Review

Bioaerosol Health Effects and Exposure Assessment: Progress and Prospects

J. DOUWES[1,2]*, P. THORNE[3], N. PEARCE[2] and D. HEEDERIK[1]

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

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, peptidoglycans, 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

*Author to whom correspondence should be addressed. IRAS, PO Box 80176, 3508 TD, Utrecht, The Netherlands. Tel: +31-30-2535400; fax: +31-30-2535077; e-mail: j.douwes@iras.uu.nl

diseases, acute toxic effects, allergies and cancer. Several new industrial activities have emerged in recent years in which exposures to biological agents can be abundant. One example is the waste recycling industry. Workers in this industry (e.g. waste sorting, organic waste collection and composting) are often exposed to very high levels of microorganisms (van Tongeren *et al.*, 1997; Douwes *et al.*, 2000a) and several studies have indicated a high prevalence of respiratory symptoms and airway inflammation in these industries (Sigsgaard *et al.*, 1994; Poulsen *et al.*, 1995; Thorn and Rylander, 1998a; Douwes *et al.*, 2000a; Wouters *et al.*, 2002). Another example is the production of highly purified biological substances such as microbial enzymes that are used particularly in the food processing industry (e.g. α-amylase in the bread making industry) and detergent industry (Sandiford *et al.*, 1994; Schweigert *et al.*, 2000). Today, there is a clear trend to increase production and use of these enzymes. Many of these enzymes are potent allergens that may cause allergic asthma and rhinitis in workers handling the enzymes and/or intermediate products that contain enzymes (Cullinan *et al.*, 2000, 2001). The increased insulation of buildings combined with poor ventilation has also created environments with elevated exposures to bioaerosols (mainly moulds) and several studies have suggested that a significant portion of building-related disease occurrence (e.g. 'sick building syndrome') is associated with these exposures (Walinder *et al.*, 2001). Finally, the widespread use of antibiotics in livestock has accelerated the development of antibiotic-resistant pathogens which may increase the risk of severe infectious diseases in workers handling and processing livestock.

Despite the recognition of the importance of bioaerosol exposure on human health, the precise role of biological agents in the development and aggravation of symptoms and diseases is only poorly understood. It is not clear (with the exception of specific pathogens and a few individual components such as bacterial endotoxin and specific allergens; see below) which specific component(s) primarily accounts for the presumed health effects. Dose–response relationships have often not been described and knowledge about threshold values is (with the exception of a few agents) not available. This relative lack of knowledge is mainly due to the lack of valid quantitative exposure assessment methods.

In this paper we will give an overview of the health effects associated with bioaerosol exposure in the occupational environment. In addition, we will review exposure assessment methods with a focus on non-culturable methods. Finally, we will discuss the potential for standard setting and identify relevant future research areas.

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, 1987; Charous *et al.*, 1994) or pre-term births or late abortions in farm women 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

Table 1. Non-infectious respiratory diseases, potential causal agents and work environments with known or suspected increased risks

Respiratory diseases	Agents	Environments
Non-allergic		
Non-allergic asthma, non-allergic rhinitis/mucous membrane irritations (MMI), chronic bronchitis, chronic airflow obstruction, organic dust toxic syndrome (ODTS)	Fungi, bacteria, actinomycetes, endotoxin, β(1,3)-glucans, peptidoglycans, mycotoxins, and probably many other currently unidentified plant and amicrobial components	Agriculture and related industries, sewage/manure treatment/handling, food and animal feed industry, vegetable and animal fibre processing, wood industry, paper production, fermentation industry, slaughterhouses, metal machining industries (contaminated metal fluids), garbage collection and composting, buildings with contaminated ventilation/humidifying systems
Allergic		
Allergic asthma, allergic rhinitis, hypersensitivity pneumonitis (HP)/extrinsic allergic alveolititis (EEA)/farmer's lung	Fungi, microbial enzymes, plant proteins (soy, wheat, pollen, latex, etc.), mammalian proteins (rat, mouse, cow, etc.), invertebrate proteins (moths, locusts, spiders, etc.)	Compost facilities, agriculture and related industries, biotechnology industry and enzyme producers, food and animal feed industry, detergent industry, bakery industry, medical and public health sector (latex), veterinarians, pet shop keepers, laboratory animal facilities, biopesticide industry (invertebrates)

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 inflam-

matory 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, fatique, 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 *Thermoactinomyces 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 $\beta(1\rightarrow3)$-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, FEV_1 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; Jagielo *et al.*, 1996; Michel, 1997; Thorn and Rylander *et al.*, 1998b). 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 airway 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)/m^3 (~5 ng/m^3) (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).

