Bioaerosol Health Effects and Exposure Assessment: Progress and Prospects

J. DOUWES¹,²*, P. THORNE³, N. PEARCE² and D. HEEDERIK¹

¹Institute for Risk Assessment Sciences (IRAS), Division of Environmental and Occupational Health, Utrecht University, The Netherlands; ²Centre for Public Health Research, Massey University Wellington Campus, Wellington, New Zealand; ³University of Iowa College of Public Health, Department of Occupational and Environmental Health, IA, USA

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Exposures to bioaerosols in the occupational environment are associated with a wide range of health effects with major public health impact, including infectious diseases, acute toxic effects, allergies and cancer. Respiratory symptoms and lung function impairment are the most widely studied and probably among the most important bioaerosol-associated health effects. In addition to these adverse health effects some protective effects of microbial exposure on atopy and atopic conditions has also been suggested. New industrial activities have emerged in recent years in which exposures to bioaerosols can be abundant, e.g. the waste recycling and composting industry, biotechnology industries producing highly purified enzymes and the detergent and food industries that make use of these enzymes. Dose–response relationships have not been established for most biological agents and knowledge about threshold values is sparse. Exposure limits are available for some contaminants, e.g. wood dust, subtilisins (bacterial enzymes) and flour dust. Exposure limits for bacterial endotoxin have been proposed. Risk assessment is seriously hampered by the lack of valid quantitative exposure assessment methods. Traditional culture methods to quantify microbial exposures have proven to be of limited use. Non-culture methods and assessment methods for microbial constituents [e.g. allergens, endotoxin, β(1→3)-glucans, fungal extracellular polysaccharides] appear more successful; however, experience with these methods is generally limited. Therefore, more research is needed to establish better exposure assessment tools and validate newly developed methods. Other important areas that require further research include: potential protective effects of microbial exposures on atopy and atopic diseases, inter-individual susceptibility for biological exposures, interactions of bioaerosols with non-biological agents and other potential health effects such as skin and neurological conditions and birth effects.

Keywords: asthma; β(1,3)-glucans; bioaerosols; cancer; endotoxin; exposure assessment; infections; microorganisms

INTRODUCTION

Bioaerosols are usually defined as aerosols or particulate matter of microbial, plant or animal origin that is often used synonymously with organic dust. Bioaerosols or organic dust may consist of pathogenic or non-pathogenic live or dead bacteria and fungi, viruses, high molecular weight (HMW) allergens, bacterial endotoxins, mycotoxins, peptido-glycans, β(1→3)-glucans, pollen, plant fibres, etc.

The interest in bioaerosol exposure has increased over the last few decades. This is largely because it is now appropriately recognized that exposures to biological agents in both the occupational and residential indoor environment are associated with a wide range of adverse health effects with major public health impact, including contagious infectious...
HEALTH EFFECTS

In most situations exposure occurs to complex mixtures of toxins and allergens (and chemicals) and a wide range of potential health effects have to be considered. Three major groups of diseases associated with bioaerosol exposure can be distinguished: ‘infectious diseases’, ‘respiratory diseases’ and ‘cancer’. Infectious and respiratory diseases are most common; however, valid incidence or prevalence data for most diseases caused by biological agents are lacking. In addition to these major disease groups (discussed in more detail below) other adverse health effects have been described [e.g. dermatitis in latex exposed workers (Turjanmaa, 1988a; Charous et al., 1994) or pre-term births or late abortions in farm workers exposed to mycotoxins with immunotoxic and hormone-like effects (Kristensen et al., 2000)]. However, to date these effects have not been studied extensively and therefore only limited information is available on these issues. In this section we will discuss the general types of health effects that may occur, and these will then be considered in more detail when examining specific exposures in the following sections. Viruses will be discussed as a cause of infections and cancer but will not be described in more detail since exposure and risk assessment for viruses have hardly been developed for the occupational environment.

