# 16. Biological hazards

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## 16.1 INTRODUCTION

Biological hazards in the workplace have been a topic of study, discussion and publications for many centuries. Notable early works include Bernado Ramzinni's 18th-century treatise on occupational diseases, *De Morbis Artificum Diatriba*; John Tyndall's (1888) *Essays on the Floating-Matter of the Air: In Relation to Putrefaction and Infection*; and *Dangerous Trades* (Oliver 1902). In our own time, outbreaks of SARS and avian flu, anthrax mail attacks, post-Hurricane Katrina mould investigations and other events involving biological hazards have led to an increased awareness of such hazards among occupational hygienists (Esswein et al. 2004; Halpin 2005; Schwab et al. 2007; Thrasher & Crawley 2009). However, there are still many areas in which our knowledge of biological hazards is limited, and there is still research to be done. In particular, complications in the relating of exposure levels to recognisable health effects, and limited knowledge on exposure–response pathways, inhibit the development of exposure standards for many biological hazards. Assessment of biological hazards is a challenging area of occupational hygiene, and this chapter presents an introductory overview of the subject. For more in-depth information on the topics discussed here, the reader is referred to the references in Section 16.9.

## 16.2 BIOLOGICAL HAZARDS

Biological hazards include bacteria (peptidoglycan, endotoxin and exotoxins), fungi (yeasts, moulds and mycotoxins), archaea, protozoans, viruses, prions, plant particles, organically derived dusts such as wood dust, insect parts and faeces, and allergens such as animal dander, dust mite or pollen. All are capable of producing adverse health effects in humans. Biological hazards may also be discussed in relation to bioaerosols, 'airborne particles that are living or originate from living organisms' (Macher 1998, p. 1–1) or aerobiology, the 'study of passively airborne microorganisms—of their identity, behaviours, movements and survival' (Gregory 1973, p. xiii). The following sections provide a brief introduction to bacteria, fungi, allergens, viruses, protozoans and prions.

### 16.2.1 *BACTERIA*

Bacteria can be defined as single-celled organisms with no nucleus, or membrane-bound organelles. They belong to the kingdom Monera, and have an average size of 500 nm. Bacteria may have a variety of external structures to help them interact with, and defend against, their environment and other cells. These include flagella (motility), glycocalyces (protective outer coating), pilli (reproduction) and fimbrae (attachment and protective biofilm/slime layer). Bacteria reproduce asexually through division (binary fission). However, some bacteria, such as *Clostridium tetani*, which causes tetanus, can also produce

hardy endospores when environmental conditions become inhospitable (Tortora, Funke & Case 2011).

A common means of differentiating bacteria is based on their response to the Gram stain. This test can identify bacteria as Gram positive or Gram negative, based on their respective outer cell-wall constituents lipopolysaccharide and peptidoglycan (Bauman 2012). Gram-positive bacteria typically stain purple (as shown in Figure 16.1a), and Gram-negative bacteria stain pink (Figure 16.1b). However, some strains of bacteria, such as *Neisseria* species and environmental isolates, will produce a Gram-variable stain that is both pink and purple.

Occupational hygienists may encounter the terms Gram positive and Gram negative when reviewing documents relating to organic dust exposures, particularly in the discussion of respiratory diseases relating to endotoxin or peptidoglycan exposures. The outer cell wall of Gram-negative bacteria is composed of lipopolysaccharide, often referred to as endotoxin. Endotoxin is a well-known proinflammatory compound that has been widely studied over the last 30 years in agricultural, waste management, textile and food industries (Doyen et al. 2012; Poole & Romberger 2012). Gram-positive bacteria have an outer cell wall composed primarily of peptidoglycan (approximately 85 per cent), which is considered an important emerging agent of inflammatory disease in agricultural environments (Poole et al. 2010; Poole & Romberger 2012). Gram-negative bacteria also contain peptidoglycan in their cell wall, but it accounts for only around 5 per cent (Poole & Romberger 2012). Various bacterial species are also capable of producing exotoxins that can cause illness and/or death in exposed persons. Exotoxins can be categorised as cytotoxins, which cause death of all cell types; neurotoxins, which interfere with nerve-cell function (botulinum and tetanus); and enterotoxins, which alter the functioning of cells in the gastrointestinal tract (cholera, diphtheria and staphylococcal food poisoning) (Popoff & Poulain 2010; Bauman 2012). Cyanobacteria (also referred to as blue-green algae) are a group of Gram-negative photosynthetic bacteria that produce toxic metabolites called cyanotoxins. During a bloom of cyanobacteria, toxins in the water can build up to levels that threaten the health of humans and livestock. Not all strains of cyanobacteria produce toxins—in some cases, a bloom will be non-toxic or of low toxicity, depending on the strains present. The most common cyanotoxin is a hepatotoxin (affecting the liver), but a number of other potent toxins, such as neurotoxin and cytotoxin, are also produced (Syrcek & Smith 2004).