$\beta(1\rightarrow3)$-glucans

$\beta(1\rightarrow3)$-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 2. Work-related health effects of bacterial endotoxin and various occupational environments in which those effects have been demonstrated

Occupational environment	Health effects
Cotton industry	Acute and chronic respiratory symptoms including asthma
Farming (livestock, particularly pigs and poultry; grain)	
Animal feed and grain industry	Acute and chronic decline in lung function (FEV1, PEF, FVC, MMEF)
Potato processing industry	
Fibreglass industry	Non-allergic (neutrophilic) airway inflammation
Slaughter houses	Increased bronchial hyperresponsiveness
Waste and compost industry	Organic dust toxic syndrome (acute and short-lived flu-like symptoms with fever and airway symptoms)
Vegetable fibre production	
Automobile industry	Chronic bronchitis (?)

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 garbage 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.

Mycotoxins

Mycotoxins 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 mycotoxins 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 mycotoxins and respiratory health effects. Mycotoxins of *Fusarium*, *Aspergillus* and *Penicillium* genera are known to be present in the inhalable fraction of airborne corn dust (Sorenson, 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-Fahrländer *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 microorganisms 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 $\beta(1\rightarrow3)$-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 taxanomically 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 10^6–10^{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.

Table 3. Assessment methods for constituents of microorganisms in bioaerosol samples

Microorganisms	Aetiological agent	Marker	Analytical method[a]
Gram-negative bacteria	Endotoxin (LPS)		LAL
		3-Hydroxy fatty acids	GC-MS
Gram-positive and Gram-negative bacteria	Peptidoglycans	Muramic acid	GC-MS
Fungi	$\beta(1\rightarrow3)$-Glucans		LAL, ELISA
		Ergosterol	GC-MS
		EPS	ELISA
		mVOC	GC-MS
Fungi/bacteria	Allergens		ELISA
	Mycotoxins		TLC, HPLC, GC-MS, RIA, ELISA
		DNA	PCR

[a]LAL, *Limulus* amoebocyte lysate; GC-MS, gas chromatography–mass spectrometry; ELISA, enzyme-linked immunosorbant assay; TLC, thin layer chromatography; RIA, radioimmunoassay; PCR, polymerase chain reaction.

ASSESSMENT METHODS FOR BIO-ALLERGENS

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).

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 (Douwes 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

(Hollander *et al.*, 1993; Douwes *et al.*, 1995; Thorne *et al.*, 1997, 2003; Duchaine *et al.*, 2001) it was decided to adopt the European Standardization Organisation (CEN) draft protocol for measurement of endotoxin (CEN, 2001). However, the CEN protocol does not describe extraction and measurement procedures very specifically, thus potentially resulting in significant variations in exposure assessment between laboratories. Several international round robin tests have been conducted showing good correlations between laboratories but significant differences in absolute levels (Chun *et al.*, 2000; Reynolds *et al.*, 2002). Therefore for standard setting purposes further validation and standardization of sampling, extraction and analytical procedures are urgently needed.

The possibility of establishing exposure limits for allergen concentrations in the air has only been explored in some isolated cases. Subtilisins are bacterial enzymes usually produced from *Bacillus subtilis* and used in detergents. They are well-recognized respiratory sensitizers, and a TLV of 60 ng/m^3 (ceiling concentration) for workplace airborne exposure has been adopted by the ACGIH (2002). However, there is considerable doubt about the underpinning of this TLV, and the proposed value rationale for the TLV seems determined mainly by analytical limitations, i.e. by the detection limits of some of the earlier methods for exposure measurements. ACGIH (2002) now lists subtilisins as a substance under study. The Health and Safety Executive (HSE) in the UK is proposing to withdraw the British limit (OES, 60 ng/m^3, TWA), which is based on the ACGIH TLV, because no safe exposure limit for subtilisins could be identified. The ACGIH has also adopted an exposure standard of 0.5 mg/m^3 inhalable flour dust (8-TWA) based on the published exposure–response relationship for wheat allergens (ACGIH, 1990). For several other allergens, exposure–response relationships have now been established (Heederik *et al.*, 1999). This has been made possible due to use of newly developed immunoassays to measure the allergens directly instead of crude exposure proxies such as dust levels. It is to be expected that these relationships will be used for the development of exposure standards. However, before this is possible, standardization of immunoassays for measurement of allergens is urgently required.

RESEARCH NEEDS

As described above, bioaerosol exposure is associated 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,

there is a clear need for method development, particularly 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, allergens) 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. Moreover, a clear need is developing for rapid and direct reading assays for bioaerosols for immediate evaluation 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 bioaerosols and individual components thereof.

Other important areas that require further research include: (i) the potential protective effect of endotoxin 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, neurological 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, selection of the most appropriate method(s) is highly dependent on the specific goals of the study. Most culture methods provide important qualitative information 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

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 assessment 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 assessment tools and to validate newly developed methods.

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