>23 poultry workers</td>
<td>0.4-15.3 mgm$^{-3}$</td>
<td>No association between exposure to dust and respiratory symptoms or lung function.</td>
<td></td>
<td>Concurrent exposure to 4 x 10$^{-4}$ x 10$^{7}$ cfum$^{-3}$ bacteria, mainly coagulase-negative staphylococcal strains, and 500-4000 cfum$^{-3}$ of fungi.</td>
<td>Hagmar et al (1990)</td>
<td></td>
</tr>
<tr>
<td>Study population</td>
<td>Exposure</td>
<td>Health effects</td>
<td>Threshold</td>
<td>Exposure-response</td>
<td>Comments</td>
<td>Study</td>
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</tr>
<tr>
<td>Pig workers</td>
<td></td>
<td>Cross shift decline in lung function</td>
<td>A 10% decline in FEV&lt;sub&gt;1&lt;/sub&gt; (Forced Expiratory Volume in 1 second) was associated with an exposure-concentration of 2.8 mgm&lt;sup&gt;−3&lt;/sup&gt;.</td>
<td>The threshold concentration associated with a 10% decline in FEV&lt;sub&gt;1&lt;/sub&gt; increased with increasing duration of exposure suggesting either the development of tolerance or a healthy worker effect with those susceptible to respiratory illness leaving the industry.</td>
<td>Donham &lt;i&gt;et al&lt;/i&gt; (1995)</td>
<td></td>
</tr>
<tr>
<td>53 pig farm employees</td>
<td>3.03-14.05 mgm&lt;sup&gt;−3&lt;/sup&gt;</td>
<td>Symptoms consistent with ODTS were reported by 31 (58.5%) of the workers; No abnormalities in lung function were detected.</td>
<td>Concentrations of airborne micro-organisms that ranged from 613.7-1246.7 x 10&lt;sup&gt;3&lt;/sup&gt; cfum&lt;sup&gt;−3&lt;/sup&gt; (mean value 930.6 x 10&lt;sup&gt;3&lt;/sup&gt; cfum&lt;sup&gt;−3&lt;/sup&gt;) combined with extremely high levels of exposure to endotoxin (mean 22.8 ugm&lt;sup&gt;−3&lt;/sup&gt;, range 1.88-31.25 ugm&lt;sup&gt;−3&lt;/sup&gt;).</td>
<td>Mackiewicz (1998)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort of 207 swine producers</td>
<td></td>
<td>Cross-shift reductions in lung function (FEV&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>significant correlation with personal exposures to total dust, total endotoxin, respirable endotoxin, and ammonia</td>
<td>Dust was specifically associated with lung function changes in workers with more than 6 years of exposure whereas endotoxin was associated with lung function effects in workers with shorter exposures.</td>
<td>Reynolds &lt;i&gt;et al&lt;/i&gt; (1996)</td>
<td></td>
</tr>
<tr>
<td>29 pig farmers</td>
<td>1.66 to 21.04 mgm&lt;sup&gt;−3&lt;/sup&gt;, (ammonia - 1.50 to 13.23 ppm; airborne micro-organisms (mostly gram-positive bacteria) ranged from 10&lt;sup&gt;5&lt;/sup&gt; to &gt; 10&lt;sup&gt;7&lt;/sup&gt; cfum&lt;sup&gt;−3&lt;/sup&gt;)</td>
<td>23 reported work-related respiratory symptoms, typically chest tightness/wheeze and nasal and eye irritation</td>
<td>3 farmers had specific IgE to pig squames or urine and 8 to feed components but none to the microbial extracts. Specific IgG to pig squames or urine and to feed components was demonstrated in 14 and 9 workers, respectively. Specific IgE responses occurred mainly in subjects with chest tightness or wheeze. Specific IgG responses were not related to symptoms. Concentrations of endotoxin were low.</td>
<td>Crook &lt;i&gt;et al&lt;/i&gt; (1991)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Workers in a grass seed quality inspection laboratory</td>
<td>Symptoms resembling OTDS</td>
<td>Symptoms associated with high levels of endotoxin exposure (geometric mean of 1800 EUM&lt;sup&gt;−3&lt;/sup&gt; with levels up to 274 000 EUM&lt;sup&gt;−3&lt;/sup&gt; determined using the LAL assay). On SEM examination, microbial infestation was found in almost all seed samples</td>
<td></td>
<td>Smit &lt;i&gt;et al&lt;/i&gt; (2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study population</td>
<td>Exposure</td>
<td>Health effects</td>
<td>Threshold</td>
<td>Exposure-response</td>
<td>Comments</td>
<td>Study</td>
</tr>
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</tr>
<tr>
<td>240 workers in two Ukrainian fodder production plants</td>
<td>Plant 1: 48.2 mgm(^3) Plant 2: 6.8 mgm(^3) Endotoxin levels were 240 ngm(^3) and 1.8 ngm(^3) at the two plants respectively</td>
<td>Chronic bronchitis ODTS Chronic bronchitis ODTS 47.9 +/- 7.2% of workers with ODTS also had chronic bronchitis.</td>
<td>Prevalence 26.4 +/- 4.0% 39.7 +/- 4.4% 8.8 +/- 4.8% 14.7 +/- 6.0% Lung function clearly decreased with increasing duration of employment.</td>
<td>The length of service at the 1st plant was more than twice that at the 2nd. Respiratory symptoms were more strongly associated with dust exposure than with smoking. Examination of lung function revealed obstructive changes, particularly in exposed workers at 1st plant. The earliest signs of respiratory illness were obstruction of small bronchi and bronchial hyper responsiveness (registered in 74.7% of workers).</td>
<td>Kuchuk et al (2000)</td>
<td></td>
</tr>
<tr>
<td>Workers at an animal feed mill</td>
<td>0.2-150 mgm(^3)</td>
<td>Reduced lung function</td>
<td>Association with dust concentrations</td>
<td>Relationship between dust and lung function weaker than between endotoxin and lung function</td>
<td>Smid et al (1992)</td>
<td></td>
</tr>
</tbody>
</table>
### Table A5.3: Experimental exposure of human volunteers to organic dust

<table>
<thead>
<tr>
<th>Exposed population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>38 healthy volunteers with no previous exposure to farm dust</td>
<td>Exposure to swine dust while weighing swine for 3 hours; The median (25th to 75th percentile) concentration of inhalable dust was 21 (16 to 25) mg/m³ (endotoxin 1.2 (0.9 to 1.4) ug/m³; peptidoglycan at 6.5 (2.7 to 13) ug/m³)</td>
<td>Systemic inflammatory response assessed from an increase in serum interleukin-6</td>
<td>The association with symptoms was substantially stronger for endotoxin than dust. Peptidoglycan, but not endotoxin was correlated with an increase in the blood granulocyte concentration (a non-specific immune response) and in body temperature.</td>
<td>Zhiping <em>et al</em> (1996)</td>
<td></td>
</tr>
<tr>
<td>14 healthy, nonatopic, nonasthmatic, never-smoking volunteers</td>
<td>On 4 separate occasions subjects were exposed to inhalation challenges of LPS or Corn dust extract, each containing either a high (6 micrograms/mL) or low (0.9 microgram/mL) endotoxin concentration</td>
<td>Chest tightness, cough, dyspnea, and sputum production</td>
<td>A greater inflammatory response (as assessed from concentrations of inflammatory mediators and cells in blood) was observed in the high dose versus low dose experiments</td>
<td>Physiological and inflammatory response to inhaled corn dust extract was indistinguishable from the response to an equivalent dose of lipopolysaccharide (LPS)</td>
<td>Jagielo <em>et al</em> (1996)</td>
</tr>
<tr>
<td>6 normal subjects and 8 subjects with mild asthma</td>
<td>Exposure to ammonia (16-25 ppm) and/or endotoxin-rich grain dust (4 mg/m³)</td>
<td></td>
<td>No significant change in pulmonary function in the normal subjects following any of the exposure conditions. Among asthmatics, increased bronchial hyperreactivity and a significant transient decrease in lung function (FEV₁) were induced by grain. Concurrent exposure to ammonia did not have an important effect.</td>
<td>Sigurdarson <em>et al</em> (2004)</td>
<td></td>
</tr>
</tbody>
</table>
### A5.2 BACTERIA

**Table A5.4:** Exposure-response information for bacteria in studies of the waste industry; NAL – nasal lavage; CI – confidence interval, FVC – Forced Vital Capacity, a measure of lung function

<table>
<thead>
<tr>
<th>Exposed population</th>
<th>Control population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Threshold</th>
<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 waste collection workers involved in collecting food and garden waste - Norway</td>
<td>-</td>
<td>Median $0.84 \times 10^6$ Range 0.06-3.8$\times 10^6$ Total bacteria $m^{-3}$; Median 0, range 0-1.1 $\times 10^6$ actinomycetes spores $m^{-3}$</td>
<td>No relationship between measures of bacterial exposure (other than endotoxin) and effects on NAL parameters and symptoms</td>
<td>No relationship</td>
<td>Total bacteria by fluorescence microscopy; Actinomycetes by SEM Not possible to relate NAL parameters to symptoms; bacteria dominated by spherical species (median concentration $0.82 \times 10^6$ versus $0.06 \times 10^6$ for rod shaped);</td>
<td>Heldal et al (2003b)</td>
<td></td>
</tr>
<tr>
<td>25 waste collection workers involved in collecting food and garden waste - Norway</td>
<td>-</td>
<td>AM 1.2 $\times 10^6$ GM 0.8 $\times 10^6$ Range 0.06-3.8 $\times 10^6$ Total bacteria $m^{-3}$; AM 0.03 $\times 10^6$ GM 0.00 $\times 10^6$ Range 0-0.4 $\times 10^6$ actinomycetes spores $m^{-3}$</td>
<td>Significant decline in FEV over working week; no relationship between exposure and symptoms</td>
<td>&gt; $1.2 \times 10^6$ total bacteria</td>
<td>Not demonstrated that airborne bacteria specifically related to effects on symptoms or lung function, relationship between endotoxin and IL8 in induced sputum</td>
<td>Role of bacteria not described in detail; bacteria dominated by spherical species as above</td>
<td>Heldal et al (2003a)</td>
</tr>
<tr>
<td>Questionnaire study of 22 waste collection workers - Norway</td>
<td>-</td>
<td>AM 0.81 $\times 10^6$ GM 0.4 $\times 10^6$ Range 0.12-5.6 $\times 10^6$ Total bacteria $m^{-3}$, assessed fluorescence microscopy;</td>
<td>Significant association of total bacteria with nose irritation; significant association rod shaped bacteria with nose irritation, eye irritation, unusual tiredness; nonsignificant associations total bacteria and runny nose, eye irritation, unusual tiredness, headache, nausea</td>
<td>Nose irritation $0.9 \times 10^6$ total bacteria $m^{-3}$; 0.02 $\times 10^6$ “rod shaped” bacteria; eye irritation 0.01 $\times 10^6$ “rod shaped” bacteria; unusual tiredness 0.1 $\times 10^6$ “rod shaped” bacteria</td>
<td>Not demonstrated</td>
<td>Bacteria dominated by spherical species; bacteria highly correlated with endotoxin</td>
<td>Heldal &amp; Eduard, (2004)</td>
</tr>
<tr>
<td>Exposed population</td>
<td>Control population</td>
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<td>2303 waste collectors – 70% response rate - Denmark</td>
<td>1430 male municipal workers in mainly outdoor jobs (including unskilled gardeners)</td>
<td>7.8x10^4 cells/m^3 (total micro-micro-organisms minus fungi)</td>
<td>Nausea and diarrhoea</td>
<td>NOEL &lt; 6x10^5 cells m^-3 total micro-organisms; LOEL between 6 x 10^5 and 6 x10^6 cells m^-3 total micro-organisms</td>
<td>No clear relationship with total micro-organisms</td>
<td>Exposure assessment was based on measurement plus modelling based on questionnaire response on job tasks and time spent on different tasks; Nausea and gastrointestinal symptoms most prevalent in the summer; apparent link with odour intensity; Role of bacteria not specifically investigated, but exposure-response relationship found for endotoxin; comparison group likely to have had exposure to bioaerosol</td>
<td>Ivens et al (1997, 1999)</td>
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| Investigation of impacts of commercial composting of waste on health of local residents – 82 within 150-200m, 76 >200-400m, 55-400-500m - Germany | 142 controls; | Total bacteria in ambient air – filter based culture (R2A agar, oxoid), 25°C; Upwind, 500m, 8.4x10^2-1.8x10^5cfu m^-3; Downwind 200m 2.2x10^4-5.1x10^5 cfu m^-3 | Respiratory symptoms, limited range of other health endpoints – gastrointestinal symptoms, tiredness, shivering/fever; No relationship with joint trouble or muscular pain | >10^6 TOTAL MICRO-ORGANISMS about 10^2 cfu m^-3 for total bacteria, about 10^2 for thermophilic actinomycetes | OR (95% CI) for >10^6 cfu m^-3 – TOTAL MICRO-ORGANISMS (150-200m from site) Bronchitis 3.59 (1.40-9.47); Waking up due to coughing 6.59 (2.57-17.73); Coughing on rising/ during day 3.18 (1.24-8.36); Excessive tiredness 4.27 (1.56-12.15); Current medication 2.64 (1.08-6.60) | Exposure categorised by distance bands, 150-200m >10^6 cfum^-3 (Fig. 2.3) analysis did not separate fungi and bacteria – relative importance of fungi and bacteria in causation of effects uncertain; Effects greater in those residing at current address for more than 5 years (OR, 95% CI, Itching eyes 2.85 (1.31-6.50); nausea/vomiting 4.10 (1.28-18.44) shivering 3.67 (1.32-12.20); no relationship between odour complaints and respiratory symptoms | Herr et al (2003) |

<p>| Cross sectional study – 58 compost workers, 53 biowaste collectors, 40 controls - Germany | Controls newly employed compost workers (7) and waste collection workers (24) | Composting plants 10^3 cfu m^-3; biowaste collection 10^2 cfu m^-3; reference area &lt;10^3 cfu m^-3 Significantly increased antibody concentrations (IgG) against fungi and actinomycetes in workers at composting plants but not in biowaste collectors | Compost workers had significant raised risks of diseases of the airways, gastrointestinal symptoms, diseases of the skin but lowered risks of allergic rhinitis and atopy in family when compared with the controls; | No increase in respiratory, gastrointestinal or skin effects in waste collection workers implying 10^5 cfu is effectively a NOEL and the threshold for effects is between 10^5 and 10^6 cfu m^-3 | Significant association between the diseases and increased antibody concentrations in the compost workers; effects not specifically related to bacterial exposure as opposed to other bioaerosol components; | Mean duration of employment for compost workers was 3 years compared with 1.5 years for waste collection workers who were also younger; healthy worker effect indicated by the under representation of atopic diseases among the compost workers compared with biowaste collectors and the control group. | Burger et al (2000) |</p>
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<tr>
<th>Exposed population</th>
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<tbody>
<tr>
<td>218 compost workers at 41 composting plants; 5 year follow up study - Germany</td>
<td>66 full time office employees not exposed to organic dust</td>
<td>Increased incidence of mucous membrane irritation; Significant decline in lung function (FVC) and increase in prevalence of chronic bronchitis over 5 years;</td>
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<td></td>
<td></td>
<td>Pattern of health effects differs from those at other workplaces with organic dust exposure, possibly due to high levels of actinomycetes and filamentous fungi;</td>
<td>Bunger et al (2007)</td>
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<tr>
<td>15 workers in a new plant producing RDF pellets - Denmark</td>
<td>dust concentrations of 8.1 mgm⁻³ in September 1986 and microbes of up to 3 x 10⁹ cfu m⁻³</td>
<td>Over 8 months, 8/15 employees developed respiratory symptoms: 7 with bronchial asthma and one with chronic bronchitis; 4 had initial symptoms of the OTDS. 6 months later another case of asthma arose</td>
<td></td>
<td>Not assessed</td>
<td></td>
<td>Only 2/9 had previously had asthma or atopic disease; no evidence of type-I allergy; 6/9 had specific antibodies to refuse. In spring 1989, from 6-18 months after the onset of symptoms, 6 had dyspnoea on exertion, 3 had positive histamine-provocation tests 7/9 had left the plant.</td>
<td>Sigsgaard et al (1990)</td>
</tr>
</tbody>
</table>
## A5.3 ENDOTOXIN