Infectious diseases

Infectious diseases arise from viruses, bacteria, fungi, protozoa and helminths and involve the transmission of an infectious agent from a reservoir to a susceptible host through direct contact, a common vehicle, airborne transmission or vector-borne transmission. Since the focus of our paper is on bioaerosols we will discuss occupational infectious diseases caused by airborne exposures only. These may be attributable to: (i) occupation-specific exposures such as may occur in, for example, health workers (tuberculosis, winter stomach flu, measles), farmers, abattoir workers, veterinarians (Q-fever, swine influenza, anthrax) and forestry workers (tularaemia); (ii) clustering of people in the workplace such as in the case of office, military or aviation workers (influenza, winter stomach flu, TB, etc.) (Driver et al., 1994; Van den Ende et al., 1998). In addition, Legionnaires disease and Pontiac fever are high profile bioaerosol transmitted infections that are caused by occupational (as well as non-occupational) exposures to Legionellae (particularly Legionella pneumophila). Legionellae are Gram-negative bacteria that inhabit many water environments including man-made water systems (often in bio-films in, for example, cooling towers, air conditioning systems, etc.) that can cause pneumonia which may be fatal, particularly in susceptible subjects (e.g. elderly or immuno-compromised subjects). Legionellae become airborne often as a...
result of active aerosolizing processes (e.g. aeration of contaminated water). Outbreaks have been reported in a variety of workplaces including those associated with cooling towers (Castellani Pastoris et al., 1997; Brown et al., 1999), hospital bathrooms (Kool et al., 1999), meat packers (Osterholm et al., 1983), workplaces where water mist systems are used (fruit and vegetable stores; Mahoney et al., 1992) or whirlpool spas and sprinklers (Den Boer et al., 2002). Finally, several diseases may arise from inhalation of fungal spores in the course of handling decaying matter, faeces, compost or soil. These diseases include aspergillosis, histoplasmosis, blastomycosis, coccidioidomycosis and adiaspiromycosis (MMWR, 1993, 1999; NIOSH, 1997).

Thus, high-risk occupations for occupational infectious diseases due to bioaerosol exposure include farmers, veterinarians, health care workers and biomedical workers studying infectious agents. For a more complete overview of occupational infections the reader is referred to textbooks (Garibaldi and Janis, 1992; Gerberding and Holmes, 1994) and the NIOSH website (National Occupational Research Agenda: infectious diseases at www.cdc.gov/niosh/nrinfo.html).

Respiratory diseases

Respiratory symptoms and lung function impairment are probably the most widely studied among organic dust-associated health effects. They can range from acute mild conditions that (at least initially) hardly affect daily life, to severe chronic respiratory diseases that require specialist care. Generally, occupationally related respiratory symptoms result from airway inflammation caused by specific exposures to toxins, pro-inflammatory agents or allergens. Based on the underlying inflammatory mechanisms and subsequent symptoms, a distinction between allergic and non-allergic respiratory diseases can be made. Non-allergic respiratory symptoms reflect a non-immune-specific airway inflammation, whereas allergic respiratory symptoms reflect an immune-specific inflammation in which various antibodies (IgE, IgG) can play a major role in the inflammatory response. In occupational medicine it has long since been recognized that a substantial proportion of work-related asthma symptoms are non-allergic. This type of asthma is often referred to as ‘asthma-like disorder or syndrome’ or ‘irritant-induced asthma’ (Bernstein et al., 1999) and is highly prevalent in farmers and farm-related occupations and is in these occupations assumed to be caused by bioaerosol exposures (particularly endotoxin) (Anonymous, 1998). Although it meets the clinical criteria of asthma (i.e. reversible airways obstruction) it has been shown in some populations (e.g. swine farmers) that these symptoms are not only associated with a cross-shift reversible decrease in lung function (asthma) but also with an accelerated chronic decline in lung function (COPD, chronic obstructive pulmonary diseases) (Vogelzang et al., 1998). This is clearly different from allergic asthma (caused by allergen exposure) where a chronic lung function decline is generally only moderate. In addition, cross-shift decline in lung function is usually smaller than observed in typical type I allergic asthma. Table 1 gives an overview of allergic and non-allergic respiratory diseases with potential causal agents of biological origin. Pre-existing respiratory conditions or other host factors (e.g. atopy, smoking) may modify the risk of developing work-related respiratory symptoms (Cullinan et al., 1999). For instance, an asthmatic worker with pre-existing asthma may experience work-related exacerbation of asthma.
symptoms due to organic dust exposure at levels that do not normally induce any symptoms in other ‘healthy’ workers.

In addition to asthma and COPD (see above), organic dust exposed workers may develop hypersensitivity pneumonitis (HP) and organic dust toxic syndrome (ODTS) (Table 1). ODTS is an acute febrile non-allergic illness, characterized by an increase in body temperature (fever), shivering, dry cough, chest tightness, dyspnea, headache, muscle and joint pains, fatigue, nausea and general malaise (Donham and Rylander, 1986; Von Essen et al., 1990; Rylander, 1997c). The symptoms resemble those of influenza, but symptoms usually disappear the following day. The disease is common among workers heavily exposed to organic dust. HP is a generic term used to describe a serious pulmonary condition with delayed febrile systemic symptoms, manifested by an influx of inflammatory cells to the lung parenchyma and the formation of granulomas there (Bourke et al., 2001). HP is also known as extrinsic allergic alveolitis (EAA) and, depending on the specific work environment where the disease has been observed, various other names have been introduced to describe the disease, e.g. farmer’s lung, pigeon breeder’s lung, mushroom grower’s lung, maple bark strippers disease, etc. Symptoms characteristic for HP are very similar to those described for ODTS but are more serious and in its chronic stage may lead to permanent lung damage and work disability (Pickering and Newman Taylor, 1994). The underlying immunological mechanisms of HP are complex and only partly understood but both allergic and non-allergic immunological responses are believed to play a role (Salvaggio, 1997; Bourke et al., 2001).