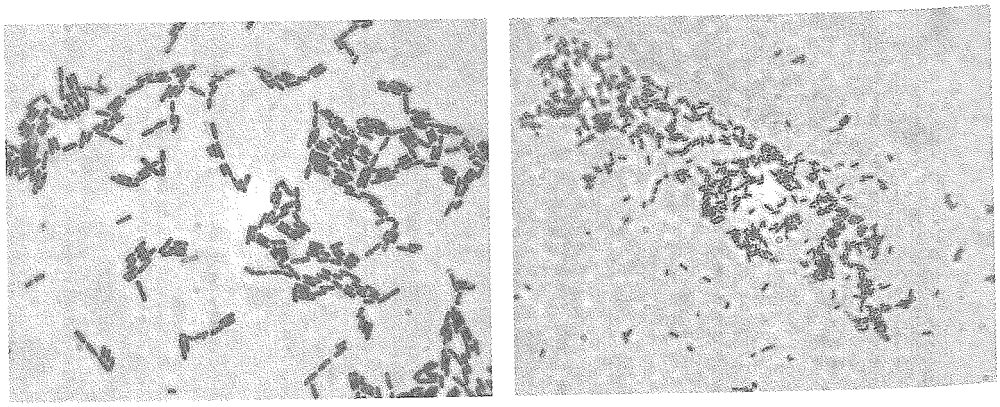


**Figure 16.1** Bacterial Gram stains
(a) Gram-positive sample; (b) Gram-negative sample

### 16.2.2 FUNGI

Fungi can be described as single and multicellular organisms with cell walls that typically contain chitin. They are non-motile and non-photosynthetic, and reproduce both sexually and asexually. They can be classified as yeasts, which are unicellular and reproduce by budding or spores; and moulds, which may be either unicellular or multicellular and reproduce by spores or gamete and spore formation (Tortora, Funke & Case 2011). Fungal spores typically range between 2–50 μm in size—bigger than bacteria, but usually smaller than pollens (Madelin & Madelin 1995).

Direct fungal growths on animal hosts are called mycoses, while diseases associated with ingestion, dermal contact and inhalation of toxic fungal metabolites are called mycotoxicoses (Bennett & Klich 2003). Moulds of occupational health significance include *Aspergillus, Penicillium, Cladosporium, Fusarium,* and *Alternaria* species, as well as the more notorious *Stachybotrys chartarum* (black/toxic mould) (Semple 2010; Dutkiewicz et al. 2011). These moulds are potential sources of mycotoxins, microbial volatile organic compounds (MVOCs), allergenic mycelia (hyphae) and cell wall constituents such as β-(1,3)-D-glucans. More recently there has been an interest in *Botrytis cineria,* which is reported to cause adverse health conditions at low concentrations, including the hypersensitivity pneumonitis variant 'Wine grower's lung' (Dutkiewicz et al. 2011).

Mycotoxins can be described as low-weight, naturally occurring secondary metabolites produced by filamentous fungi, which are toxic to animals and humans (Bennett & Klich 2003). Mycotoxins have also been associated with an increased risk of respiratory disease in agriculture (Dutkiewicz et al. 2011). Notable mycotoxins include carcinogenic aflatoxins, produced by various *Aspergillus* species; citrinin, from a variety of *Penicillium* and several *Aspergillus* species; ergot alkaloids, which have been associated with human disease since antiquity; fumonisins from *Fusarium* species; ochratoxin from *Aspergillus* species; patulin from *Penicillium* species; zearalenone from *Fusarium graminearum*; and trichothecenes from multiple fungi genera, which include the *Stachybotryis* toxin satratoxin.