### A5.3.1 Workplace studies

**Table A5.5:** Exposure-response information for endotoxin from the waste industry; LAL - Limulus Amoebocyte Lysate Chromogenic Assay using the kinetic rather than endpoint protocol; 1 ng endotoxin is equivalent to about 9 EU; where concentrations are reported in ng

<table>
<thead>
<tr>
<th>Exposed population</th>
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<tbody>
<tr>
<td>47 waste collectors, 22% employed for &lt;6 months - Netherlands</td>
<td>15 office workers from same facilities employed for &gt; 6 months</td>
<td>GM: 39.4 EU m(^{-3}), LAL</td>
<td>Changes in NAL parameters, nonsignificant increase in total cells, significant increase in IL8</td>
<td>Not determined, lowest exposure &lt;0.2 EU m(^{-3})</td>
<td>Increase in inflammation in waste collection workers during week; no significant difference between workers employed for &lt; or &gt; 6 months;</td>
<td>Waste collectors showed significantly more cough, phlegm and itchy nose than controls but prevalence of chronic respiratory symptoms higher in controls (smaller proportion of controls were current smokers); no evidence of habituation; stronger relationships between NAL parameters and dust than with endotoxin</td>
<td>Wouters et al (2002)</td>
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<tr>
<td>31 waste collection workers involved in collecting food and garden waste - Norway</td>
<td></td>
<td>median 13 EU m(^{-3}), Range 4-180 EU m(^{-3}), LAL</td>
<td>Evidence of mild upper airways inflammation in NAL but no relationship with respiratory symptoms</td>
<td>Nonsignificant increase in inflammatory markers in NAL at 25 EU m(^{-3})</td>
<td>Significant exposure-response relationship between endotoxin and inflammatory markers in NAL</td>
<td>Not possible to relate NAL parameters to symptoms; authors suggest low level endotoxin exposure may increase susceptibility to effects of beta (1-&gt;3) glucan;</td>
<td>Heldal et al (2003b)</td>
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<tr>
<td>25 workers involved in collecting food and garden waste - Norway</td>
<td></td>
<td>AM 31 EU m(^{-3}), GM 13 EU m(^{-3}), Range 4-180 EU m(^{-3}), LAL</td>
<td>Significant decline in FEV(_1) over working week; no relationship between exposure and symptoms</td>
<td>&lt; 50 EU m(^{-3})</td>
<td>SS exposure-response relationship between median exposure to endotoxin and IL8 in induced sputum</td>
<td>Not possible to relate IL8 in induced sputum to symptoms; significant decline in FEV(_1) over working week not specifically linked to endotoxin</td>
<td>Heldal et al (2003a)</td>
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<tr>
<td>Questionnaire study of 22 waste collection workers - Norway</td>
<td></td>
<td>AM 2.5 EU m(^{-3}), GM 1.8 EU m(^{-3}), Range 0.04-0.91 EU m(^{-3}), LAL</td>
<td>Significant association with nasal irritation; nasal irritation association with runny nose, eye irritation, cough, shortness of breath, unusual tiredness, headache, diarrhoea</td>
<td>Nose irritation reported at 4.5 EU m(^{-3})</td>
<td>Exposure monitoring may have influenced reporting of symptoms; endotoxin highly correlated with dust and with bacteria</td>
<td>Heldal &amp; Eduard (2004)</td>
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<tr>
<td>Exposed population</td>
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<tr>
<td>2303 waste collectors – 70% response rate – Denmark</td>
<td>1430 male municipal workers in mainly outdoor jobs (including unskilled gardeners)</td>
<td>0.1-500 EU m⁻³; based on measurement plus modelling based on job tasks and time spent on different tasks</td>
<td>Nausea and diarrhoea, prevalent in the summer</td>
<td>NOEL &lt;0.1 EU m⁻³; LOEL – 0.1-100 EU m⁻³</td>
<td>Exposure-response relationship demonstrated for diarrhoea and nausea; approx 5 fold increase in risk of diarrhoea at 500 EU m⁻³</td>
<td>Bioaerosol characterisation limited to endotoxin and fungi; effects appear more strongly associated with endotoxin than with fungi; possibility endotoxin marker of exposure to infectious agents; beta (1 &gt;3) glucan not measured;; apparent link with odour intensity</td>
<td>Ivens et al. (1999, 1997)</td>
</tr>
<tr>
<td>Investigation of impacts of indoor storage of organic waste on residents health – Germany</td>
<td>Poorly characterised – raised levels of endotoxin in settled dust</td>
<td>Significant increase in diarrhoea, fatigue, skin rash, doctor diagnosed allergy (except atopic dermatitis), increased risks greater in atopic individuals</td>
<td>Effects not specifically linked to endotoxin exposure</td>
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<td>Herr et al. (2004)</td>
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<td>Workers in 2 RDF plants with &gt;5 years service (Plants A and B) - US</td>
<td>GM (GSD) 2000 Survey A 22.5 (8.3) B 34.5 (5.5) 1995 Survey A 20.7 (2.0) B 38.4 (3.2) EU m⁻³ LAL 26/147 measurements &gt;100 EU m⁻³</td>
<td>Lung function – American Thoracic Society protocol – no significant excess decrement in lung function over 5 years</td>
<td>&gt; 38 EU m⁻³;</td>
<td>No correlation between duration of exposure and decline in lung function across group as a whole, significant decline in lung function in smokers employed for &gt;15 years</td>
<td>No evidence of serious long term impact on respiratory health associated with exposure to endotoxin concentrations of &lt; 38 EU m⁻³, authors commented on the stability of the workforce and the possibility that the absence of effects was partly due to a healthy worker effect – only those relatively resistant to respiratory illness remain in the industry</td>
<td>Mahar et al. (2002)</td>
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<td>As above</td>
<td>21.4-38.7 EU m⁻³</td>
<td>Sinus trouble, headaches, nose irritation, and diarrhoea reported by &gt;50% of employees. Small, statistically significant, cross-shift decrements in lung function</td>
<td>&lt;38 EU m⁻³</td>
<td>Workers employed seven years or more had significantly larger cross-shift decrements in lung function than those employed for a shorter period.</td>
<td>Threshold for short term effects on respiratory health</td>
<td>Mahar et al. (1999)</td>
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<td>Study of about 175 workers in 9 MRFs in England and Wales (159 participated)</td>
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<td>Plant means ranged from 1.86 – 31 ng m⁻³; highest measurement 198 ngm⁻³</td>
<td>Significant increase in cough with phlegm, hoarse/parched throat; Nonsignificant increase in nasal irritation/sneezing, stuffy nose, dry cough, chest tightness, nausea, stomach problems and skin rash</td>
<td>&lt;70 EU m⁻³</td>
<td>OR for &gt; 8 ngm⁻³ (approx 70 EU m⁻³) for a range of respiratory symptoms was about 3 (range from 1.6 for stuffy nose to 2.6 for hoarse throat) 95% CI typically 0.8-5); OR for nausea 2.9 (95% CI 0.8-10)</td>
<td>25% of workers had been employed for &lt;6 months in MRF and only 30% with &gt;2 years employment; tendency for self-reported respiratory and gastrointestinal symptoms to increase with length of employment</td>
<td>Gladding et al (2003)</td>
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<tr>
<td>Compost workers 14 in 1st survey 15 in 2nd survey - Netherlands</td>
<td>University staff/students 1st survey 6 2nd survey 9</td>
<td>GM 1st survey (2nd survey) Bulldozer – 75 (29) Tech personnel – 373 (108) Supervisors – 475 (242) Production workers 527 (285) EUm⁻³</td>
<td>1st survey – NAL parameters suggested higher level of chronic upper airways inflammation in compost workers; no significant difference between compost workers and controls in 2nd study</td>
<td>NOEL – 2nd survey 29-285 EU m⁻³; LOEL – 1st survey 75-527 EU m⁻³</td>
<td>Higher prevalence of nasal symptoms, cough and cough with phlegm during first survey relative to second, low incidence of shortness of breath, wheezing and chest tightness in both surveys; in 1st survey pre-shift inflammatory markers greater at &gt;461 EU m⁻³ (median exposure); but no correlation with cross shift changes</td>
<td>Two surveys in composting plant were carried out at an interval of one year during which time industrial hygiene at the plant was much improved; results may suggest potential recovery from chronic inflammation of the upper airways, but sample size extremely small; endotoxin and beta (1-3) glucan moderately correlated; limited evidence that exposure to &gt; 461 EU m⁻³ associated with development of chronic inflammation, although little evidence for acute effects at this level of exposure</td>
<td>Douwes et al (2000)</td>
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<td>8 collectors of compostable waste; 17 collectors of unsorted waste - Sweden</td>
<td>24 workers in local municipal administration</td>
<td>AM (SD) Compostable waste – 0.5 (0.3) ngm⁻³; unsorted waste – 0.7 (0.3) ngm⁻³</td>
<td>Significant increase in congested nose, cough with phlegm, unusual tiredness and diarrhoea; Nonsignificant difference between waste workers and controls for other respiratory symptoms; no effects on FEV or airways responsiveness (metacholine)</td>
<td>10 EUm⁻³ = NOEL for lung function and airways responsiveness; LOEL for congested nose, cough with phlegm, unusual tiredness and diarrhoea &lt;10 EUm⁻³</td>
<td>Limited bioaerosol characterisation, small study population, effects not clearly linked to endotoxin, lower macrophage counts and lymphocyte counts in induced sputum and lower levels of inflammatory markers in blood of exposed workers versus controls (authors attributed this to the ongoing nature of the exposure).</td>
<td>Thorn et al (1998)</td>
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<tr>
<td>218 compost workers at 41 composting plants; 5 year follow up study - Germany</td>
<td>66 full time office employees not exposed to organic dust</td>
<td>Median 16 ngm⁻³; Range 0.8-34 ngm⁻³; LAL</td>
<td>Increased incidence of mucous membrane irritation; Significant decline in %FVC predicted over 5 years; significant increase in 5 years in compost workers with chronic bronchitis;</td>
<td>LOEL between 0.8 and 34 ngm⁻³ (approx 7 and 300 EU m⁻³)</td>
<td>DR relationship not investigated</td>
<td>Specific role of endotoxin not highlighted, high levels of actinomycetes and filamentous fungi may be an important factor in development of reported effects</td>
<td>Burger et al (2007)</td>
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Table A5.6: Summary of exposure-response relationships for endotoxin reported in studies of other industry sectors