Cancer

Cancer can be caused by a variety of factors including oncogenic viruses and other biological agents. To date the only clearly established non-viral biological occupational carcinogens are the mycotoxins. These occur in industries in which mould-contaminated materials are handled (Anonymous, 1998). Perhaps the best-known carcinogenic mycotoxin is aflatoxin from Aspergillus flavus, which is an established human carcinogen particularly with regard to liver cancer (Hayes et al., 1984; Sorenson et al., 1984; Bray and Ryan, 1991). Ochratoxin A is also considered a possible human carcinogen (National Toxicology Program, 1991). The most relevant route of exposure to aflatoxin and ochratoxin is by ingestion, but exposure can also occur by inhalation in industries such as peanut processing or livestock feed processing and in industries in which grain dust exposure occurs (Sorenson et al., 1984; Autrup et al., 1993). Workers in livestock feed processing have an increased risk of liver cancer as well as cancers of the biliary tract, salivary gland and multiple myeloma (Olsen et al., 1988). Farmers are at increased risk for certain specific cancers, including haematological cancers, lip, stomach, prostate, connective tissue and brain cancer (Blair et al., 1992; Khuder et al., 1998). Hypothesized explanations involve exposure to pesticides or exposure to oncogenic viruses or other biological agents carried by farm animals. Studies have shown a consistent excess of lung cancer associated with abattoir workers and butchers (Reif et al., 1989). Others also indicated increased leukaemia risks with employment in the meat industries (Bethwaite et al., 2001). Direct contact with animals was determined to be an important factor suggesting that biological exposures (probably zoonotic viral exposures such as herpes, avian leucosis and papilloma viruses) are likely to be responsible. In addition, a number of studies have found associations between exposure to wood dust and various specific cancers, in particular sinonasal cancer in furniture making, cabinet making, carpentry and joinery and in other wood-related jobs including sawmills (Demers and Boffetta, 1998). Finally, workers in several other industries that process biological materials are at risk of developing various cancers, such as workers in the rubber, textile, leather and boot and shoe industries. However, it is currently unknown whether these excess risks occur from exposures to biological agents or are due to various chemicals used in these industries.

SPECIFIC EXPOSURES

Fungi and bacteria

Fungi and thermophilic bacteria are well-known sources of allergens that play a role in the development of HP. The species involved include many common genera such as Penicillium and Aspergillus, which occur in some work environments usually at very high levels (e.g composting facilities, farms, etc.). Hay contaminated with thermophilic bacteria such as Saccharopolyspora rectivirgula or Thermoactinomycetes vulgaris is the source of allergens causing farmer’s lung or HP (Pepys et al., 1990; Reboux et al., 2001), and similar disorders have been observed among mushroom growers (Van den Bogart et al., 1993) and, incidentally, among compost workers (Weber et al., 1993; Allmers et al., 2000). A specific exposure with high risk of occupational disease is that to Aspergillus fumigatus, a fungus that not only induces allergic sensitization and symptomatic allergic lung disease, but can also cause an infectious mycosis (broncho-pulmonary aspergillosis), especially in immuno-compromised subjects. Many fungal species have also been described as producers of type I allergens (IgE binding allergens), and IgE sensitization to common outdoor and indoor fungal genera like Alternaria, Penicillium, Aspergillus and Cladosporium spp. is strongly associated
with allergic respiratory disease, especially asthma (Halonen et al., 1997; Ostro et al., 2001). However, there is very little evidence that supports an important role for type I allergy to fungi in occupational respiratory disease. Fungi are also a source of β(1→3)-glucans which are suspected to cause non-allergic respiratory symptoms (see below).

Most bacteria or bacterial agents are not very potent allergens, with the exception of the spore-forming actinomycetes described above. Bacterial cell wall components, such as endotoxin (present only in Gram-negative bacteria; see below) and peptidoglycans (most prevalent in Gram-positive bacteria), are agents with important pro-inflammatory properties that may induce respiratory symptoms. The effects of peptidoglycans are assumed to be very similar to those observed with endotoxin exposure; however, this has not been systematically studied.

Finally, bacteria and fungi include a number of well-known pathogenic infectious microorganisms that may after inhalation cause specific diseases as described above.

**Endotoxin**

Endotoxin is composed of lipopolysaccharides (LPS) and is a non-allergenic cell wall component of Gram-negative bacteria with strong pro-inflammatory properties. It is commonly present in many occupational environments (Table 1) but also in the general environment, and particularly in house dust (Peterson et al., 1964; Douwes et al., 2000b).