In the process of growth, fungi produce a number of metabolites, mainly carbon dioxide and water. Many fungi, as well as bacteria, also produce microbial volatile organic compounds (MVOCs). Fungi produce MVOCs through the degradation of the substrate on which they are growing. MVOCs may be strong smelling, and humans may detect the smell at very low concentrations. However, whether or not they are detrimental to human health is a subject of debate. The presence of volatile organic compounds (VOCs) in buildings also does not necessarily correlate with microbial growth; there may be many other sources of VOCs, such as cleaning products or new furniture.

β–1,3-D-glucans is a glucose polymer, including cellulose, which is a cell wall component of fungi, plants and some bacteria (Rylander 2010; Dutkiewicz et al. 2011). Like endotoxin, β-(1,3)-D-glucans is a biological agent of interest in research into occupational respiratory diseases. Other beta-glucans, including β-(1,6)-D-glucans, may also be present in occupational environments; however, less is known about their immunogenic properties (Noss et al. 2012). While animal and human laboratory studies have demonstrated the inflammatory properties of beta-glucan (Goto, Yuasa & Rylander 1994; Wan et al. 1999; Ormstad et al. 2000; Sigsgaard et al 2000; Straszek et al. 2007; Rylander 2010; Bellanger et al. 2011), occupational assessments have had conflicting results. Some studies found that

beta-glucan had an effect on respiratory function and inmmune response (Rylander et al. 1999; Beijer et al. 2003; Heldal et al. 2003), while others found either weak or no response between exposure and symptoms, lung function or inflammatory markers (Rylander 1997; Eduard et al. 2001; Douwes 2005; Wouters et al. 2006; Stuurman et al. 2008; Sykes et al. 2011). β-(1,3)-D-glucans exposure has also been identified as having a protective effect on lung-function variation (Riedler et al. 2001).

### 16.2.3 ALLERGENS

Allergens encompass a wide range of biological contaminants that cause asthma and other allergic responses in workers. These include substances of low and high molecular weight, such as animal dander and hair, pollens, fungal spores and hyphae, bacteria, protozoa, bird droppings, feathers, and insects such as house dust mites and cockroaches (Macher 1998). Important occupational allergens include flour dust in bakeries and mills, enzymes used in washing powder and food production, latex from rubber gloves, animal proteins in agriculture and laboratory animal handling facilities, and wood dust (Illi et al. 2012).

### 16.2.4 VIRUSES

Viruses consist only of protein and nucleic acid, which may be either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). They vary in size from 20–450 nm and can only replicate inside other living cells, making them obligate intracellular parasites (Cowan 2012). There has been a steady increase in the study of airborne viruses in occupational environments, which has evolved alongside molecular detection methods such as real-time polymerase chain reaction, and in response to recent viral pandemics (Cao et al. 2011; Christensen et al. 2011; Lv et al. 2011; Ziros et al. 2011). However, sampling for airborne viruses is not routinely conducted in occupational environments. At present, it would be considered only in response to an outbreak—that is, to provide clearance for an area.

### 16.2.5 OTHER BIOLOGICAL HAZARDS

Other biological hazards that could be present in occupational environments include organisms from the kingdom Protista (algae, protozoa and amoebae), which includes the protozoans that cause giardia and cryptosporidiosis. Prions are another biological hazard. Exposure to prions can cause neurodegenerative transmissible spongiform encephalopathy diseases such as classical Creutzfeldt-Jakob Disease (CJD) and variant CJD (vCJD) in humans, and bovine spongiform encephalopathy (BSE) in animals. These diseases are always fatal. Prions are infectious proteins that replicate by acting as a template that causes normal cellular proteins to reshape into prion proteins. Classical CJD has been recorded in Australian since 1970; however, there have been no reported cases of vCJD. Australia is considered a negligible-risk country for BSE (NSW Health 2007; DHA 2012).