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<tr>
<th>Study population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Threshold</th>
<th>Exposure-response relationship</th>
<th>Comments</th>
<th>Study</th>
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<tr>
<td>Cross sectional study in 1032 UK workers in 9 industries exposed to organic dusts</td>
<td>&lt;5-50000 ngm(^{-3})</td>
<td>respiratory symptoms, organic dust toxic syndrome</td>
<td>exposures to &gt;5 ngm(^{-3}) are associated with more symptoms than baseline levels</td>
<td>exposure-response relationship shown for respiratory symptoms (log scale)</td>
<td>prevalence of symptoms ranged from 3% at 1 ngm(^{-3}), 10% at 10 ngm(^{-3}), 18% at 100 ngm(^{-3}), 25% at 1000 ngm(^{-3}); pooling of data across a number of industries may reduce the confounding effects of other dust components</td>
<td>Simpson et al (1998)</td>
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<tr>
<td>Dairy farms; comparison of farms where 11 farmers had experienced febrile reactions or hypersensitivity pneumonitis with farms where 17 farmers reported no symptoms</td>
<td>Highest measured endotoxin values: &lt;0.01 to &gt; 50 ugm(^{-3}) for symptom farms (median 6.4 ugm(^{-3}), GM 2.2 ugm(^{-3})); &lt; 0.01 to 50 ugm(^{-3}) for reference farms (median 42 ugm(^{-3}), GM 29 ugm(^{-3})). Background values in reference farms were 1.3 (median) and 0.4 (GM) ugm(^{-3}).</td>
<td>No relationship with hypersensitivity pneumonitis or fever was observed in farmers exposed to 10-50000 ngm(^{-3})</td>
<td>&gt;50 ugm(^{-3}) for some individuals, much lower for others</td>
<td>Concentrations of endotoxin on dairy farms where farmers had experienced febrile reactions or hypersensitivity pneumonitis were not significantly different from comparison farms. There were weak, but statistically significant, correlations between endotoxin concentrations and total spore count or dust concentrations. The authors expressed concern that the LAL assay might be sensitive to other components in the dust rather than endotoxin.</td>
<td>Rask-Andersen et al (1989)</td>
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<tr>
<td>22 workers exposed to swine dust for 3 hours during a period of work in a swine confinement building</td>
<td>Total inhalable dust 20.5 (14.6-30.0) mgm(^{-3}); endotoxin 1.2 (0.8-1.4) ugm(^{-3}), 3-OH fatty acid 3.5 (2.2-4.5) ugm(^{-3}), muramic acid 0.9 (0.3-1.9) ugm(^{-3}).</td>
<td>Fever (&gt; 38 degrees C) in three subjects, and approximately 25% of the subjects experienced symptoms</td>
<td>&gt;1.2 ugm(^{-3})</td>
<td>Bronchial responsiveness to methacholine increased by 3.5 (1.6-4.8) doubling doses (median (25th-75th percentile)). There was a significant correlation between the inflammatory marker IL-6 in BAL fluid and exposure to dust endotoxin activity and 3-OH fatty acids (p &lt; 0.05).</td>
<td>Wang et al (1997)</td>
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<tr>
<td>29 UK pig farmers</td>
<td>mean inhalable dust concentrations in 20 pig houses ranged from 1.7 to 21.0 mgm(^{-3}); inferred airborne endotoxin concentrations ranged up to 25-50 ngm(^{-3});</td>
<td>23 of 29 workers complained of at least one of: nasal and eye irritation, cough, chest tightness/wheeze</td>
<td></td>
<td>5 of 11 workers with chest tightness showed specific IgE to meal extract and 3 other workers with specific IgE to meal extract had symptoms of irritation; Bacterial concentrations were high with up to 10(^7) cfum(^{-3}) but fungal concentrations were relatively low (up to 10(^7) cfum(^{-3})). co-exposure to ammonia and dust would have contributed to irritative response</td>
<td>Crook et al (1991)</td>
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<tr>
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<td>207 piggery workers with two years of follow up</td>
<td>GM concentrations in 2 surveys: total dust- 3.73 and 3.32 mgm⁻³; respirable dust- 0.2 and 0.3 mgm⁻³; total endotoxin - 133 and 130 EUm⁻³; respirable endotoxin -18.5 and 5.6 EUm⁻³; ammonia - 5.35 and 4.17 ppm</td>
<td>Endotoxin strongly associated with cross shift changes in lung function in workers with less than 6 years exposure but no association in workers with longer exposure</td>
<td>&lt;130 EUm⁻³</td>
<td>Magnitude of cross-shift change in lung function was significantly correlated with personal exposures to total dust, total endotoxin, respirable endotoxin, and ammonia.</td>
<td>Authors suggest that the correlation of dust with FEV₁ changes in workers with more than 6 years of indicates that dust exposure is an important factor in chronic respiratory disease and that similarly the correlation of endotoxins with FEV₁ changes in the group with less than 6 years exposure suggests endotoxins may have more significance for subacute respiratory effects.</td>
<td>Reynolds et al (1996)</td>
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<tr>
<td>38 healthy subjects exposed to swine dust while weighing swine for 3 hours</td>
<td>The median (25th to 75th percentile) concentration of inhalable dust was 21 (16 to 25) mgm⁻³. LPS (LAL) was 1.2 (0.9 to 1.4) ugm⁻³; LPS (GC-MS) was 3.9 (2.5 to 4.9) ugm⁻³; and the peptidoglycan concentration in airborne dust was 6.5 (2.7 to 13) ugm⁻³.</td>
<td>LPS(LAL) correlated with symptoms, an increase in bronchial responsiveness, reduced lung function (vital capacity) and the inflammatory mediator interleukin-6 in serum</td>
<td>&lt;9000 EUm⁻³</td>
<td>All exposure markers correlated significantly with an increase in serum interleukin-6. LPS(LAL) showed the highest correlation (r² = 0.29) and total inhaled dust the lowest (r² = 0.09). Peptidoglycan, estimated from muramic acid measured with GC-MS correlated with an increase in the blood granulocyte concentration and in body temperature.</td>
<td>Zhiping et al (1996)</td>
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<tr>
<td>57 workers on 30 swine farms in southern Sweden and 55 matched controls.</td>
<td>Swine workers reported significantly higher frequencies of respiratory symptoms, more frequent colds and absence due to chest illness, and a history of pneumonia.</td>
<td>Adverse effects on lung function at 40-350 ngm⁻³; 80 ngm⁻³ identified as a threshold for effects</td>
<td>Multiple regression analysis of the relation between 16 environmental parameters and 5 lung function parameters revealed significant dose-response relation between endotoxin and FEV₁</td>
<td>Increased frequency of symptoms of respiratory disease was related to the number of years and percent of the day spent working with swine. Symptoms were also associated with respirable dust, total dust, endotoxin in total dust, and number of microbes in the air of the work environment.</td>
<td>Donham (1989)</td>
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<td>Study population</td>
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<td>Review of 14 epidemiologic studies from four countries, involving 2,786 piggery workers</td>
<td></td>
<td>Prevalence of cough and phlegm: 12 to 55%; prevalence of tightness of chest and wheezing: 12 to 33%; ODTS reported; Decrements in flow rates, but not volumes of baseline lung function; over-shift changes showed small decrements in both flows and volumes.</td>
<td>Limited exposure response information, endotoxin associated with decrements in lung function and respiratory symptoms.</td>
<td></td>
<td>Acute symptoms, directly associated with work, were from 1.5 to 2 times more prevalent than chronic symptoms. IgG antibodies to swine house antigens were common but were not associated with symptoms</td>
<td>Donham (1990)</td>
</tr>
<tr>
<td>23 poultry workers</td>
<td>Mean level of total dust was 6.3 mgm⁻³ (range 0.4-15.3) and of endotoxins 0.40 µgm⁻³ (range 0.02-1.50). Total levels of 4 x 10⁴⁻⁴ x 10⁶ cfum⁻³ of airborne bacteria, mainly coagulase-negative staphylococcal strains, 500-4000 cfum⁻³ of fungi; cross-shift increase in respiratory symptoms and decrease in VC (3.1%) and FEV₁ (4.1%)</td>
<td>No effects on lung function at 20-1500 ngm⁻³ function</td>
<td>No associations between these over-shift decreases and the individual time-weighted average breathing zone levels of either total dust or of endotoxins.</td>
<td></td>
<td>None of the workers had experienced any symptoms of hypersensitivity pneumonitis or ODTS</td>
<td>Hagmar et al (1990)</td>
</tr>
<tr>
<td>Population-based, cross-sectional investigation among grain handlers and postal workers</td>
<td>Mean total endotoxin of grain workers 2859 EUm⁻³ (std 7209); respirable endotoxin 83 EUm⁻³ (std 278)</td>
<td>Significantly higher prevalence of work-related (cough, phlegm, wheezing, chest tightness, and dyspnoea) and chronic (usual cough or phlegm production) respiratory symptoms in grain workers</td>
<td>Exposure-response for respiratory symptoms (Fig. 2.10)</td>
<td>After controlling for age, gender, and cigarette smoking status, work-related respiratory symptoms, reduced lung function (measured as air flow) and bronchial reactivity were strongly associated with endotoxin. Total dust concentration had little effect on spirometric measures of airflow (FEV₁, FEV₁/FVC, and FEF₂₅₋₇₅) and airway reactivity and was only weakly associated with respiratory symptoms</td>
<td>Schwartz et al (1995)</td>
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<tr>
<td>Study population</td>
<td>Exposure</td>
<td>Health effects</td>
<td>Threshold</td>
<td>Exposure-response</td>
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<td>Comparison of 97 male paper mill workers with 55 control workers</td>
<td>Median (Max-Min) 69 (370-6) EU m⁻³ at the wet-end of the paper machines; 6 (19-16) in the pulping area.</td>
<td></td>
<td>&gt;200 EU m⁻³</td>
<td>No evidence that lung function decrements increased with increased endotoxin exposure</td>
<td>No evidence that exposures &lt; 200 EU m⁻³ had any impact on lung function decline over an 11 year period. A greater prevalence of respiratory symptoms in persons lost to follow up suggests possible health worker effect</td>
<td>Sigsgaard et al (2004)</td>
</tr>
<tr>
<td>Study of 77 workers in 9 workplaces (slaughter houses, grain/vegetable storage, animal feed, garbage handling, cotton mill, printing plant, wood industry, metal working)</td>
<td>Median personal exposure concentration (ng m⁻³)</td>
<td>Half of the surveyed 77 workers reported respiratory symptoms, 27% eye symptoms, and 10% fever or shivering.</td>
<td>Greater proportion of workers with respiratory symptoms at &gt;25 ng m⁻³.</td>
<td></td>
<td>Biologically-active endotoxins as assessed with the LAL assay were more closely related to self-reported symptoms in workers than the total amount of endotoxins analysed as 3-hydroxy (OH) fatty acids with GC-MS. Specific 3-OH 14:0 fatty acid in the total endotoxin samples associated better with the symptoms than other 3-OH fatty acids.</td>
<td>Laitinen et al (2001)</td>
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<tr>
<td>Animal feed mill workers</td>
<td>effects on lung function at 0.2-470 ng m⁻³.</td>
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<td>Smid et al (1992)</td>
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<tr>
<td>443 cotton workers from 2 factories in Shanghai and 439 control subjects from a nearby silk mill</td>
<td>Total elutriated dust concentration (range: 0.15 to 2.5 mg m⁻³) and endotoxin (range: 0.002 to 0.55 ug U.S. Reference Endotoxin/m3)</td>
<td>Reduced lung function, byssinosis, chronic bronchitis</td>
<td>A dose-response trend was seen with the current endotoxin level and FEV₁, delta FEV₁%, and the prevalence of byssinosis and chronic bronchitis, except for the highest exposure level group in which a reversal of the trend was seen.</td>
<td>No dose-response relationships were demonstrated comparing dust concentration to any pulmonary function or symptom variable. The regression coefficients for current endotoxin exposure were significant (p less than 0.05) in the models for FEV₁ and chronic bronchitis but not in the models for delta FEV₁% (i.e., acute change in FEV₁) or byssinosis prevalence.</td>
<td>Kennedy et al (1987)</td>
<td></td>
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<tr>
<td>Cross-sectional study of respiratory disorders and atopy in Danish textile industry workers at cotton mills, a wool mill, and a man-made fibre (MMF) mill; 409/445 workers participated</td>
<td>Respirable endotoxin: Cotton mill: 9-126 ng m⁻³ Wool mill: 11-49 ng m⁻³ MMF: 6 ng m⁻³ Total endotoxin: Cotton mill: 33-325 ng m⁻³ Wool mill: 32-109 ng m⁻³ MMF: 3 ng m⁻³</td>
<td>Effects on lung function, development of byssinosis in cotton workers at exposure levels of 33-325 ng m⁻³</td>
<td>Exposure-response relationships reported for lung function, byssinosis and chronic bronchitis</td>
<td>See Figures 2.1 and 2.2</td>
<td>Sigsgaard et al (1992)</td>
<td></td>
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<tr>
<td>Study population</td>
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</table>
| Study of longitudinal changes in lung function and respiratory symptoms from 1981 to 2001 in 447 cotton textile workers, along with 472 silk textile controls | Mean (std) EUm$^{-3}$ years 49123 (45284) 
Equivalent concentration over 20 years – EUm$^{-3}$ 2456 (2264) | Cotton workers had more persistent respiratory symptoms and greater annual declines in forced expiratory volume in one second (FEV1) and forced vital capacity as compared with silk workers. | | | After exposure cessation, in the final 5-yr period, the rate of FEV1 decline tended to slow in nonsmoking males, but not in nonsmoking females. Workers who reported byssinotic symptoms more persistently suffered greater declines in FEV1. Chronic loss in lung function was more strongly associated with exposure to endotoxin than to dust. | Wang et al (2005) |
| Cotton workers | Median endotoxin concentration 450 (range 4-7177) EUm$^{-3}$; concurrent exposure to mould 52-4029 cfum$^{-3}$ and bacteria 4293 cfum$^{-3}$ | Significant cross shift impacts on lung function consistent with an obstructive ventilation pattern associated with current exposures exceeding 450 EU m$^{-3}$. | | | Exposure-response relationship found for endotoxin but not other exposure parameters (moulds, bacteria and dust). | Oldenburg et al (2007) |
| Australian sawmill workers | The geometric mean and standard deviation of endotoxin concentrations averaged across 3 plants were 6.61 and 5.22 ngm$^{-3}$ respectively. Mean concentrations of inhalable dust were 6.3 mgm$^{-3}$ and mean concentrations of gram negative bacteria and fungi in the three plants ranged from 7.81-11.65 x10$^{3}$ cfum$^{-3}$ and 41.2-54.2 cfum$^{-3}$ respectively. | Significantly increased prevalence of regular cough, chronic bronchitis, regular blocked nose, regular sneezing, sinus problems, flu-like symptoms, and eye and throat irritation | Significant association between endotoxin and health endpoints including chronic bronchitis, blocked nose and headache | The prevalence of blocked nose, chronic bronchitis and headache in exposed workers was 36, 49 and 55% versus 12, 29 and 18% in the controls. Inhalable dust and gram –live bacteria were also correlated with the prevalence of frequent headache. Significant positive correlations were found among endotoxin and Gram (-)ve bacteria, beta (1--->3) glucan and fungi, and endotoxin and beta (1--->3) glucan exposure levels. The relative importance of endotoxin versus other bioaerosol components in giving rise to effects is difficult to determine from the published study. | Mandryk et al (2000) |
| Printing factory workers where humidifier was contaminated with Pseudomonas | 0.13-0.39 ugm$^{-3}$. | 20/50 workers in a printing factory reported typical symptoms of fever, chills, and chest tightness when the humidifiers were operating | | | | Rylander & Haglind (1984) |
### A5.3.2 Experimental exposure of humans to endotoxin