Endotoxin has been recognized as an important factor in the aetiology of occupational lung diseases including (non-allergic) asthma (Douwes and Heederik, 1997) and ODTS (see above). Subjects exposed to endotoxin in inhalation experiments experience clinical effects such as fever, shivering, arthralgia, influenza-like symptoms (malaise), blood leukocytosis, neutrophilic airway inflammation, asthma symptoms such as dry cough, dyspnea and chest tightness, bronchial obstruction, as well as dose-dependent lung function impairment (FVC, FEV₁, and flow-volume variables) and decreased lung diffusion capacity (Pernis et al., 1961; Castellan et al., 1987; Michel et al., 1992, 1996, 1997; Clapp et al., 1994; Jägielo et al., 1996; Michel, 1997; Thorn and Rylander et al., 1998). Many occupational studies have shown positive associations between endotoxin exposure and health effects including both reversible (asthma) and chronic airway obstruction, respiratory symptoms (symptoms of asthma, bronchitis and byssinosis) and increased airways responsiveness (Table 2). This was consistently observed in a large variety of occupational environments (listed in Table 2) characterized by different exposure levels and different compositions of the bioaerosol exposures (Kennedy et al., 1987; Milton et al., 1996; Douwes and Heederik, 1997). Several of these studies reported clear exposure–response relationships (Smid et al., 1992; Vogelzang et al., 1998). One study in the potato processing industry showed that acute airway obstruction was already apparent at levels of ~50 endotoxin units (EU)/m³ (~5 ng/m³) (Zock et al., 1998). Subjects with increased bronchial hyperresponsiveness and/or asthma are more sensitive to develop symptoms (Michel et al., 1989) but interestingly large differences in airway responsiveness to inhaled endotoxin also exist in healthy (non-allergic) subjects suggesting that potentially only susceptible individuals are at risk (Kline et al., 1999). Several studies in the indoor environment have suggested a causal association between endotoxin and asthma exacerbation in children and adults (Michel et al., 1996; Park et al., 2001).

**β(1→3)-glucans**

β(1→3)-glucans are glucose polymers with variable molecular weight and degree of branching (Williams, 1997) that originate from most fungi, some bacteria, most higher plants and many lower plants (Stone and Clarke, 1992).

<table>
<thead>
<tr>
<th>Occupational environment</th>
<th>Health effects</th>
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<tbody>
<tr>
<td>Cotton industry</td>
<td>Acute and chronic respiratory symptoms including asthma</td>
</tr>
<tr>
<td>Farming (livestock, particularly pigs and poultry; grain)</td>
<td>Acute and chronic decline in lung function (FEV₁, PEF, FVC, MMEF)</td>
</tr>
<tr>
<td>Animal feed and grain industry</td>
<td>Acute and chronic decline in lung function (FEV₁, PEF, FVC, MMEF)</td>
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<tr>
<td>Potato processing industry</td>
<td>Non-allergic (neutrophilic) airway inflammation</td>
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<tr>
<td>Fibreglass industry</td>
<td>Increased bronchial hyperresponsiveness</td>
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<td>Slaughter houses</td>
<td>Organic dust toxic syndrome (acute and short-lived flu-like symptoms with fever and airway symptoms)</td>
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<tr>
<td>Waste and compost industry</td>
<td>Chronic bronchitis (?)</td>
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<td>Vegetable fibre production</td>
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<td>Automobile industry</td>
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Results of several studies in which subjects were exposed to airborne β(1→3)-glucans suggest that these agents play a role in bioaerosol-induced inflammatory responses and resulting respiratory symptoms (Rylander et al., 1992; Fogelmark et al., 1994; Rylander, 1996). In a small group of garlic workers only minor inflammatory responses in the nasal mucosa were demonstrated after nasal instillation with β(1→3)-glucan (Sigsgaard et al., 2000). However, in a whole blood assay measuring cytokine release after in vitro exposure to high concentrations of β(1→3)-glucans, a significant increase in all measured cytokines (TNFα, IL-1β, IL-6 and IL-8) was found (Sigsgaard et al., 2000), thus demonstrating the pro-inflammatory potential of β(1→3)-glucans. Several, mostly small, field studies have been performed in the home environment, day care centres, an office building, schools and among household waste collectors and paper mill workers, suggesting a relation with respiratory symptoms, airway inflammation, lung function and atopy in exposed individuals (Rylander et al., 1994, 1998, 1999; Rylander, 1997a,b; Thorn and Rylander, 1998a,b; Wan and Li, 1999). A recent study in The Netherlands showed an association between peak flow variability and β(1→3)-glucan levels in house dust among children (n = 159) with respiratory symptoms (Douwes et al., 2000b). Animal studies showed that β(1→3)-glucan may act synergistically with endotoxin in causing airway inflammation (Fogelmark et al., 1992, 1994), and it was further suggested that β(1→3)-glucan may enhance the production of specific IgE. However, the results of those studies were mixed (Rylander and Holt, 1998; Wan and Li et al., 1999; Fogelmark et al., 2001). Health effects of β(1→3)-glucan exposure in the occupational environment thus seem plausible but the evidence is currently still weak since most studies were small and not always appropriately controlled for other potential causal exposures.