## 16.3 OCCUPATIONAL DISEASE

Occupational diseases can be grouped into infectious diseases, respiratory diseases and cancer. Infectious diseases are those that may be transferred from one human or animal

to another, including zoonotic diseases, which are passed between humans and animals. Respiratory diseases are complex responses by the body to various triggers, and may be divided into allergic and non-allergic diseases. In the longer term, the body may respond to an occupational stimulus by developing cancer, and a number of occupationally linked cancers are now accepted by the scientific community. All these groups of occupational diseases will be discussed in greater detail later in this section.

A recent study found that 19 per cent of workers surveyed were exposed to biological hazards in the workplace (de Crespigny 2011). Workers in the following fields are at significant risk of acquiring an occupational disease through exposure to biological hazards:

- health care
- social assistance
- veterinary medicine
- waste management
- biomedical research
- agriculture forestry and fishing.

Other occupations at increased risk for exposure to biological hazards include microbial enzyme production for the food and detergent industries, and bioterrorism research (Shapiro & Schwartz 2002; Rusnak et al. 2004; Rusnak et al. 2005). Consideration also needs to be given to military and charitable aid workers deployed in foreign countries, who may be exposed to various exotic infectious diseases (Smoak, Magill & Sharp 2006), and to the targeting of government agencies in bioterrorism attacks (Fennelly et al. 2004). However, it must also be noted that, owing to the ubiquitous nature of biological aerosols, any worker can be at risk of acquiring an occupational disease if workplace health and safety are ignored.

### 16.3.2 *INFECTIOUS DISEASE*

Infectious diseases, otherwise referred to as communicable diseases, are transmitted through various routes, including direct human or animal contact, accidental auto-inoculation (needlestick), ingestion of contaminated food or water, inhalation of contaminated aerosols and/or contact with contaminated objects (fomites). Bites from disease vectors such as mosquitoes or ticks represent a significant health risk to outdoor workers because Australia has many endemic mosquito- and tick-borne viral diseases, including Ross River virus, Murray Valley encephalitis and Barmah Forest virus, which can cause severe illness or even death. Sporadic cases of dengue and Japanese encephalitis also occur in Northern Australia (Russell & Doggett 2012). Australia uses a mandatory reporting system of notifiable infectious diseases. Further information and a full list of notifiable diseases are available from the National Notifiable Disease Surveillance System at <www.health.gov.au> (DHA 2012). Since 2000–01 in Australia, occupational workers' compensation data has reported workplace cases of Brucellosis, human immunodeficiency virus (HIV), leptospirosis, mycosis, Q fever and viral hepatitis (SWA 2012). A list of infectious diseases that have been reported in Australia is presented in Table 16.1.

Microorganisms of particular importance to the industrial hygienist include types of bacteria and viruses that have the potential to cause illness in otherwise healthy humans.

**Table 16.1** Infectious diseases in Australian occupational environments

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| **Viral** | | | | |
| *Arboviral infections* | | | | |
| Barmah Forest virus | Alphavirus | Mosquitoes and humans | Mosquitoes | Outdoor workers on South Coast of NSW |
| Dengue | Flavivirus (DEN1, DEN2, DEN3 or DEN4) | Human–*Aedes aegypti* cycle | | Outdoor workers in northern Queensland |
| Japanese encephalitis | Flavivirus | Mosquito (*Culex annulirostiris*), wild and domestic birds and pigs | | Outdoor workers in northern Australia |
| Kunjin virus infection | Flavivirus | Mosquito (*Culex annulirostris*) and water birds | | Outdoor workers in northern Australia |
| Murray Valley encephalitis | Flavivirus | Mosquitoes and water birds | | Outdoor workers in northern Australia |
| Ross River fever | Alphavirus | Mosquitoes and humans | | |
| Australian bat lyssavirus infection | Lyssavirus | Insectivorous bats, flying foxes | | Bat carers, wildlife workers and veterinarians |