#### Table A5.7: Inhalation experiments in human volunteers were exposed to endotoxin

<table>
<thead>
<tr>
<th>Exposure Regime</th>
<th>Exposed population</th>
<th>Exposure</th>
<th>Exposure concentration averaged over 24 hours</th>
<th>Reported effects</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Repeated exposure to LPS (four exposures to LPS at 2 weekly intervals)</td>
<td>20 young healthy volunteers</td>
<td>Successive doses of LPS that were increased every 30 minutes to give a cumulative exposure of 100 ug/exposure</td>
<td>5 ug m⁻³, approximately 4.5x10⁴ EU m⁻³</td>
<td>During 71 provocations, 13 episodes of clinical complaints were observed in 10 subjects with 11 local reactions (15.5%, e.g., cough), and 6 systemic reactions (8.5%, e.g., fatigue). All adverse events resolved within 10 hours. Changes of FEV₁ and expired NO showed no significant differences between visits. Most subjects (88.2% on visit 1-3, 76.5% on visit 4) showed a rise in body temperature (&gt;0.5°C) that normalised within 24 hours.</td>
<td>Kitz et al (2007)</td>
</tr>
<tr>
<td>Subjects exposed to successively greater doses of LPS during the course of each exposure session. FEV₁, determined after each provocation and up to 24 hours later; the procedure was stopped when FEV₁ declined more than 12.5%.</td>
<td>43 adult volunteers (13 asthmatics, 30 healthy controls)</td>
<td>LPS inhaled every 30 minutes up to a cumulative dose of 100 ug (2.5, 10.5, 42, 45 ug)</td>
<td>5 ug m⁻³, approximately 4.5x10⁴ EU m⁻³</td>
<td>LPS induced a similar reduction in lung function in healthy and asthmatic subjects, but in both groups there were some subjects who showed a substantially greater response than less sensitive subjects. Both healthy and asthmatic volunteers showed a similar systemic response to LPS inhalation. Significant increases were found for polymorphonuclear neutrophils (inflammatory cells), C-reactive protein and LPS binding protein. LPS had no impact on expired NO in healthy subjects. Bronchial expired NO increased only temporarily in asthmatics. Sensitive asthmatics had lower expired NO than less sensitive but showed a greater increase in expired NO on challenge with LPS.</td>
<td>Kitz et al (2006)</td>
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<tr>
<td>Exposure Regime</td>
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<tr>
<td>Retrospective analysis of data from volunteer experiments (see exposure response information in Figure 5.12, Main report)</td>
<td>119 subjects</td>
<td>Up to 41.5 ug</td>
<td>Up to 2.08 ug m⁻³, approximately 2 x 10⁵ EUm⁻³</td>
<td>Fever occurred in 30% of subjects and was associated with a higher cumulative dose of LPS. Two subjects showed fever following exposure to only 6.5 ug (approximately equivalent to inhalation of 3200 EUm⁻³ over 24 hours). Maximum body temperature arose more than 3 hours following exposure. A dose related reduction in mean arterial pressure occurred in 21% of subjects. Fever and decreased arterial pressure were unrelated to airway responsiveness to inhaled LPS. Reported symptoms included: chills (64%), malaise (56%), cough (56%), chest tightness (49%), headache (43%), and myalgias (27%).</td>
<td>Sundy et al (2006)</td>
</tr>
<tr>
<td>LPS inhalation combined with isotonic saline challenge</td>
<td>18 healthy non-atopic human</td>
<td>either 15 ug (n=10) or 50 ug (n=8) Escherichia coli LPS by inhalation</td>
<td>0.75 or 2.5 ug m⁻³, approximately 7 x 10⁷ or 2x10⁸ EUm⁻³</td>
<td>Acute flu-like symptoms, fever and inflammatory markers in blood and induced significantly greater in the 50 ug than 15 ug LPS group. These changes were resolved at one week. In the 50 ug dose group, there was a reduction in the proportion of peripheral blood interferon (IFN)-gamma-producing CD4+ and CD8+ T cells at 6h followed by an increase at 1 week after inhaled LPS.</td>
<td>Loh et al (2006)</td>
</tr>
<tr>
<td>Study of effects of inhaled LPS on allergic airways responses to air pollution through its effects on dendritic cell maturation and associated loss of phagocytic activity.</td>
<td>9 healthy volunteers</td>
<td>Clinical Center Reference Endotoxin (CCRE; 20,000 EU)</td>
<td>1000 EU m⁻³</td>
<td>Elevated neutrophils in the airways, blunted phagocytosis (monocytes, macrophages) that was negatively correlated with PMN influx and associated with increased levels of GM-CSF and IL-1beta, potent dendritic cell maturation agents.</td>
<td>Alexis et al (2005)</td>
</tr>
<tr>
<td>Recovery of sputum and peripheral blood after saline and LPS challenge.</td>
<td>10 atopic asthmatic subjects</td>
<td></td>
<td></td>
<td>Inhalation of endotoxin at levels adequate to induce a neutrophil influx to the airways (but not systemic symptoms) resulted in decreased phagocytosis in both airway and circulating cells</td>
<td>Alexis et al (2003)</td>
</tr>
<tr>
<td>Investigation of CD14, the principal receptor mediating LPS responses in vivo. Induced sputum was collected at 24 hours before and 6 hours after inhalation.</td>
<td>10 atopic asthmatic subjects and 8 healthy control subjects</td>
<td>to 0.9% saline and LPS (Escherichia coli 026:B6, 5 ug)</td>
<td>0.25 ug m⁻³; approximately 2000 EU m⁻³</td>
<td>Significant associations were found between the LPS-induced neutrophilic response in sputum and both constitutive sCD14 and membrane-bound CD14. Asthmatic subjects demonstrated significantly higher levels of constitutive sCD14 than control subjects and baseline eosinophils were significantly associated with baseline sCD14 and LPS-induced inflammatory response.</td>
<td>Alexis et al (2001)</td>
</tr>
<tr>
<td>Exposure Regime</td>
<td>Exposed population</td>
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<td>Exposure concentration averaged over 24 hours</td>
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<tr>
<td>Inhalation exposure to increasing concentrations of inhaled LPS</td>
<td>9 volunteers</td>
<td>0.5 ug, 5.0 ug, and 20 ug</td>
<td>0.025, 0.25 and 1 ug m⁻³; approximately 200, 2000 and 10^4 EUm⁻³</td>
<td>2 individuals with a marked clinical response developed marked monocyte cell surface CD14 (expressed as mean linear fluorescence) upregulation. Otherwise upregulation of CD14 was not significantly different that observed with the placebo.</td>
<td>Fishwick et al (2004)</td>
</tr>
<tr>
<td>Weekly inhalation</td>
<td>15 healthy subjects</td>
<td>saline solution or LPS (0.5, 5, or 50 ug).</td>
<td>0.025, 0.25 and 2.5 ug m⁻³; approximately 200, 2000 and 2x10³ EUm⁻³</td>
<td>Significant increase in body temperature in 7 subjects was associated with a greater systemic inflammatory response (blood neutrophils and concentrations of C-reactive protein and LPS binding proteins). A significant increase in airway responsiveness in 8 subjects was associated with an increase in the sputum concentration of eosinophil cationic protein. The amplitude of the systemic response and decrease in FEV₁ were inversely associated with the atopic status.</td>
<td>Michel et al (2001)</td>
</tr>
<tr>
<td>weekly by inhalation with saline and 3 doses of LPS</td>
<td>9 healthy subjects</td>
<td>0.5, 5, and 50 ug LPS (Escherichia coli).</td>
<td>0.025, 0.25 and 2.5 ug m⁻³; approximately 200, 2000 and 2x10³ EUm⁻³</td>
<td>Inhalation of 0.5 ug LPS induced dose-related systemic inflammation marked by changes levels of inflammatory cells and mediators in blood</td>
<td>Michel et al (1997)</td>
</tr>
<tr>
<td>exposed by inhalation of a solution containing LPS a week after bronchial challenge with control solution</td>
<td>6 normal non-atopic subjects</td>
<td>20 ug LPS (from Escherichia coli 026:B6)</td>
<td>1 ug m⁻³; approximately 9000 EUm⁻³</td>
<td>No response in lung function was observed for 6 hours after the LPS inhalation. An increase in the level of luminal enhanced-chemiluminescence (luminol-CL, as a marker of the degree of activation of neutrophils) occurred at 120 minutes before changes in neutrophil counts at 300 and 360 minutes. After 24 and 48 hours the acute-phase C-reactive protein was significantly raised with other proteins C3 and haptoglobin being unchanged. A slight increase in adrenocorticotropic hormone was observed 240 and 360 minutes.</td>
<td>Michel et al (1995)</td>
</tr>
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<td>Exposure Regime</td>
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<tr>
<td>Volunteers subjected to bronchial challenge tests, in a single-blind trial, on Day 1 with control solution and on Day 7 with 20 micrograms endotoxin of Escherichia coli (026:B6).</td>
<td>8 patients with mild asthma</td>
<td>20 ug LPS (from Escherichia coli type 026:B6)</td>
<td>1 ugml-3 approximately 9000 EUm-3</td>
<td>A significant bronchial obstructive response was demonstrable 45 minutes after LPS inhalation, lasting 5 hours; a significant increase in airways hyper-responsiveness at 6 hours LPS, partially normalized at 24 and 48 hours. A short peak in TNF-alpha at 60 minutes, an increase in total white blood cells and neutrophil polymorphonuclear neutrophils at 360 min and C-reactive proteins at 24 and 48 hours were significant.</td>
<td>Michel et al (1992a)</td>
</tr>
<tr>
<td>Inhalation study in subjects with hyperresponsive airways as determined by a fall in specific airway conductance of 40% (PD40sGaw) after inhaling up to 900 ug histamine</td>
<td>16 subjects with hyperresponsive airways</td>
<td>Dust mite antigen and endotoxin (Escherichia coli 026:B6) at doses of 100 AU and 1000 ng, respectively</td>
<td>0.05 ugml-3 approximately 450 EUm-3</td>
<td>Significant increases in the total and differential inflammatory cell counts was observed in NAL at 8 hours after dust mite/endotoxin exposure.</td>
<td>Michel et al (1992b)</td>
</tr>
<tr>
<td>Inhalation of endotoxin and allergen Volunteers with atopic asthma</td>
<td>10 atopic asthmatic subjects and 6 normal subjects</td>
<td>saline and 0.1, 0.3 and 1.0 ug of LPS</td>
<td>LPS (1.0 ug) increased the percent of eosinophils in nasal lavage fluid 4 hours after challenge in atopic subjects; correlation between constitutive nasal GM-CSF and eosinophil response to LPS in atopic subjects.</td>
<td>No change in cardiovascular parameters or lung function; significant rise in temperature in asthmatic patients with a peak of 0.6 °C at seven hours; no change in absolute cell counts at any time point in asthmatics; no change in neutrophil counts in the atopic subjects; significant rise in sputum interleukin 8 (IL-8) concentrations in normal subjects at 6 hours and in asthmatics at 24 hours; no changes in sputum concentrations of tumour necrosis factor alpha or granulocyte macrophage colony stimulating factor at any time.</td>
<td>Peden et al (1999)</td>
</tr>
<tr>
<td>Nasal exposure to LPS</td>
<td>11 non-atopic normal subjects, seven atopic, non-asthmatic individuals, and eight atopic, asthmatic patients</td>
<td>60 ug</td>
<td>3 ugml-3 approximately 2.7x106 EUm-3</td>
<td>No change in cardiovascular parameters or lung function; significant rise in temperature in asthmatic patients with a peak of 0.6 °C at seven hours; normal subjects, significant rise in absolute neutrophil counts at 24 hours in normal subjects but no change in differential counts. significant rise in differential neutrophil counts at 6 hours but no change in absolute cell counts at any time point in asthmatics; no change in neutrophil counts in the atopic subjects; significant rise in sputum interleukin 8 (IL-8) concentrations in normal subjects at 6 hours and in asthmatics at 24 hours; no changes in sputum concentrations of tumour necrosis factor alpha or granulocyte macrophage colony stimulating factor at any time.</td>
<td>Nightingale et al (1998)</td>
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<td>Exposure Regime</td>
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<td>Exposure by inhalation with examinations before and 24 hours after exposure</td>
<td>21 healthy subjects</td>
<td>40 micrograms LPS; Eleven of the subjects inhaled hypertonic saline without endotoxin exposure as controls</td>
<td>2 ug m⁻³ approximately 1.8x10⁴ EUm⁻³</td>
<td>A significantly higher proportion of subjects reported respiratory and general symptoms after endotoxin inhalation. MPO and the number of neutrophils in the blood were higher and spirometrical values were decreased after the LPS challenge. In the sputum MPO, ECP, and the numbers of neutrophils and lymphocytes were higher after the LPS.</td>
<td>Thorn and Rylander (1998)</td>
</tr>
<tr>
<td>LPS inhalation followed by bronchoalveolar lavage</td>
<td>8 healthy nonsmoking subjects</td>
<td>100 ug LPS from E. coli dissolved in 2 ml isotonic NaCl, aerosolized with a jet nebulizer calculated dose delivered to the lung approximately 25 ug.</td>
<td>1.25 ug m⁻³ = approximately 1.1 x10⁴ EU m⁻³</td>
<td>An approximate 100-fold increase in neutrophils and 3-fold increase in lymphocytes in BAL; recovered alveolar macrophage showed significantly reduced phagocytosis of opsonized yeast particles in vitro, increase in fibronectin. Without changes BAL albumin, indicating that the elevated level of fibronectin could not be explained by an increased permeability, but rather by a local production.</td>
<td>Sandstrom et al (1992)</td>
</tr>
<tr>
<td>Subjects subjected to serial testing to demonstrate that the response to inhaled LPS was reproducible.</td>
<td>72 volunteers</td>
<td>Exposure to increasing doses of LPS: 0.5 µg, 1.0 µg, 2.0 µg, 3.0 µg, 5.0 µg, 10 µg, and 20 µg to a total of 41.5 µg of LPS</td>
<td>≤3000 EUm⁻³, 20000 EUm⁻³</td>
<td>8 &quot;sensitive&quot; subjects had at least 20% decline in their FEV₁, after inhaling ≤5.5 ug, whereas 11 &quot;hyporesponsive&quot; subjects maintained an FEV₁ &gt;90% of their baseline after inhaling 41.5 ug LPS. Sensitive subjects were more commonly female and hyporesponsive subjects were more often male (p = 0.016). Peripheral blood monocytes from hyporesponsive subjects, compared with sensitive subjects, released less interleukin (IL)-6 and IL-8.</td>
<td>Kline et al (1999)</td>
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<tr>
<td>Subjects exposed to respirable dust generated by the carding of unwashed and washed cottons from the 3 major growing regions of the US in an experimental cardroom,</td>
<td>30 volunteers</td>
<td>18 x 6 hour exposures Dust concentrations of 0.59 mgm⁻³ +/- 0.04 for all exposures</td>
<td></td>
<td>Significant correlation between endotoxin exposure and acute decrease in FEV₁; Effect on FEV₁ per ng of airborne endotoxin was greater for Mississippi cotton than for cotton from the other regions; Water washing of cotton results in reduced airborne endotoxin and less bronchoconstriction.</td>
<td>Petersen et al (1986)</td>
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<tr>
<td>Subjects exposed to respirable dust experimental cardroom. Cotton from different geographical locations with varying amounts of endotoxin were used</td>
<td>Workers from cotton mills</td>
<td>dust: 0.6 to 3.8 mgm⁻³; endotoxin: 0.1 to 8.0 ug m⁻³ (900 to 72000 EUm⁻³)</td>
<td>225 to 180000 EUm⁻³</td>
<td>Airborne endotoxin correlated with decrease in FEV₁; and increase in blood neutrophils; Significant reduction in FEV₁ at all exposure levels for endotoxin; no relationship between the decrease in FEV₁ and airborne dust; FEV₁ decrease was more pronounced among smokers</td>
<td>Rylander &amp; Haglind (1986)</td>
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<td>Exposure Regime</td>
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<td>Exposure in an experimental cardroom</td>
<td>68 students and 39 cotton mill workers</td>
<td>Thresholds for no effects on FEV(_1) were 0.58 mgm(^{-3}) dust and 0.17 ugm(^{-3}) endotoxin for students; 0.43 mgm(^{-3}) and 0.08 ugm(^{-3}) for smoking workers.</td>
<td>about 4000 EUm(^{-3}) Dose related decrease found for FEV(_1) which was more pronounced in smoking workers</td>
<td>Haglind &amp; Rylander (1984)</td>
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<td>6 hours of exposure to card-generated cotton dust from 7 different cottons (of several grades and growing regions).</td>
<td>52 healthy humans, selected for their acute airway responsiveness to cotton dust</td>
<td>Significant correlations between concentrations of airborne bacteria (total and gram negative), dust and endotoxin</td>
<td>Concentrations of airborne bacteria (total and gram negative), vertically elutriated gravimetric dust, and vertically elutriated endotoxin were associated with exposure-related acute changes in lung function with endotoxin being the most highly correlated and gravimetric dust was the least correlated</td>
<td>Castellan et al (1984)</td>
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<tr>
<td>Exposure to cotton dusts in an experimental cardroom</td>
<td>Pooling of data from a total of 108 separate sessions of exposure to dust and 32 different cottons.</td>
<td>Average concentrations of airborne dust ranged from 0.12 to 0.55 mgm(^{-3}) and of endotoxin ranged from 6 to 779 ngm(^{-3})</td>
<td>Group mean percentage change in FEV(_1) was unrelated to dust concentrations but significant exposure-response relation between endotoxin and FEV(_1). Logarithmic transformation of endotoxin values improved the correlation</td>
<td>Castellan et al (1987)</td>
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Table A5.8: Summary of experiments in which volunteers were systemically exposed to endotoxin by injection

<table>
<thead>
<tr>
<th>Exposure Regime</th>
<th>Exposed population</th>
<th>Exposure</th>
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</thead>
<tbody>
<tr>
<td>Intravenous administration</td>
<td>healthy adults</td>
<td>Escherichia coli LPS (4 ng/kg)</td>
<td>14 ngm$^{-3}$; 126 EU m$^{-3}$</td>
<td>Increased plasma concentrations of pro-inflammatory cytokine and increased cortisol levels that remained two- to threefold above baseline throughout the protocol.</td>
<td>Lang et al (1997)</td>
</tr>
<tr>
<td>Study of effect of effects of injected endotoxin</td>
<td>six healthy subjects</td>
<td>Escherichia coli endotoxin (2 ng/kg body weight)</td>
<td>7 ngm$^{-3}$; 63 EU m$^{-3}$</td>
<td>Increased plasma concentrations of inflammatory cytokines after 30 to 45 minutes that reached a maximal level after 60 to 90 minutes. A transient increase in body temperature and pulse rate occurred simultaneously with the cytokine increases, whereas a significant decrease in blood pressure occurred after 120 minutes. Coagulation activation (increased prothrombin fragments and thrombin-antithrombin III complexes) also occurred at 120 minutes. No complement activation was detected.</td>
<td>Van Daventer et al (1990)</td>
</tr>
<tr>
<td>Study of effects of endotoxin on sleep in which endotoxin administration was followed by a 4-hour period of quiet wakefulness in bed (light intensity &lt; 200 lux). Unlimited sleep was allowed after 2300 hours (lights off) until the next morning</td>
<td>17 healthy men (including unexposed controls)</td>
<td>Injection of endotoxin, at 0.4 or 0.8 ng/kg body weight at 1900 hours</td>
<td>1.4 ngm$^{-3}$ or 2.8 ngm$^{-3}$; 12 or 25 EU m$^{-3}$</td>
<td>Increased body temperature and heart rate from approximately 2 hours administration that persisted through most of the sleep period. Sleep latency remained unchanged, rapid eye movement (REM) sleep latency increased from 60.3 to 89.0 minutes, Stage 2 sleep increased from 45.5 to 49.0% of time in bed, total nonrapid eye movement (NREM) sleep increased from 64.2 to 69.1% but slow-wave sleep (SWS, stages 3 and 4) was unchanged.</td>
<td>Trachsel et al (1994)</td>
</tr>
<tr>
<td>Single-blind placebo-controlled crossover design investigation of the effects of Salmonella abortus equi endotoxin administered intravenously in the morning on the primary response and on daytime sleep by use of a multiple napping protocol in healthy volunteers.</td>
<td>0.8 ng/kg body weight at 1900 hours</td>
<td></td>
<td>2.8 ngm$^{-3}$; 25 EU m$^{-3}$</td>
<td>The effects on body temperature and heart rate achieved by 0.8 ng of endotoxin per kg of body weight given at 0900 h were comparable to those associated with 0.4 ng/kg at 1900 h. However, sleep was only slightly influenced. Endotoxin reduced the amount of REM sleep and increased REM latency. Subjective tiredness, sleep onset latency, total sleep time, and the amounts of slow-wave and non-REM sleep were not affected by endotoxin.</td>
<td>Korth et al (1996)</td>
</tr>
</tbody>
</table>
A5.3.3 Animal studies

Overview

A large number of animal studies have investigated the specific effects of endotoxin on respiratory health and the mechanisms by which endotoxin influences respiratory health. The results of these studies confirm the potential harmfulness of inhaled toxin but do not provide much information on exposure-response relationships. There is a complex interplay between endotoxin exposure and allergen response and it is apparent that the toxicity of endotoxin is substantially modified by co-exposure to other agents.