Mycoxotins

Mycoxotins or fungal toxins are low molecular weight biomolecules produced by fungi that are toxic to both animals and humans. Some (e.g. aflatoxin from Aspergillus) can be potent carcinogens (see above). Numerous other mycoxotins have been classified (Nelson et al., 1983; Krough, 1984) possessing distinct chemical structures and reactive functional groups, including primary and secondary amines, hydroxyl or phenolic groups, lactams, carboxylic acids and amides. Very little is known about occupational airborne exposures to mycoxotins and respiratory health effects. Mycoxotins of Fusarium, Aspergillus and Penicillium genera are known to be present in the inhalable fraction of airborne corn dust (Sorensen, 1990), cotton dust (Salvaggio et al., 1986) and grain dust (Lacey et al., 1994). It is not clear, however, whether these components contribute to the frequently reported respiratory symptoms in the cotton and grain industries.

Allergens

Allergens can comprise a large variety of macro-molecular structures ranging from low (mainly chemicals such as di-isocyanates) to high molecular weight sensitizers, which are most often proteins of biological origin. Most potent occupational IgE binding allergens include enzymes derived from fungi and bacteria produced by biotechnological companies for use in, for example, washing powders and both the human and animal food industries (Sandiford et al., 1994; Schweigert et al., 2000; Cullinan et al., 2000, 2001). Populations at risk are therefore not only workers in the enzyme producing industries, but also those workers in for instance food processing industries where enzyme preparations are used. Other well-known IgE binding allergens are plant pollens, which may cause allergies in greenhouse workers (van der Zee et al., 1999). Latex allergens have received extensive attention during the last decade with high numbers of health and hospital workers being sensitized due to the use of latex gloves produced from sap from the rubber tree Hevea brasiliensis (Turjanmaa et al., 1996). Finally, several animal proteins (dust mite, cat, mouse and rat allergens) are known to have strong allergenic properties. In particular, it is well established that laboratory animal workers are at risk of developing occupational type I (IgE-mediated) allergy to mice and rat allergens (Cullinan et al., 1999). In addition to IgE binding allergens workers may be exposed to IgG binding allergens. These allergens are assumed to be involved in the pathogenesis of HP or farmer’s lung and are produced by moulds and actinomycetes (see above). However, although an allergic immune response is suspected in HP the exact pathology is not known and other mechanisms are assumed to play a role as well.

PROTECTIVE EFFECTS OF MICROBIAL EXPOSURE?

In recent years the ‘hygiene hypothesis’ has shifted attention from the adverse health effects to the potential beneficial effects of microbial agents (Martinez, 1999). This postulates that growing up in a more hygienic environment may enhance atopic (Th2) immune responses (Holt et al., 1997; Martinez, 1999). It is believed that microbial exposures, and particularly exposure to endotoxin early in life, may protect from developing atopy and allergic asthma; however, mechanisms are not well understood (Douwes et al., 2002). This potential protective effect is remarkable since it is well known from occupational studies that these agents may cause non-
allergic respiratory symptoms (see above). Some animal and in vitro work (Tulic et al., 2000) and some population studies (Gereda et al., 2000; Braun-Fahrlander et al., 2002) appear to support this theory. In addition, several studies in farmers’ children have shown that growing up on a farm protects against atopy and asthma (Braun-Fahrlander et al., 1999; Riedler et al., 2001). The reasons for this are not established but one possible explanation is that high endotoxin exposures in this environment may play a role (Von Mutius et al., 2000). (Most of the studies in farmers’ children were conducted on small family farms that may not be representative of large industrialized farms.) It is not clear whether these same protective effects operate in the working population. Although respiratory symptoms including (non-allergic) asthma are generally more prevalent in working populations exposed to high levels of microbial agents and endotoxins such as, for example, farmers (Anonymous, 1998), some studies indicate that atopy is less prevalent (Van Hage-Hamsten et al., 1987; Iversen and Pedersen, 1990) thus suggesting that these agents may protect from atopy and atopic symptoms also in the working population. However, this lowered prevalence might be the result of exposures in childhood that are sustained up to adult age, although studies among farmers’ apprentices with an urban background suggest otherwise (Portengen et al., 2002). Few studies have addressed this important area and the results are inconclusive, studies in working populations without an exposure history to endotoxins in childhood are therefore urgently needed.