Airways inflammation

Exposure of guinea pigs to aerosols of Salmonella typhosa endotoxin at 100 mgm$^{-3}$ for 2 or 4 hours had significant effects on the number of inflammatory cells in cardiac blood and in the airways (as assessed from bronchiolavage fluids) but there was no evidence of the recruitment of inflammatory cells to the gas-exchange region of the lungs (Hudson et al, 1977). In a more recent study in guinea pigs, Gordon et al (1991) reported that 3 hours exposure to endotoxin led to the leakage of protein from cells lining the airways accompanied by decrements in pulmonary function and by an influx of inflammatory cells (neutrophils) into the airway wall. Exposure of guinea pigs to concentrations of 0.03 to 50.5 ugm$^{-3}$ aerosolized endotoxin over 4 hours was associated with a significant increase in total cell count and lactate dehydrogenase levels in bronchoalveolar lavage fluid except at the lowest dose of 0.03 ugm$^{-3}$ (about 300 EUm$^{-3}$; Gordon et al, 1992). In mice exposed to an estimated alveolar dose of < 10 ng LP$S$/mouse over a 10 minute period, neutrophils in bronchoalveolar lavage fluid were increased by 6.94, 32.7, and 38.8% after 2, 6, and 24 hours, respectively with an accompanying increase in a range of proinflammatory cytokines (Johnston et al, 1998). Brass et al (2003) demonstrated substantial differences in the inflammatory response in endotoxin resistant and endotoxin sensitive mice.

Airways reactivity

In studies in guinea pigs, Gordon et al (1991) reported significant decrease in airways conductance (ie airways constriction), 60-90 minutes into exposure to endotoxin and Gordon (1992) reported that significant decreases in specific airway conductance were observed following two hours exposure to 9.6 and 50.5 ugm$^{-3}$ endotoxin but not at lower levels of exposure. Increased airways reactivity, as measured by intravenous acetylcholine administration, was observed in guinea pigs exposed by inhalation to bacterial LPS (Nagai et al, 1991). In guinea pigs exposure to 30 ug ml$^{-1}$ for 1 hour, airways hyperreactivity to inhaled histamine occurred at 1 hour after LPS challenge and was resolved by 4 hours (Toward and Broadley, 2001). This coincided with reduction and recovery, respectively, of NO levels in bronchiolavage fluids. After two exposures, the airways hyperreactivity and NO deficiency were extended to 4 hours. Repeated LPS exposures, 48 hours apart, initially caused persistent dilation of the airways, whereas later exposures produced progressively persistent constriction of the airways. Airways hyperreactivity was detected 24 hours after the eighth challenge. Twenty-four hours after the ninth LPS exposure, macrophages, neutrophils, eosinophils, and NO metabolites were elevated in bronchiolavage fluids consistent with an inflammatory response.

In experiments with endotoxin tolerant and endotoxin sensitive strains of mice, Brass et al (2003) reported that after 5 days or 8 week of LPS exposure, only the endotoxin sensitive strain showed elevated airway hyperreactivity to inhaled methacholine.

Effects on the cells lining the airways

In rats exposed to saline or endotoxin aerosols for 3 hours/day for 3 days, exposure to endotoxin produced a dose-dependent increase in stored mucosubstances in cells lining the lung in animals exposed to as little as 0.3 ugm$^{-3}$ endotoxin (Gordon & Harkema, 1994). Similar effects were
observed in the trachea only after exposure to ≥ 3.1 ugm⁻³ endotoxin, whereas no significant changes were observed in the nasal airways even at concentrations as high as 52.4 ugm⁻³. In rats exposed to aerosols of 0.05, 0.5, and 5.0 ugm⁻³ for 3 hours /day, 5 days/week for 4 weeks increases in stored mucosubstances were observed in the lung walls of animals exposed to 0.5 or 5.0 ugm⁻³ but there was no evidence of inflammation (Gordon et al, 1996). In animals repeatedly exposed to 5.0 ugm⁻³ endotoxin and allowed to recover for 1 month, a persistent mucous cell metaplasia (abnormality) was observed in the lung.

In a comparison of endotoxin-sensitive and endotoxin-resistant mice, Brass et al (2003) reported that an 8-wk exposure to LPS resulted in expansion of the submucosal area and increased rates of cell proliferation only in the endotoxin sensitive strain.

**Adaptive response to endotoxin**

Lantz et al (1985) demonstrated that hamsters could be rendered endotoxin tolerant by pre-injection of LPS. On exposure for 5 hours to LPS derived from Enterobacter agglomerans, a bacterium found in cotton and cotton mill dust as an aqueous aerosol (effective LPS concentration 4 ugm⁻³), the pre-treated hamsters failed to develop the inflammatory response observed in hamsters that had not undergone pre-treatment.

An adaptive response to LPS was found in rats and mice exposed to low-dose LPS aerosols (predicted lung deposition approximately 20 ng in rats and approximately 5 ng in mice) for 10 minutes on 3 consecutive days and a high dose (rats approximately 200 ng; mice approximately 25 ng) on day 4. Significantly less airways inflammation was observed in the adapted animals than in those exposed on only day 4, as assessed from the neutrophil content of BAL fluids. Adaption occurred in both young and old animals (21 months of age) although there was a greater variability in the response of older animals.

**Effects of co-exposure to other pollutants including effects on allergen response**

Several investigators have demonstrated that co-exposure of animals to ozone and endotoxin gives rise to a greater response than observed with either agent alone (Harkema et al, 2005; Wagner et al, 2001). The results of other studies indicate that inhaled LPS may enhance the response to inhaled allergens. In a study in ovalbumin (OVA)-sensitised mice primed with 10 ug LPS by intranasal administration 24 hours before the start of OVA challenges (20 minutes on 3 consecutive days), more severe inflammation, as assessed from eosinophil inflammation and higher nitrite formation, was found in LPS-primed than in non-primed sensitised mice. Similarly Slater et al (1998) demonstrated that LPS enhanced the immune responses of mice to inhaled latex allergen with stimulation of all 3 immunoglobulin types. Rylander & Holt (1998) reported that LPS caused a stimulation of the OVA-induced antibody production in guinea pigs which was abolished by simultaneous exposure to beta(1→3) glucan. Animals were exposed to 2 ng LPS and/or 8 pg beta(1→3) glucan over a period of 4 hours/day, 5 days/week for 5 weeks. Wan et al (2000) demonstrated that mice pre-exposed to airborne endotoxin mounted significantly higher OVA-specific IgE antibody responses to inhaled OVA than those observed in OVA-only sensitized mice. The authors suggested that inhaled endotoxin may potentiate allergen-specific airway inflammation through the down regulation of repeated airway antigen exposure-induced IgE isotype-specific tolerance. Gerhold et al (2002) reported that systemic LPS administration before OVA sensitization of mice reduced OVA-specific IgE serum levels, T(H)2 cytokine production by splenic mononuclear cells and the extent of airway inflammation compared with that in OVA-sensitized mice not pretreated with LPS. Neither systemic nor local LPS exposure affected airways responsiveness. Mizoguchi et al (1986) demonstrated that serum antibody responses of mice to repeatedly inhaled protein antigens such as OVA were enhanced by inhalation of LPS simultaneously with the antigens. Both mouse strain and antigen played a role in the severity of response. Oral administration of antigen or antigen plus LPS-K did not induce any detectable antibody response.
In an *in vitro* study, Shahan *et al* (1994) demonstrated that LPS potentiated the production of superoxide anion production in response to fungi by macrophages recovered from guinea pigs.

*Differences in toxicity of endotoxin from different sources*

Baseler *et al* (1983) reported that when mouse B lymphocytes were stimulated with LPS preparations from different gram-negative bacteria, distinctly different dose-response curves were obtained.

Mizoguchi *et al* (1986) demonstrated a difference in toxicity of LPS derived from different organisms. Inhalation of LPS extracted from *Klebsiella O3* (LPS-K) but not LPS from *Escherichia coli O55* (LPS-E), at the time of initial inhalation of antigen in mice, significantly intensified the priming for the secondary antibody response to the antigen subsequently inhaled. Both LPS-K and LPS-E, however, caused an enhanced response when they were inhaled repeatedly together with the antigen. Oral administration of antigen or antigen plus LPS-K did not induce any detectable antibody response.
### A5.4: HEALTH EFFECTS OF EXPOSURE TO AIRBORNE FUNGI

#### A5.4.1 Workplace studies

Table A5.8: Exposure-response information for exposure to airborne fungi emitted during waste management activities

<table>
<thead>
<tr>
<th>Exposed population</th>
<th>Control population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Threshold</th>
<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 waste collection workers involved in collecting food and garden waste - Norway</td>
<td>-</td>
<td>Median 0.17 x 10^6 Range 0-2.0 x10^6 spores m^-3, assessed by SEM</td>
<td>Evidence of mild upper airways inflammation in NAL but no relationship with respiratory symptoms</td>
<td>Significant increase in nasal congestion and neutrophils in NAL &gt;0.3 x 10^6 spores m^-3</td>
<td>Significant exposure-response relationship between fungal spores and nasal congestion measured by acoustic rhinometry; Significant increase in neutrophils in NAL</td>
<td>Not possible to relate NAL parameters to symptoms;</td>
<td>Heldal et al (2003b)</td>
</tr>
<tr>
<td>25 waste collection workers involved in collecting food and garden waste - Norway</td>
<td>-</td>
<td>AM 0.32 x 10^6 GM 0.2 x 10^6 Range 0-2.0 x10^6 spores m^-3, assessed by SEM</td>
<td>Significant decline in FEV1 over working week; no relationship between exposure and symptoms</td>
<td>&gt; 0.4 x 10^6 spores m^-3</td>
<td>Not demonstrated that fungal spores specifically related to effects on symptoms or lung function</td>
<td>Role of fungal spores not described in detail; relationships between induced sputum parameters and endotoxin and beta (1-&gt;3) glucan discussed</td>
<td>Heldal et al (2003a)</td>
</tr>
<tr>
<td>Questionnaire study of 22 waste collection workers - Norway</td>
<td>-</td>
<td>AM 0.4 x 10^6 GM 0.12 x 10^6 Range 0-2.3 x10^6 spores m^-3, assessed by SEM</td>
<td>Significant association with cough, unusual tiredness, headache; Nonsignificant association with nasal irritation, runny nose, eye irritation, cough with phlegm</td>
<td>Cough – mean exposure 0.28; unusual tiredness 0.27; headache 0.28</td>
<td>Not demonstrated</td>
<td>Exposure monitoring may have influenced reporting of symptoms; fungal spores not correlated with bacteria or endotoxin; no relationship with diarrhoea, nausea</td>
<td>Heldal &amp; Eduard, (2004)</td>
</tr>
<tr>
<td>2303 waste collectors – 70% response rate - Denmark</td>
<td></td>
<td>3x10^4-1.9x10^5 cfu m^-3; 10^3 – 4.9x10^6 cells m^-3; based on measurement plus modelling</td>
<td>Nausea</td>
<td>NOEL &lt;10^5 cfu m^-3; LOEL 10^5 cfu m^-3; for total fungal cells NOEL &lt; 2x10^5 cells m^-3</td>
<td>Exposure-response relationships not clearly demonstrated for nausea; clear exposure-response relationship with diarrhoea (Fig. 2.6)</td>
<td>Bioaerosol characterisation limited to endotoxin and fungi; role of infectious agents in observed relationship not investigated; workers in control group may have had bioaerosol exposures; nausea and gastrointestinal symptoms most prevalent in the summer; apparent link with odour intensity</td>
<td>Ivens et al (1997, 1999)</td>
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<tr>
<td>Exposed population</td>
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<tr>
<td>Case study of organic waste collection worker - Germany</td>
<td></td>
<td>Conc's of Aspergillus fumagatus in air behind garbage truck were greater than 10^5 cfu</td>
<td>ABPA including asthmatic responses and hypersensitivity pneumonitis; high levels of IgG antibodies to aspergillus fumagatus</td>
<td>&gt;10^6 TOTAL MICRO-ORGANISMS fungi - about 10^4 cfu m^-3</td>
<td>Significant relationships between TOTAL MICRO-ORGANISMS (150-200m from site) and bronchitis, waking up due to coughing, coughing on rising/ during day, excessive tiredness, current medication (see Table 2.3)</td>
<td>Two years after cessation of exposure, levels of IgE had fallen but shortness of breath and exercise induced asthma persisted</td>
<td>Allmers et al (2000)</td>
</tr>
<tr>
<td>Investigation of impacts of commercial composting of waste on health of local residents – 82 within 150-200m, 76 &gt;200-400m, 56-400-500m - Germany</td>
<td></td>
<td>500 m upwind, 1.9x10^3, 3.5x10^2 cfu m^-3 200m downwind 7.7x10^2-1.3x10^3 cfu m^-3 Collection on filter, culture (dichloran-glycerine- (DG18)-(oxoid), 25°C);</td>
<td>Respiratory symptoms, limited range of other health endpoints – gastrointestinal symptoms, tiredness, shivering/fever; No relationship joint trouble or muscular pain</td>
<td>&gt;10^6 TOTAL MICRO-ORGANISMS fungi - about 10^4 cfu m^-3</td>
<td>Exposure categorised by distance bands, 150-200m &gt;105 cfum^-3; analysis did not separate fungi and bacteria – relative importance of fungi and bacteria in causation of effects uncertain; Effects greater in those residing at current address for more than 5 years; no relationship between odour complaints and respiratory symptoms</td>
<td>Herr et al (2003)</td>
<td></td>
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<td>Cross sectional study – 58 compost workers, 53 biowaste collectors, 40 controls - Germany</td>
<td></td>
<td>Composting plants 10^3 cfu m^-3; biowaste collection 10^5 cfu m^-3; reference area &lt;10^3 cfu m^-3</td>
<td>Compost workers had significant raised risks of diseases of the airways and gastrointestinal symptoms, diseases of the skin; no effects in waste collection workers</td>
<td></td>
<td>Effects not specifically related to fungal exposure as opposed to other bioaerosol components; lack of correlation between IgG and respiratory illness; increase in IgG in compost workers with duration of employment</td>
<td>Mean duration of employment for compost workers was 3 years compared with 1.5 years for waste collection workers who were also younger; 20/58 compost workers had significantly raised IgG antibodies to aspergillus fumagatus compared with 3 waste collection workers and 1 control; 13% workers had previous bioaerosol exposure from farming; lower incidence allergic rhinitis and atopy in compost workers attributed to healthy worker effect</td>
<td>Bunger et al (2000)</td>
</tr>
<tr>
<td>218 compost workers at 41 composting plants; 5 year follow up study - Germany</td>
<td>66 full time workers, 60% exposed to organic dust</td>
<td>Increased incidence of mucus membrane irritation; Significant decline in %FVC predicted over 5 years; significant increase in 5 years in compost workers with chronic bronchitis</td>
<td></td>
<td></td>
<td>Pattern of health effects differs from those at other workplaces with organic dust exposure, possibly due to high levels of actinomycetes and filamentous fungi; allergic alveolitis in 2 compost workers (IgG to actinomycetes and filamentous fungi found in plant air)</td>
<td>Bunger et al (2007)</td>
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<td>Exposed population</td>
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<td>Canadian study of 226 employees at 36 randomly selected liquor stores with bottle recycling and in-house glass breaking</td>
<td></td>
<td>GM concs 0.18 mgm$^-3$ inhalable dust; 3.6 EUm$^-3$ endotoxin (270 personal samples); viable fungi 1064 cfum$^-3$ (648 area samples).</td>
<td>Employees reported more work related chronic chest tightness and chronic nasal symptoms than unexposed controls.</td>
<td>Acute chest symptoms were associated with breaking visibly moldy bottles, but not with measured fungal counts.</td>
<td>Inhalable particulate matter levels &gt;0.2 mgm$^-3$ were associated with acute upper airway irritation. Somatic symptoms were associated with measures of psychosocial job strain.</td>
<td>Kennedy et al (2004)</td>
<td></td>
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</table>

**Table A5.9:** Studies of the health effects of exposure to airborne fungi in other industries

<table>
<thead>
<tr>
<th>Exposed population</th>
<th>Control population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Threshold</th>
<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
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</thead>
<tbody>
<tr>
<td>11 Swedish farmers with a confirmed diagnosis of allergic alveolitis from ten farms, 16 farmers with symptoms of ODTS from 12 farms, and 17 reference farmers</td>
<td>Farms with: allergic alveolitis - 2.6 +/- 1.8 x 10$^9$ (SD) spores/m$^3$ (averaged over 10-30 minutes); OTDS - 13 +/- 13 x 10$^9$ spores/m$^3$; Reference farms - 0.12 +/- 0.20 x 10$^9$ spores/m$^3$</td>
<td>The daily spore dose associated with allergic alveolitis was 2 x 10$^10$ spores/day which was ten times higher than on reference farms. The average dose associated with ODTS was 2 x 10$^10$ spores.</td>
<td></td>
<td>It was included that allergic alveolitis was associated with high exposure levels on most weekdays for weeks, and ODTS was associated with extreme exposure occurring on a single day. There was no correlation with individual spore types and disease.</td>
<td>Malmberg et al (1993)</td>
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<td>189 workers from 14 peat moss processing plants (3 all-year mixing plants and 11 seasonal plants),</td>
<td>Seasonal plants were more contaminated with mould than all-year mixing plants</td>
<td>28% of the workers had a positive serum reaction to at least one of the tested moulds</td>
<td>The proportion of sensitised workers varied from none in 4 plants to 14/21 for 1 plant, but was not correlated with the airborne levels of moulds.</td>
<td>FEV generally lower in workers with positive antibodies. IgG positive frequency higher and FEV/FVC lower in workers in all-year plants than in seasonal plant workers. Duration of exposure may trigger more sensitization than the level of exposure.</td>
<td>Meriaux et al (2006)</td>
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<tr>
<td>Exposed population</td>
<td>Control population</td>
<td>Exposure</td>
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<tr>
<td>11 sawmill workers</td>
<td></td>
<td>2 x 10^7 spores/m^3 to 1.5 x 10^8 spores/m^3 (mean 7 x 10^7)</td>
<td>No differences between work and vacation in concentrations of inhalable dust, endotoxins, and terpenes were relatively low</td>
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</tr>
<tr>
<td>Finnish study of 521 adults with newly diagnosed asthma and 932 controls</td>
<td></td>
<td>SEM - 1,300 x 10^4 Rhizopus microsporus spores/m^3; 130 x 10^5 Paecilomyces variotii spores/m^3 (AM) Exposure assessed using antibody levels to R. microsporus and P. variotii</td>
<td>Risk of asthma related to visible and/or odour in the workplace (odds ratio, 1.54; 95% CI 1.01-2.32) but not to water damage or damp stains</td>
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<tr>
<td>A longitudinal study (1985 to 1988) of wood trimmers exposed to spores and planing operators from 2 sawmills (N = 303 and 170).</td>
<td></td>
<td></td>
<td>Higher antibody levels, MMI, chronic nonspecific lung disease, hypersensitivity pneumonitis, and OTDS in wood trimmers than planing operators.</td>
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<tr>
<td>Swedish sawmill workers</td>
<td></td>
<td>Short-lasting alveolitis-like symptoms had a prevalence of about 5-10% and occurred mainly in nonsmokers with high levels of antibodies to Rhizopus.</td>
<td>10^6 Rhizopus cfum^3 (Rcfu m^-3)</td>
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</table>

Inflammatory markers in NAL were lower than in workers at a mouldy school building; Concentrations of the inflammatory mediator TNFalpha in the NAL fluid were positively correlated with the concentration of terpenes in the working environment (r = .768; p = .006). Terpenes also emitted from some waste management activities.

76 (7.5%) controls with a history of asthma excluded from study; fraction of asthma attributable to workplace exposure estimated as 35.1% (95% CI, 1.0-56.9%) in exposed workers.

Antibody levels to R. microsporus and P. variotii were higher in wood trimmers than in other sawmill workers with lower exposure to spores. Antibody levels were significantly elevated after periods with high exposure compared to antibody levels in the same wood trimmers after periods with low exposure. Antibodies were also found in newly employed wood trimmers.

Other upper and lower airways symptoms and a mild, reversible decrement in lung function over one week’s work shift was also suspected to be linked to Rhizopus exposure.
<table>
<thead>
<tr>
<th>Exposed population</th>
<th>Control population</th>
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<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung function study in 66 wood trimmers exposed to organic dust in 2 sawmills</td>
<td></td>
<td>1-10 cfum⁻³</td>
<td>Restrictive pulmonary dysfunction associated with high exposure to moulds; effects observed after one month of no exposure</td>
<td>Effects on lung function at 10 cfum⁻³ but not at 1 cfum⁻³</td>
<td>Repeated measurements immediately after one month’s vacation, three months later on a Monday morning after two days of no exposure showed a continuing reduction in FVC and FEV₁ in a sawmill with high exposure to moulds (10 cfum⁻³)</td>
<td></td>
<td>Hedensteina et al (1986)</td>
</tr>
<tr>
<td>Australian sawmill workers</td>
<td></td>
<td>10⁰ cfum⁻³</td>
<td>Green mill workers had significantly high prevalence of regular cough, chronic bronchitis, regular blocked nose, regular sneezing, sinus problems, flu-like symptom</td>
<td></td>
<td></td>
<td>The levels of exposure to endotoxin, beta (1→3) glucan, bacteria and fungi were high in green mills compared with dry mills. Significant positive correlations were found among endotoxin and Gram (-)ve bacteria, beta (1→3) glucan and fungi, and endotoxin and beta (1→3) glucan exposure levels.</td>
<td>Mandryk et al (2000)</td>
</tr>
<tr>
<td>Measurement of cross-shift lung function changes in 15 wood trimmers and 26 sawmill workers with a follow-up time of 27 months.</td>
<td>highest concentration of spores for the wood trimmers was 10⁰ cfum⁻³, (exposure of sawmill workers several times lower)</td>
<td>At the follow-up, wood trimmers had a lower forced vital capacity (FVC) on average, after adjustment for age and height, compared to the sawmill workers.</td>
<td>Across-week change in FEV₁ correlated with the decline in FEV₁ between the first and the second survey, after adjusting for normal aging in nonsmoking wood trimmers</td>
<td></td>
<td></td>
<td>Dahlqvist &amp; Ulfvarson (1994)</td>
<td></td>
</tr>
<tr>
<td>95 current and 17 ex-sawmill workers, divided into high and low exposure groups depending on place of work</td>
<td>58 workers from a nearby light engineering factory</td>
<td>Raised prevalence of work related wheeze in high exposure and comparison groups versus the low exposure group. Raised prevalences of chronic bronchitis and symptomatic bronchial hyper-reactivity in high and low exposure groups versus the comparison group.</td>
<td>The prevalence of symptoms suggestive of hypersensitivity pneumonitis was 4.4% in the high exposure group, 0% in the low exposure group and 1.9% in the comparison group.</td>
<td>Serum concentrations of specific IgG against extracts of sawdust and Trichoderma koningii were significantly higher in the high exposure group than in the other two groups.</td>
<td></td>
<td>Halpin et al (1994)</td>
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</table>
A5.4.2 Respiratory symptoms in children

There have been a large number of studies of respiratory symptoms in children living in damp homes or attending damp schools but few of these studies provide information that links observed effects to concentrations of airborne fungi.

A UK study of 1000 children found a significant excess of wheeze among children whose homes were reported to be mouldy, although airborne mould counts were not related to reports of visible mould, or to a history of wheeze in the index child. (Strachan et al, 1990). The heterogeneous group of non-sporing fungi (mycelia sterilia) were the only airborne fungi present at significantly higher concentrations in the homes of wheezy children (geometric mean 2.1 v 0.7 cfu m\(^{-3}\)) than in children without wheeze. Total airborne fungal counts varied from 0 to 41,000 cfu m\(^{-3}\), but were generally in the range 50-1500 cfu m\(^{-3}\), much lower than the concentrations found outdoors in summer. There was a non significant relationship between higher counts at home and a 10% or greater decline in lung function (FEV\(_1\)) in children after exercise (geometric mean 354 v 253 cfu m\(^{-3}\)). Similarly a Canadian study found a 25 to 50% relative increase in the prevalence of respiratory symptoms in school children who reported exposure to mould, but neither symptoms nor recorded cough were related to objective measures of mould (airborne ergosterol and viable fungi in dust; Dales et al, 1999).

In a study of Taiwanese children, Lee et al (2003) concluded that parental atopy contributed more to childhood asthma than indoor or outdoor environmental factors.

In a study of 148 Australian children, Garret et al (1998) reported that Penicillium exposure was a risk factor for asthma, while Aspergillus exposure was a risk factor for atopy. Fungal allergies were more common among children exposed to Cladosporium or Penicillium in winter or to musty odour. Respiratory symptoms were marginally more common with exposure to Cladosporium or total spores in winter. Actual measurements of fungal spores predict health outcomes better than reported dampness. The odds ratio for physician-diagnosed asthma associated with a 100 cfu m\(^{-3}\) increase in Penicillium spores was 1.43 (95% CI 1.03-2.00) and the odds ratio for atopy (a positive response to at least one skin prick test) associated with a 10 cfu m\(^{-3}\) increase in Aspergillus spores was 1.48 (95% CI 1.10-1.99). Other studies have also linked exposure to fungal spores to increased risks of asthma and/or allergy in children. Muller et al (2002) reported that Aspergillus exposure was associated with allergic rhinitis or related symptoms in children and that there was a significant association between exposure to Penicillium spores and respiratory tract infections. In a study of IgE antibodies to 24 moulds in 93 children from three moisture problem schools and 33 children from a reference school, Taskinen et al (2002) reported that antibodies to moulds common in moisture damaged buildings were associated with allergic diseases, as well as with mould-specific immunoglobulin E (IgE) or skin prick test (SPT) findings. Aspergillus fumigatus and A. versicolor were the moulds with the most consistent findings. In contrast, the association between asthma, wheezing or cough symptoms, and IgG to moulds was not significant and there were no significant differences in mould-specific IgG concentrations between exposed and non-exposed school-children. Seven (39%) of the 18 children with multiple (> 7) elevated IgG findings, however, suffered from asthma or wheezing. In a similar study of 181 primary school children with asthma, wheezing, or cough symptoms in 2 schools, with and without mould damage, Hyvarinen et al (2003) found that children’s microbe-specific IgG levels were often higher in the reference school. In a study in 272 German school children, Jacob et al (2002) found that after adjusting for age, sex, parental education, region of residency, and parental history of atopy, mould spore counts for Cladosporium and Aspergillus were associated with an increased risk of allergic sensitization. Sensitized children exposed to high levels of mould spores (> 90th percentile), as defined by concentrations in settled dust, were more likely to suffer from symptoms of rhinoconjunctivitis.

Perception may affect the response to mould exposure. In a study of school children, Handel et al (2004) reported an association between fungal contamination at school and symptoms including coughing/wheezing, headaches and joint pains. After the problem was publicised the perception of symptoms increased.

Overall, the results of studies in children do not provide a clear indication of a threshold level for effects and they also suggest that background levels of fungal exposure, in the absence of
waste management sources, are sufficient to give rise to adverse respiratory effects in some children including an increased risk of developing asthma. It is possible that exposure levels as low as 350 cfum\(^{-3}\) in indoor air, may be sufficient to cause mild adverse effects on respiratory health. Species of both *Penicillium* and *Aspergillus* have been identified as being particularly associated with respiratory effects.

### A5.4.3 Pulmonary haemorrhage in infants

In a study of a geographic cluster of 10 cases of pulmonary haemorrhage and haemosiderosis in infants, Etzel *et al.* (1999) reported that mean colony counts for all fungi averaged 29 227 cfum\(^{-3}\) in homes of patients and 707 cfum\(^{-3}\) in homes of controls. The mean concentration of *Stachybotrys atra* in the air was 43 cfum\(^{-3}\) in the homes of patients and 4 cfum\(^{-3}\) in the homes of controls. Viable *S atra* was detected in filter cassette samples of the air in the homes of 5 of 9 patients and 4 of 27 controls. The matched odds ratio for a change of 10 units in the mean concentration of *S atra* in the air was 9.83 (95% CI, 1.08-3 x 10\(^6\)). The mean concentration of *S atra* on surfaces was 20 x 10\(^5\) cfu/g and 0.007 x 10\(^6\) cfu/g in homes of patients and controls, respectively. The authors concluded that infants with pulmonary haemorrhage and haemosiderosis were more likely than controls to live in homes with toxigenic *S atra* and other fungi in the indoor air. A subsequent review within CDC and by outside experts of this investigation, however, identified shortcomings in the implementation and reporting of the investigation that led CDC to conclude that suggested association between acute pulmonary hemorrhage/hemosiderosis in infants and exposure to moulds, specifically *S atra*, was not proven (CDC, 2000). Even without the subsequent doubts about the findings of Etzel *et al.* (1999), their study is of limited relevance to understanding the risks associated with fungal emissions from waste management activities in the UK.