**EXPOSURE ASSESSMENT**

The assessment of exposures to bioaerosols offers distinct challenges from those for inorganic aerosols and chemical agents. Pathogenic microorganisms may be hazardous at extremely low levels while other organisms may only become important health hazards at orders of magnitude higher concentrations. Some organisms and spores are extremely resilient while others may be easily degraded in the sampling process. Certain fungal spores are easily identified and counted while many bacteria are difficult to distinguish. Sensitive and specific methods are available for the quantification of some biological agents while there are no good methods for others. Many of the newly developed methods [e.g. measurement of microbial agents such as β(1→3)-glucans or fungal extracellular polysaccharides; see below] have not been well validated and are often not commercially available. Even for some well-established methods (e.g. the LAL assay to measure bacterial endotoxin; see below) significant variations in exposure assessment between laboratories have been demonstrated (Thorne et al., 1997; Chun et al., 2000; Reynolds et al., 2002). Also, issues of storage and transport of bioaerosol samples have often not been addressed whereas it is known that these conditions may affect the activity of some biological agents, e.g. endotoxin (Thorne et al., 1994; Douwes et al., 1995; Duchaine et al., 2001). Finally, many biological agents that may cause health effects are currently not identified. For instance, sewage treatment workers have an increased risk of developing a wide range of symptoms including respiratory, gastrointestinal and neurological symptoms, whereas causal agents have not conclusively been identified (Douwes et al., 2001). Thus, in order to establish a complete picture of the bioaerosol exposure (and to appropriately assess the risks associated with it) various exposure assessment methods have to be explored.

**ASSESSMENT METHODS FOR MICROORGANISMS**

Measurement of microorganisms relies upon the collection of a sample into or onto solid, liquid or agar media with subsequent microscopic, microbiological, biochemical, immunochemical or molecular biological analysis (Eduard and Heederik, 1998). Two distinctly different approaches are being distinguished for the evaluation of microbial exposure: ‘culture-based methods’ and ‘non-culture methods’.

**Culture-based methods**

Airborne exposure to microorganisms in the environment can be studied by counting culturable propagules in air samples (or in settled dust samples). A variety of devices for microbial bioaerosol sampling have been developed and described previously (Eduard and Heederik, 1998). Sampling of culturable bioaerosols is based on impactor (microorganism are collected directly on a culture medium), liquid impinger (microorganisms are collected in liquid collection fluid) or air filtration methods (microorganisms are collected on a filter). After sample collection colonies of bacteria and fungi are grown on culture media at a defined temperature over a 3–7 day period. Colonies are counted manually or with the aid of image analysis techniques.

Counting of culturable microorganisms has some serious drawbacks including poor reproducibility, selection for certain species due to chosen culture media, temperature etc. and the fact that dead microorganisms, cell debris and microbial components are not detected, while they too may have toxic and/or allergenic properties. In addition, good methods for personal air sampling of culturable microorganisms are not available, and air sampling for a period of more than 15 min is often not possible, whereas air concentrations usually vary largely in time. On the other hand, counting of culturable microorganisms is potentially a very sensitive technique and many
different species can be identified. Traditionally used culture methods have proven to be of limited use for quantitative exposure assessment. Culture-based techniques thus usually provide qualitative rather than quantitative data that can, however, be important in risk assessment, since not all fungal and bacterial species pose the same hazard. An extensive review on techniques for sampling and culturing microorganisms has recently been published (Eduard and Heederik, 1998).

Non-culture methods

Non-culture-based methods enumerate organisms without regard to viability. Sampling of non-culturable bioaerosols is generally based on air filtration or liquid impinger methods. Microorganisms can be stained with a fluorochrome, e.g. acridine orange, and counted with an epifluorescence microscope (Thorne et al., 1994). Possibilities of classifying microorganisms taxonomically are limited because little structure can be observed. Electron microscopy (EM) or scanning EM can also be used and allow better determination (Eduard et al., 1988; Karlsson and Malmberg, 1989). Simple light microscopy may be used to count microorganisms, but counting is based only on morphological recognition, which may result in severe measurement errors. Bacteria collected with impingers or filters can be counted by flow cytometry after staining with 4',6-diamino-2-phenylindole (DAPI) or by applying fluorescent in situ hybridization (FISH) (Lange et al., 1997). FISH involves the use of fluorochrome-labelled nucleic acid probes to target rRNA within morphologically intact cells, allowing taxonomic determination from kingdom to species (Lange et al., 1997).

The main advantage of microscopy or flow cytometry is that both dead and living microorganisms are quantified, selection effects are limited, personal air sampling is possible and sampling time can be varied over a large range. Disadvantages include laborious and complicated procedures, high costs per sample, unknown validity, no detection of possibly relevant toxic or allergenic components or cell debris, while possibilities for the determination of microorganisms for most of these techniques are limited. A more extensive review on microscopy and flow cytometry methods for counting non-culturable microorganisms has recently been published (Eduard and Heederik, 1998).