### A5.5.4 Other studies of moulds in indoor air

In a literature search, Bornehag *et al.* (2001) identified 590 peer-reviewed articles relating to the health effects of "dampness" in buildings of which 61 were considered for review. They concluded that "dampness" in buildings appears to increase the risk for health effects in the airways, such as cough, wheeze and asthma. Relative risks were in the range of OR 1.4-2.2. They also found some evidence of an association between "dampness" and other symptoms such as tiredness, headache and airways infections. The mechanisms by which "dampness" gives rise to health effects are unknown.

The results of a number of studies suggest an association between exposure to moulds in indoor air and increased risks of developing allergy with or without asthma. In a study of 135 patients with possible mould-related health effects following prolonged indoor exposure, a strong correlation was found among atopy, mould sensitization, and sensitization to specific moulds identified in the patient's environmental report (Bobbit *et al.*, 2005). There were no associations, however, between the patients' presenting symptoms, atopic status, and magnitude of exposure. In a study of 49 inhabitants of 20 flats with signs of moulds identified in the patient's environmental report (Bobbit *et al.*, 2005), Elevated IgE's were found in 12 subjects (24.5%) and in three patients (6.1%) mould specific serum IgE were detected. Sensitisation to moulds as determined in a skin prick test was accompanied by allergy to other common allergens. In a nested case-referent study of adult-onset asthma in a random population sample (n = 15813), aged 20-50 years, increased adjusted odds for asthma were associated with exposure to moulds (OR 2.2, 95%, CI 1.4-3.5), environmental tobacco smoke (OR 2.4, 95%, CI1.4-4.1), and the presence of a wood stove (OR 1.7, 95% CI 1.2-2.5; Thorn *et al.*, 2001a). In a longitudinal study of indoor allergen and fungal levels in Melbourne homes between 1996 and 1998, Matheson *et al.* (2005) found that young adults whose *Cladosporium* fungal exposure doubled had 52% greater odds of having had an attack of asthma in the last 12 months. A doubling of fungal exposure was also associated with 53% greater odds of developing atopy. In a cross European study conducted across 30 centres of 1132 adults aged 20-44 years with current asthma and with skin prick test results, the frequency of sensitisation to moulds (*Alternaria alternata* or *Cladosporium herbarum*, or both) increased significantly with
increasing asthma severity (odds ratio 2.34 (95% CI 1.56 to 3.52). This association existed in all of the study areas (gathered into regions), although there were differences in the frequency of sensitisation (Zureik et al, 2002).

A number of studies have demonstrated associations between mould exposure and increased respiratory symptoms, not necessarily asthma. In a study of residential exposure to moulds, Klanova (2000) reported that the total concentrations of airborne fungi (monitored by aeroscop) were much higher in mouldy rooms than in the reference rooms. Although health complaints such as cough, headache, rhinitis and sore throat did not correlate with the total concentrations of airborne fungi, all occupants of rooms where the average concentrations was 2,476 cfum⁻³ reported health complaints. In a study of 2401 Norwegian adults, Skorge et al (2005) reported that exposure to mould was significantly associated with respiratory symptoms but only made a small contribution to the respiratory symptom burden in the population at large. In a small study in which 16 adults living in mouldy housing and complaining of chronic rhinitis were compared with 16 healthy referents without any known mould exposure, the respiratory symptoms reported by occupants of mouldy residences were apparently related to nonspecific inflammation following irritation rather than mould allergy (Ruoppi et al, 2003). In a Finnish study, Koskinen et al (1999) reported living in a house with a moisture problem was significantly associated with sinusitis, acute bronchitis, nocturnal cough, nocturnal dyspnoea, sore throat, and increased episodes of common cold and tonsillitis. Living in a house with a mould problem was significantly associated with common cold, cough without phlegm, nocturnal cough, sore throat, rhinitis, fatigue and difficulties in concentration. In a study of Dutch adults, Brunekeef (1992) reported that cough and phlegm in both men and women were strongly associated with living in a damp home whereas weaker associations were found for wheeze and asthma.

Overall these studies provide little to link airborne concentrations to effects on health.

A5.4.5 Volunteer studies

A number of experiments in human volunteers have established the potential for airborne fungi to cause respiratory symptoms in those with and without previous exposure and sensitisation or atopy.

In a volunteer experiment involving 27 subjects: 13 with occupational exposure in a moisture-damaged building, 4 atopics, and 10 controls, Stark et al (2005) demonstrated that inhalation of a commercial A. fumigatus solution led to raised levels in inflammatory markers in nasal lavage fluid (NAL) compared with that evoked by placebo challenge. In a further study Stark et al (2006) exposed 28 subjects by inhalation to commercial A. fumigatus solution. Respiratory symptoms and levels of exhaled NO but not nasal NO or nitrite were significantly increased by A. fumigatus. Previous exposure history or atopy had no significant impact on response. Each inhalation challenge was about 5 minutes duration and the inhaled doses ranged from about 0.0039-3.9 mg for each challenge. Assuming a typical inhalation volume of 20 m³ over 24 hours, the inferred exposure concentrations over 5 minutes ranged from 0.056-56 mgm⁻³.

Wallinder et al (2005) exposed 29 volunteers to 1 mgm⁻³ of 3-Methylfuran (3-MF), a common fungal volatile product produced by a wide spectrum of fungi. No subjective ratings were significantly increased during exposure but blinking frequency and the lavage biomarkers myeloperoxidase and lysozyme were significantly increased. Concentrations of 3-MF in indoor air where there is fungal contamination range from 0.001 to 0.1 mgm⁻³.

Meyer et al (2005) reported that exposure of 8 employees from three water-damaged schools with a positive histamine release test to Penicillium chrysogenum to placebo, approximately 600,000 spores/m³ air of P. chrysogenum or approximately 350,000 spores/m³ of Trichoderma harzianum for 6 minutes on three separate days gave rise to a significant increase in mucous membrane symptoms following with no significant difference being observed between the moulds or the placebo.
In a volunteer study, Licorish et al. (1985) exposed 7 patients with a history of mild asthma to 16 bronchial challenges with the fungal species to which they had been sensitized (Alternaria or Penicillium). It was found that immediate-type asthma was readily provoked by both whole spores and by their extracts, in some subjects fewer intact than extracted spores were required, delayed-type asthma occurred only after whole spore challenges.

**A5.4.7 Animal studies**

Several studies have demonstrated the toxicity of inhaled spores of various fungal species in animals but do not provide useful exposure-response information.

In a study of the potential effects of grain dust exposure, Sumi et al. (1994) reported that exposure of rats to Aspergillus versicolor led to the development of granulomatous lesions throughout the entire lung, especially around bronchioles. Thurston et al. (1975) exposed rabbits once a day for 1 to 10 consecutive days to aerosols containing spores of Aspergillus fumigatus. Precipitating antibodies were detected only in serums from rabbits that had inhaled at least $1.6 \times 10^6$ spores/g of lung tissue at each of 6 exposures or $1.8 \times 10^6$ spores at 2 exposures. A single exposure to $3.6 \times 10^5$ spores was sufficient to stimulate antibody detectable in passive hemagglutination (HA) tests. None of the rabbits died from aspergillosis, and signs of infection were transient.

Viana et al. (2002) determined that multiple exposure of mice to soluble components of Stachybotrys chartarum gave rise to airways inflammation and hyper-responsiveness typical of allergic airway disease. Intratracheal administration of either $1.4 \times 10^6$ S. chartarum spores (< or = 35 ng toxin/kg BW) or $1.4 \times 10^6$ Cladosporium cladosporioides spores to mice resulted in granuloma formation at the sites of spore impaction (Rand et al., 2003).

Jussila et al. (2002, 2003) demonstrated dose dependent inflammatory responses to moulds in mice following exposure by intratracheal instillation to the spores of Streptomyces californicus or A. versicolor originally isolated from the indoor air of a moisture-damaged building. In an *in vitro* study, the overall potency to stimulate the production of proinflammatory mediators decreased in the order *Pseudomonas fluorescens* > > *Bacillus cereus* > *Stachybotrys chartarum* > *Aspergillus versicolor* > *Penicillium spinulosum* in terms of equivalent numbers of bacteria and spores of fungi added to cell cultures (Huttunen et al., 2003). In experiments with guinea pig bronchial alveolar lavage (BAL) cells, Shahan et al. (1994) reported that fungal spores from *Aspergillus candidus*, *Aspergillus terreus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Eurotima amstelodami*, *Penicillium spinulosum*, and *Cladosporium cladosporioides* all increased increase superoxide anion production to variable degrees. LPS stimulated little superoxide anion production in BAL cells, but when cells were pretreated with LPS prior to stimulation with fungal spores, superoxide anion production was increased over that induced by either spores or LPS alone. The key findings of these animal studies are the confirmation of the potential of inhaled fungi to cause a dose-dependent inflammatory response in the respiratory system or the variable potency of different fungal species, evidence of lasting respiratory damage and progressive lung disease following a single over-exposure to some fungal species and the potential importance of endotoxin (LPS) in enhancing the respiratory response to inhaled fungal spores.
### A5.5 HEALTH EFFECTS OF BETA(1→3) EXPOSURE

#### A5.5.1 Effects in humans

**Table A5.10: Studies of the health effects of beta (1→3) glucan undertaken in the waste industry**

<table>
<thead>
<tr>
<th>Exposed population</th>
<th>Control population</th>
<th>Exposure</th>
<th>Health effects</th>
<th>Threshold</th>
<th>Exposure-response</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>47 waste collectors, 22% employed for &lt;6 months - Netherlands</td>
<td>15 office workers from same facilities employed for &gt; 6 months</td>
<td>GM: 1.29 GSD 3.6 Range &lt;0.26-30.8 ug m(^{-3})</td>
<td>NS increase in inflammatory markers (IL6, IL8 but not total cells) in NAL</td>
<td>Not determined, lowest exposure &lt;0.26 ug m(^{-3})</td>
<td>Not demonstrated, no significant difference between workers employed for &lt; or &gt; 6 months; higher prevalence of chronic respiratory symptoms in controls (smaller proportion of controls were current smokers)</td>
<td>Waste collectors showed significantly more cough, phlegm and itchy nose than controls; increase in inflammation in waste collection workers during week; no evidence of habituation; stronger associations between NAL parameters and endotoxin/total dust; exposures to fungal spores not reported</td>
<td>Wouters et al (2002)</td>
</tr>
<tr>
<td>31 waste collection workers involved in collecting food and garden waste - Norway</td>
<td>-</td>
<td>Median: 40 ng m(^{-3}); range 3-220 ELISA (details not given)</td>
<td>Evidence of mild upper airways inflammation in NAL but no relationship with respiratory symptoms</td>
<td>About 25 ng m(^{-3})</td>
<td>Significant correlation with IL6 in NAL; 5 fold increase at 220 ng m(^{-3}); also significant correlation with nasal congestion as assessed by acoustic rhinometry</td>
<td>Not possible to relate NAL parameters to symptoms; authors suggest low level endotoxin exposure may increase susceptibility to effects of beta (1-&gt;3) glucan; effects of beta (1-&gt;3) glucan independent from those of fungal spores</td>
<td>Heldal et al (2003a)</td>
</tr>
<tr>
<td>25 waste collection workers involved in collecting food and garden waste - Norway</td>
<td>-</td>
<td>AM 59 ng m(^{-3}); GM 52 ng m(^{-3}) Range 5-20 ng m(^{-3}); LAL described elsewhere</td>
<td>Significant decline in FEV1 over working week; no relationship between exposure and symptoms</td>
<td>About 25 ng m(^{-3})</td>
<td>Weak exposure-response relationship between beta (1-&gt;3) glucan and IL8 in induced sputum</td>
<td>No association between symptoms and exposure levels or markers of inflammation; SS decline in FEV1 over working week not specifically related to beta (1-&gt;3) glucan – correlated with neutrophils in induced sputum</td>
<td>Heldal et al (2003b)</td>
</tr>
<tr>
<td>Study of about 175 workers in 9 MRFs in England and Wales (159 participated)</td>
<td>Plant means ranged from 7.4 to 44 ng m(^{-3}); highest measurement 137 ng m(^{-3})</td>
<td>Significant increase in cough with phlegm, hoarse/parched throat, chest tightness, stomach problems; Non significant increase in nasal irritation/ sneezing, &lt;12 ng m(^{-3}); significantly greater impact at 26 ng m(^{-3}) than at 12 ng m(^{-3}) (approximately 2-3 times greater)</td>
<td>OR for &gt; 12 ng m(^{-3}): Nasal irritation 1.5 Stuffy nose 2.1 Cough + phlegm 2.9 Dry cough 2.2 Hoarse throat 2.9 Chest tightness 5.3 Nausea 5.1 Stomach problems 4.5</td>
<td>25% of workers had been employed for &lt;6 months in MRF and only 30% with &gt;2 years employment; tendency for self-reported respiratory and gastrointestinal symptoms to increase with length of employment; stronger effects with beta 1-&gt;3 glucan than with endotoxin; Significant increase in dry cough, hoarse throat, skin rash, unusual tiredness and vomiting in plant beta (1-&gt;3) glucan</td>
<td>Gladding et al (2003)</td>
<td></td>
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<tr>
<td>Exposed population</td>
<td>Control population</td>
<td>Exposure</td>
<td>Health effects</td>
<td>Threshold</td>
<td>Exposure-response</td>
<td>Comments</td>
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<td>Compost workers 14 in 1\textsuperscript{st} survey 15 in 2\textsuperscript{nd} survey - Netherlands</td>
<td>University staff/students 1\textsuperscript{st} survey 6 2\textsuperscript{nd} survey 9</td>
<td>GM 1\textsuperscript{st} survey (2\textsuperscript{nd} survey) Bulldozer – 0.54 (0.36) Tech personnel – 4.85 (4.44) Supervisors – 4.28 (2.14) Production workers 3.62 (3.8) ugm\textsuperscript{–3} methods not described</td>
<td>stuffy nose, dry cough, nausea, and skin rash</td>
<td>Skin rash 3.3</td>
<td>concentrations of 26 ngm\textsuperscript{–3} compared to plants with concentrations of 12 ngm\textsuperscript{–3}. Blood monocyte and RBC sedimentation rates significantly lower at 26 versus 12 ngm\textsuperscript{–3} – attributed to recruitment of inflammatory cells to the lung</td>
<td>Douwes \textit{et al} (2000)</td>
<td></td>
</tr>
<tr>
<td>8 collectors of compostable waste; 17 collectors of unsorted waste - Sweden</td>
<td>24 workers in local municipal administration AM (SD) Compostable waste – 19.1 (8.4) ngm\textsuperscript{–3}; unsorted waste – 9.2 (3.8) ngm\textsuperscript{–3}; ELISA</td>
<td>Significant increase in congested nose, cough with phlegm, unusual tiredness and diarrhoea; no significant increase of other respiratory symptoms; no effects on FEV or airways responsiveness (metacholine); reduced lymphocytes and total cells in induced sputum; increased lymphocytes and monocytes in blood</td>
<td>20 ngm\textsuperscript{–3} = NOEL for lung function and airways responsiveness; LOEL for congested nose, cough with phlegm, unusual tiredness and diarrhoea &lt;20 ngm\textsuperscript{–3}; threshold for effect on blood cell count between 1 and 9 ngm\textsuperscript{–3}</td>
<td>DR for lymphocytes in blood</td>
<td>Limited bioaerosol characterisation, small study population, effects not clearly linked to endotoxin, lower macrophage counts and lymphocyte counts in induced sputum and lower levels of inflammatory markers in blood of exposed workers versus controls (authors attributed this to the ongoing nature of the exposure); difficult to relate impact on blood cell count to health effects</td>
<td>Thorn \textit{et al} (1998)</td>
<td></td>
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</table>
A5.5.2 Investigations of the effects of beta (1→3) glucan exposure in animals

Exposure to beta (1→3) glucan

In an inhalation experiment in mice, Korpi et al. (2003) reported that beta-glucan aerosols provoked slight sensory irritation in the airways following 15 to 20 minutes exposure to 118 ug m⁻³, but the response was not concentration dependent at the levels studied (15-20 minute exposures to 37-1189 ug m⁻³). Slight pulmonary irritation was observed after repeated exposures. No effect was found on the serum total IgE levels, and no signs of inflammation were seen in the airways 6 hours after the final exposure. The results suggest that, irrespective of previous fungal sensitisation of the animals, inhaled beta (1→3) glucan may cause symptoms of respiratory tract irritation in the absence of apparent inflammation. In an earlier review, Rylander and Lin (2000) noted that although beta (1→3) glucan gave rise to an inflammatory response in animals following intraperitoneal injection and in vitro assays, inhaled beta (1→3)glucan did not appear to provoke an inflammatory response similar to that associated with endotoxin.