ASSessment METHODS FOR MICROBIAL CONSTITuENTS

Instead of counting culturable or non-culturable microbial propagules, constituents or metabolites of microorganisms can be measured as an estimate of microbial exposure. Toxic (e.g. mycotoxins) or pro-inflammatory (e.g. endotoxin) components can be measured but also non-toxic molecules may serve as markers of either large groups of microorganisms or of specific microbial genera or species. The use of advanced methods, such as polymerase chain reaction (PCR)-based technologies and immunoassays (see below), have opened new avenues for detection and speciation regardless of whether the organisms are culturable. Some markers for the assessment of fungal biomass include ergosterol measured by gas chromatography–mass spectrometry (GC-MS) (Miller and Young, 1997) or fungal extracellular polysaccharides measured with specific enzyme immunoassays (Douwes et al., 1999), allowing partial identification of the mould genera present. Volatile organic compounds produced by fungi may be suitable markers of fungal growth (Dillon et al., 1996). Other agents such as β(1→3)-glucans (Aketagawa et al., 1993; Douwes et al., 1996) and bacterial endotoxin are being measured because of their toxic potency. Endotoxin is measured by using a Limulus amoebocyte lysate (LAL) test prepared from blood cells of the horseshoe crab, Limulus polyphemus (Bang, 1956). Analytical chemistry methods for quantification of LPS have also been developed employing GC-MS (Sonesson et al., 1988, 1990). However, these methods require special LPS extraction procedures and have not been widely used. Two methods to measure β(1→3)-glucans have been described, one of which is based on the LAL assay (Aketagawa et al., 1993) and the other on an enzyme immunoassay (Douwes et al., 1996). Finally, PCR techniques have been developed for the identification and quantitation of specific species of bacteria and fungi in the air (Alvarez et al., 1994; Khan and Cerniglia, 1994). PCR allows amplification of small quantities of target DNA, typically by 10^5–10^10 times, to determine in a qualitative manner the presence of specific microorganisms. Application of quantitative PCR for analysis of air samples containing microorganisms is still under development but is expected to find applications in situations where specific infectious microorganisms may be present. Table 3 gives an overview of assessment methods for constituents of microorganisms.

Most of the methods to measure microbial constituents (with the exception of the method to measure bacterial endotoxin) are in an experimental phase and have as yet not been routinely applied and/or are not commercially available. Important advantages of these methods include: (i) the stability of most of the measured components, allowing longer sampling times for airborne measurements, and frozen storage of samples prior to analysis; (ii) the use of standards in most of these methods; (iii) the enhanced possibility to test for reproducibility.
Antibody-based immunoassays, particularly enzyme-linked immunosorbent assays (ELISA) are widely used for the measurement of aeroallergens and allergens in settled dust in buildings. To date, the house dust mite allergens, Der p I, Der f I and Der p/f II, have been most widely investigated and the methods have been well described (Luczynska et al., 1989; Price et al., 1990; Leaderer et al., 2002). Methods for assessment of exposure to allergens from animals (Swanson et al., 1985; Virtanen et al., 1986; Schou et al., 1991; Hollander et al., 1997), cockroaches (Pollart et al., 1991), storage mites (Iversen and Pedersen, 1990) and latex rubber (Miguel et al., 1996) have also been published. Assays are also available for the measurement of bio-technologically produced allergens such as fungal α-amylase (Houba et al., 1997).

**ASSESSMENT METHODS FOR BIO-ALLERGENS**

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**BIOMARKERS OF EXPOSURE**

For bioaerosols very few biomarkers of exposure or dose have been identified and the validity for exposure assessment is often not established. To our knowledge no direct methods to measure biological agents or metabolites thereof in body fluids (blood or urine) have been described. IgG antibodies in serum have been suggested as an indirect marker of recent exposure to fungi (Burell and Rylander, 1981; Eduard et al., 1992). However, little is known about the quantitative relation between serum IgG levels and airborne exposures. Therefore, IgG levels as a proxy of exposure or dose should be interpreted with caution.

IgE and inflammatory markers in blood, sputum, nasal lavage and exhaled breath condensate have been suggested as biomarkers of exposure, but these are more appropriately addressed as markers (or intermediates) of effect since they play a major role in the pathophysiological events leading to symptoms and disabling disease. Therefore these should not be considered markers of exposure.

**STANDARD SETTING**

Although health risks associated with bioaerosols have been identified, exposure–response relationships have been described only for a few agents, often with dust exposure as the proxy of exposure to the aetiological agent. ‘No effect levels’ (NEL) are therefore available only for some well-identified exposures. For some agents such as wood dust, standards have been adopted in several countries [e.g. 5 mg/m³ in the USA and 2 mg/m³ in The Netherlands, based on 8 h time weighted averages (8-TWA) of inhalable dust]. In a recent literature review by Demers et al. (1997) a standard of 1 mg/m³ for softwoods was suggested to protect workers from non-malignant effects. The American Conference of Governmental Industrial Hygienists (ACGIH) has established a ‘threshold limit value’ (TLV) for 8-TWA of 4 mg/m³ total grain dust (wheat, oats, barley) since 1980 (ACGIH, 1980). The Ad Hoc Committee on Grain Dust of the Canadian Thoracic Society Standards Committee considered a ‘permissible exposure level’ (PEL) of 5 mg/m³ advisable to control short-term effects, even if these effects are transient (Becklake et al., 1996).

For endotoxin ‘no observed effect levels’ (NOEL) for various health endpoints have been reported in the literature ranging from 50 to several hundred EU/m³ (Dowues and Heederik, 1997; Rylander, 1997c). A health-based exposure limit has been proposed in The Netherlands by the Dutch Health Council of 50 EU/m³ (8-TWA) (Dutch Expert Committee on Occupational Standards, 1998). The Minister of Social Affairs is now considering adopting a legally binding limit of 200 EU/m³ since a limit of 50 EU/m³ was found not to be feasible because of economic effects for some sectors of the industry. Since differences in storage, extraction and analysis of endotoxin samples may result in large differences in exposure estimates...
also adopted an exposure standard of 0.5 mg/m$^3$
for subtilisins could be identified. The ACGIH has
on the ACGIH TLV, because no safe exposure limit
(Heederik
response relationships have now been established
(ACGIH, 1990). For several other allergens, exposure–
ments. ACGIH (2002) now lists subtilisins as a
some of the earlier methods for exposure measure-
analytical limitations, i.e. by the detection limits of
underpinning of this TLV, and the proposed value
exposure has been adopted by the ACGIH (2002).
However, there is considerable doubt about the
primarily involved and even for known pathogenic
agents clear dose–response relationships have not
been established. This is mainly due to a lack of valid
methods to assess exposure quantitatively. Thus,
there is a clear need for method development, parti-
cularly based on non-culture techniques since culture
methods have been proven to be of limited use in
population-based studies. Secondly, existing methods
need rigorous validation and subsequent further
development to make them more suitable for large-
scale epidemiological studies or for industrial hygiene
purposes. Validation of methods is particularly
needed for those agents where occupational exposure
limits have been established (e.g. endotoxin, aller-
gens) resulting in internationally accepted protocols
that should include concise and uniform guidelines
on sampling, storage, extraction and analytical
procedures. Recurrence of infectious diseases and the
recent threat of bio-terrorism have accelerated the
development of measurement methods for specific
microorganisms using DNA-based technology. More-
over, a clear need is developing for rapid and direct
reading assays for bioaerosols for immediate evalu-
ation of the presence of health risks. This is a new
development that will certainly have an important
spin-off for the occupational and environmental
health fields. Application of new and better methods
will allow a more valid risk assessment for bioaero-
sols and individual components thereof.

Other important areas that require further research
include: (i) the potential protective effect of endo-
toxin and other microbial agents on atopy and atopic
diseases, for which studies should address the issue
of timing and dose of endotoxin exposure with
respect to both these potentially protective effects
and adverse non-atopic health effects; (ii) the shape
of exposure–response relationships for allergens and
development of tolerance; (iii) the issue of individual
susceptibility for allergens, endotoxin and other
biological exposures; (iv) the interaction effects
between various allergenic and non-allergenic agents
in causing health effects; (v) the identification of
other biological agents that may cause adverse (or
protective) health effects; (vi) more research into
other health effects (e.g. skin conditions, neuro-
logical symptoms, pre-term births or late abortions)
and exposure routes (e.g. skin, gastro-intestinal
system).

CONCLUSIONS

Potential health effects of bioaerosol exposures are
diverse including infectious diseases, acute toxic
effects, allergies and cancer. Methods to assess
bioaerosol exposures are available; however, selec-
tion of the most appropriate method(s) is highly
dependent on the specific goals of the study. Most
culture methods provide important qualitative infor-
mation but are at best only semi-quantitative and they
have proven to be of limited use in population-based
studies. Several non-culture methods have been
developed with promising results in epidemiological

RESEARCH NEEDS

As described above, bioaerosol exposure is associ-
ated with a large variety of symptoms and diseases.
However, it is often not clear which agents are
primarily involved and even for known pathogenic
agents clear dose–response relationships have not
been established. This is mainly due to a lack of valid
methods to assess exposure quantitatively. Thus,
studies, however, the experience with those new
assays is still limited and they are generally not
widely available. Even some of the more established
methods to measure specific biological agents (e.g.
endotoxin with a LAL assay or allergens with an
enzyme immunoassay) are only poorly validated.
Therefore, interpretation of exposure results is
impossible without detailed information about the
sampling and analytical procedures. Thus, due to
large uncertainties in exposure assessment (because
of poorly developed quantitative exposure assess-
ment tools) risk assessment is complicated, hampering
legal exposure limits being developed (with the
exception of a few specific components such as
specific allergens and endotoxin). Therefore, more
research is needed to establish better exposure assess-
ment tools and to validate newly developed methods.

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