In a series of experiments Young et al. (2001, 2003, 2006) observed a dose-dependent pulmonary response to beta (1→3) glucan (zymosan A) in rats following intratracheal instillation (IT) (dose range 0-5 mg/kg body weight). At doses of 1-2.5 mg/kg body weight, a significant dose-dependent pulmonary response was reported for pulmonary irritation (assessed from breathing frequency) and inflammatory markers in bronchoalveolar lavage fluid. The equivalent human dose on a mass for mass basis would be 70 to 175 mg, corresponding to inhalation of 3.5-17.5 mg m⁻³ over a 24 hour period. These levels of exposure are several orders of magnitude greater than reported levels of workplace exposure and there are also uncertainties as to the appropriateness of IT administration as a model for inhalation exposure. The authors reported that a similar inflammatory response had been observed in guinea pigs exposed by IT or by inhalation to Zymosan. In guinea pigs, instillation of 2.5 mg/kg was found to give a similar response to inhalation exposure to 6 mg m⁻³ for 4 hours. Recovery in rats and guinea pigs was time dependent with effects in rats being largely resolved within four days but lymphocytes recovered from lung-associated lymph nodes continued to proliferate and reached a maximum on day 6, accompanied by a lowering of the CD4⁺/CD8⁺ T cell ratio. Particulate zymosan A induced greater pulmonary inflammation and damage in rats than the soluble form of this beta-glucan. However, in an earlier study, Fogelmark et al. (1992) reported that inhalation of the water insoluble form of beta-1,3-glucan caused a delayed response in terms of a decrease in macrophages and lymphocytes in the lung wall, 1 to 7 days after exposure but no invasion of neutrophils into the airways. When solubilised in 0.02 N NaOH, the cell response was the same as that observed after exposure to endotoxin.

Mixed exposures

Beta (1→3) glucan may have an important role on respiratory health through the enhancement of the allergic response to inhaled allergens. Ormstad et al. (2000) studied the effect of beta (1→3) glucan from the fungus Sclerotinia sclerotiorum on the response to the model allergen ovalbumin (OVA) in mice. The combined exposure to beta (1→3) glucan and OVA gave rise to a significantly greater inflammatory response than either agent alone, significantly increased anti-OA IgE and IgG1 levels relative to OVA alone and significantly increased OVA-specific IgE and IgG1 levels compared with beta (1→3) glucan alone. A similar increase was not found for IgG2a. The findings of earlier investigations of the interaction between beta (1→3) glucan and immune response to OVA were mixed with one study reporting a potentiation of OVA-induced inflammation of the airways in mice whereas an earlier study had found a suppression of OVA-induced airways inflammation in guinea pigs (Rylander and Lin, 2000).

Fogelmark et al. (2001) reported a complex interaction between beta (1→3) glucan and endotoxin in guinea pigs exposed daily to an aerosol of pure beta (1→3) glucan (30 ugm⁻³, giving a dose of about 1.5 ng/animal/day) and pure endotoxin (75 ugm⁻³, giving a dose of about 4 ng/animal/day) for 4 hours/day, 5 days/week for five weeks. They reported an inflammatory response marked by an increase in eosinophil numbers in lung lavage, lung interstitium, and the airway epithelium of animals exposed to beta (1→3) glucan. In animals
simultaneously exposed to endotoxin, there was no increase in eosinophils. In the lung interstitium, beta (1→3) glucan exposure caused an increase in lymphocytes, which was not found after endotoxin exposure. Endotoxin exposure caused an increase in neutrophils and macrophages in lung lavage, which was not found after beta (1→3) glucan exposure. Rylander and Holt (1988) exposed guinea pigs by inhalation to the ovalbumin (OVA), beta (1→3) glucan (8 pg) and LPS (2 ng). LPS caused a stimulation of the OVA-induced antibody production which was abolished by simultaneous exposure to beta (1→3) glucan. An increase of eosinophils (white blood cells associated with inflammation) after OVA exposure was decreased by co-exposure to beta (1→3) glucan. The inferred exposure concentrations for beta (1→3) glucan and LPS are 160 ngm$^{-3}$ and 32.5 ugm$^{-3}$. 
APPENDIX 6

Principles of good regulation

The UK government has a long standing initiative that aims to improve regulatory processes in the UK that is currently co-ordinated by the Better Regulation Commission (www.brc.gov.uk). In 1997 the Better Regulation Task Force outlined the 5 principles of good regulation (http://www.brc.gov.uk/upload/assets/www.brc.gov.uk/principlesleaflet.pdf):

Proportionality – measures should be necessary, appropriate to the risk and the costs identified and minimised;
Accountability – decisions must be justifiable and subject to public scrutiny;
Consistency – rules and standards must be consistent and fairly implemented;
Transparency – regulators should be open and regulations should be simple and user friendly; and
Targeted – regulation should be focussed on the problem and minimise side effects.

In terms of the regulation of bioaerosol emissions there is perhaps a need to demonstrate that regulation is necessary and there will be a requirement to demonstrate that the costs of any regulatory measures proposed are proportionate to risk. Any measures such as the development of air quality guidelines or emissions limits must be based on a strong evidence base. There is a need to ensure that any measures introduced to control bioaerosol exposure are consistent with other measures required to protect other environmental receptors and also measures required to ensure that compost, recyclate or other products of waste processes are of an appropriate quality for subsequent use.

In addition the Better Regulation Task Force indicated that regulations must:

- Be balanced and avoid knee-jerk reactions;
- Seek to reconcile contradictory policy objectives;
- Balance risks, costs and benefits;
- Avoid unintended consequences;
- Be easy to understand;
- Have broad public support;
- Be enforceable;
- Identify accountability; and
- Be relevant to the current conditions.

Contradictory policy objectives could impinge on the regulation of bioaerosol emissions if the process conditions required to minimise emissions to land or water or to optimise product quality are inconsistent with optimal control of bioaerosol emissions. The risks of current or foreseeable levels of exposure to bioaerosols emitted from the waste industry are not well quantified and it will be important to understand the costs and quantify the benefits that might arise on implementation of different policy options. Unintended consequences of applying unattainable standards to the waste industry such as the contraction of recycling or composting capacity in the UK need to be investigated. The benefits of proposed regulatory measures must be clearly demonstrable to the waste industry and the wider public. A key issue in the regulation of bioaerosol emissions is that any proposed measures must be enforceable. For example, if air quality guidelines are proposed, it has to be possible to both achieve and demonstrate compliance. Whatever regulatory framework is developed for bioaerosol emissions, it must clearly indicate the responsibilities of operators and regulatory authorities and be suitable for implementation in the UK waste industry.

The Better Regulation Commission has been tasked with the reduction of regulatory burdens and the securing of a smarter regulatory environment through deregulation, consolidation, rationalisation and reducing administrative burdens (http://www.brc.gov.uk/upload/assets/www.brc.gov.uk/principlesleaflet.pdf). The aim is to "maintain proper limits to protect rights and standards while allowing business to be more productive and competitive, public services to be effective and affordable, voluntary
organisations to thrive without bureaucracy and citizens to exercise freely their rights and responsibilities."

Specific components of the Better Regulation initiative that might apply to the regulation of bioaerosol exposures include:

- Developing a framework for a risk based approach to inspection;
- Developing of proposals to ensure consistency of enforcement;
- Reducing the number of targets imposed on frontline staff;
- Reducing the volume of communications to frontline staff;
- Revising codes of practice and guidance;
- Streamlining data requirements;
- Reducing the frequency of inspection; and
- Reductions in burdens resulting from the introduction of information and communications technology initiatives.

In short, any proposed measures for the regulation of bioaerosol emissions should be proportionate to risk, be amenable to consistent enforcement, not overburden the staff of regulators, optimise the level of data collection and reporting required to address the level of risk of health, facilitate confidence that ongoing good management will be sustained between inspections and use appropriate information technology to ease data reporting and other administrative burdens for both the regulated and the regulator.
APPENDIX 7

Outline of process by which air quality standards are derived

A7.1 BASIS FOR STANDARD SETTING

The aim of the standard setting process is to determine a level of exposure in ambient air that would not be expected to give rise to adverse effects in the vast majority of exposed individuals. The standard setting process includes consideration of both the intensity of exposure (concentration) and the duration of exposure.

For most substances the overall process involves the establishment of the level of exposure at which no adverse effects have been observed in animals and/or humans (no observed effects level or NOEL) and the lowest level of exposure at which adverse effects have been observed (lowest observed effects level or LOEL). The adverse effects on which a standard may be based range from minor but measurable changes in physiological or neurological function of unknown significance to serious disease leading to death. More than one standard may be set for an individual substance to address different effects. For example, separate standards may be set to address respiratory irritation arising from short-term exposure and systemic toxicity arising from long term exposure. Standards are usually set for the health endpoints that have the lowest NOEL/LOELs.

For most substances, standard recommendations are based on the derivation of a NOEL and the use of uncertainty factors to provide a margin of safety between permissible levels of exposure and the NOEL. For substances, such as genotoxic carcinogens, where no threshold has been observed for adverse effects, a different approach is taken. Some authorities (including EPAQS; Defra’s Expert Panel for Air Quality Standards) have established standards based on a level of exposure that would be predicted to give rise to no observable effects in a well conducted epidemiological study. Other authorities have established standards based on a benchmark level of risk such as a $10^{-5}$ increase in lifetime cancer risk (an approach taken by the Defra and the EA in deriving soil quality standards).

A7.2 DATA USED IN STANDARD SETTING

There are a number of different types of study that may inform standard setting:

- Epidemiological studies of populations exposed to the substance of interest in ambient air;
- Epidemiological studies of workers exposed to the substance of interest;
- Experimental studies in human volunteers (short term effects only);
- Experimental studies in animals (short term or long effects);
- Studies undertaken in cell cultures.

Relevant studies are critically reviewed to assess the strength of evidence linking the substance of concern to particular health endpoints and underlying any reported NOEL or LOEL. The review process includes consideration of how well the study was designed and performed, its relevance to the prediction of human health effects in ambient air and the extent to which observed effects could be specifically attributed to the substance of interest. Generally more weight is given to the results of studies in humans than those of animal studies, although consideration is given to study quality, such that the results of a well conducted animal study may carry more weight than those from a poorly conducted human study.

The results of studies of short and longer term health effects of populations exposed via ambient air are the most relevant to the setting of ambient air quality standards but high quality studies, suitable for standard setting, are only available for a relatively small number of

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4 Text taken from a note that I prepared for EPAQS (DEFRA’s Expert Panel for Air Quality Standards) in July 2006
substances. In addition, as populations are exposed to a wide range of substances in ambient air, it can be difficult to attribute effects to individual pollutants.

The results of workplace studies may be highly informative about effects arising at relatively high levels of exposure but there can be uncertainties in exposure estimation, in the effects of concurrent exposure to other agents (confounding) and in the relevance of effects seen at high levels of workplace exposure for the prediction of effects at much lower levels of exposure in ambient air.

The results of human volunteer studies may be highly informative about effects arising during short exposure periods and exposure concentrations are usually well characterised. The exposed population is likely, however, to be unrepresentative of the general community and often only a narrow range of health endpoints are assessed.

Animal studies have been used to investigate the effects of both short term and long term exposure to a wide range of substances and over the last two decades, there has been an increasing tendency to use standardised protocols that allow for ready cross study comparison of results. Exposure concentrations are usually well characterised and experiments generally investigate the effects of single agents. There are differences in physiology and metabolism between species, however, that can give rise to uncertainties about the relevance of effects seen in some animal experiments for the prediction of human health effects. The animal studies that have been conducted with bioaerosol components may not be particularly relevant to standard setting.

Tests involving cell cultures can be informative about the toxicity of a substance and the underlying mechanisms of toxicity but are not yet useful in the prediction of the level of exposure that is likely to give rise to toxic effects in humans.

A7.3 ADJUSTMENT FOR EXPOSURE DURATION

Air quality standards are often derived from data obtained in studies in which exposures were for only part of the week. Where effects arise as a result of prolonged exposure, then it is appropriate to adjust the exposure data to allow for continuous exposure in ambient air. For workplace studies, a factor of 5 is usually allowed for the difference between the number of hours exposed at work during a given year and the total number of hours in that year. For example, for exposure to 1 mgm\(^{-3}\) at work for one year would be equivalent to exposure to 0.2 mgm\(^{-3}\) in ambient air for one year. For effects related to life time exposure, exposure to 1 mgm\(^{-3}\) at work for 40 years would be equivalent to exposure to 0.1 mgm\(^{-3}\) in ambient air for 80 years. The exposure regimes in animal experiments are variable, but 6 hours per day, 5 days per week is not uncommon. An exposure concentration of 1 mgm\(^{-3}\) in such an experiment would be equivalent to continuous exposure to about 0.18 mgm\(^{-3}\) (= (1 mgm\(^{-3}\) x 6 hours x 5 days)/(24 hours x 7 days)).

For effects that arise as a result of short term exposure (eg respiratory irritation), no time weighting of exposure concentration would be appropriate. Consideration should, however, be given to the length of exposure giving rise to observable adverse effects.

A7.4 UNCERTAINTY FACTORS

Uncertainty factors are used to derive a standard from the NOEL (or LOEL, if no NOEL available). Typically these might be x10 to allow for interspecies differences, x10 to allow for interindividual differences and x5 to allow for the difference between a LOEL and a NOEL. Other uncertainty factors might be used to address differences in duration of exposure (for example, if extrapolation from medium term animal studies to life time effects) or differences in the route of exposure (for example, if basing an air quality standard on data from oral studies in animals, if no inhalation data are available). Expert judgement is used to determine the overall magnitude of the margin of safety between the NOEL or LOEL and the recommended standard. In general, a much larger margin of safety is allowed for severe irreversible effects than for relatively minor reversible effects.
A7.5 AVERAGING TIME

For short term effects, the recommended averaging time is based on the length of exposure believed to give rise to the health effect of concern. For example, the effects of sulphur dioxide in causing respiratory irritation are believed to be almost instantaneous and EPAQS selected 15 minutes as an averaging time on the basis that this was the shortest period over which concentrations could be measured. The short term effects of ozone and nitrogen dioxide on respiratory health have been observed in epidemiological studies that employed averaging times of 8 hours and 24 hours respectively and these averaging times were incorporated into the standards for these substances. For long term effects on health, it is customary to take a 1 year averaging time (for example benzene). In some cases it may be appropriate to take an intermediate averaging time such as 1 week or 1 month where effects arise as a result of medium term exposure.