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| *Blood-borne viruses* | | | | |
| Hepatitis A | Picornaviridae family RNA virus | Humans | Faecal/oral transmission (Hep A), contact with infected blood and other bodily fluids, sexual intercourse and mother-to-child (i.e. vertical transmission) | Health-care workers, sex industry and waste-management workers |
| Hepatitis B | Hepadnavirus | | | |
| Hepatitis C | Flavivirus | | | |
| Human immunodeficiency virus infection | HIV 1, HIV 2 | | | |
| *Hendra virus infection* | | | | |
| Hendra virus disease | Henipavirus | Horses, flying foxes, dogs and cats | Contact with infected fruit bats or horses in eastern Australia | Horse industry workers, veterinarians and government inspectors |
| *Influenzas and para-influenzas* | | | | |
| Seasonal influenza | Influenza virus | Humans | Contact with contaminated people and objects, or inhalation of aerosolised viruses | Health-care workers, educators |
| Swine flu | H1N1v virus | Swine and humans | Contact with infected animals, less often contact with infected humans | *Swine handlers, government inspectors, veterinarians, abattoir workers* |

**Table 16.1** Infectious diseases in Australian occupational environments *continued*

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| Menangle virus infection | Rubulavirus | Swine, humans and fruit bats | Contact with stillborn piglets and bats in NSW | Swine handlers, veterinarians and government inspectors |
| Newcastle disease | Avulavirus | Birds | Contact with infected birds | Poultry workers, lab staff, veterinarians |
| **Bacterial** | | | | |
| Anthrax | *Bacillus anthracis* | Domestic and wild animals | Inhalation, ingestions or contact of broken skin with diseased animals, hides, hair or offal | Veterinarians, wool sorters, abattoir workers, farmers and stock handlers. |
| Brucellosis | *Brucella suis* | Feral pigs in Queensland | Contact with infected tissues and bodily fluids, inhalation of contaminated aerosols | Feral animal hunters, agricultural workers and veterinarians. *Brucella abortis* was eradicated from Australia in 1989 |
| Campylobacteriosis | *Campylobacter jejuni*, *C. upsaliensis* | Wild birds and domestic animals and birds | Contact with infected animals and objects, contaminated water or faecal/oral transmission | Veterinarians, agricultural workers, child-care, waste-management and health-care workers |

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| Erysipeloid | *Erysipelothrix rhusiopathiae* | Fish, shellfish, mammals and poultry | Contact with contaminated meat | Fishermen, abattoir workers, farmers and butchers |
| Haemophilus influenza | *Haemophilus influenzae* | Humans | Inhalation of respiratory droplets from infected person. Rarely, contact with infected mucal discharges | Health-care and child-care workers |
| Legionellosis/ Legionnaires' disease | *Legionella pneumophila*, *L. longbeachae*, *L. micdadei*, *L. bozemanni* | Soil, water, mulch, wood chips and hot springs | Inhalation of contaminated water and dust aerosols | Office and health-care workers, horticulturalists, agricultural workers |
| Leptospirosis (Weil's disease) | *Leptospira interrogans* | Wild and domestic animals including rats, cows and pigs | Contact with infected animal urine/ flesh, ingestion of contaminated water or soil | Farmers, veterinarians, waste management and abattoir workers, sugar cane and banana farmers and miners |
| Listeriosis | *Listeria monocytogenes* | Wild and domestic animals, sewage, silage and birds | Ingestion of contaminated food and other materials | Agricultural workers, veterinarians and lab workers |
| Methicillin-resistant *Staphylococcus aureus* (MRSA) | *Staphylococcus aureus* | Swine, dogs, cats, horses, humans and wild animals | Contact with infected animals or people | Farmers, animal handlers, veterinarians, health-care and abattoir workers |

**Table 16.1** Infectious diseases in Australian occupational environments *continued*

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| *Mycobacterial infections* | | | | |
| Tuberculosis | *Mycobacterium tuberculosis* | Humans | Inhalation of infectious droplets | Health-care and child-care workers, prison wardens |
| Non-tuberculosis | *M. avium-intracellulare*<br>*M. kansasii*<br>*M. scrofulaceum*<br>*M. fortuitum*<br>*M. marinum*<br>*M. chelonae* | Groundwater, dust and soil | Rarely determined, possibly inhalation or ingestion of soil, dust or water, *M. marinum* skin inoculation | Recreational industry, agriculture, forestry and fishing workers |
| Pasteurellosis | *Pasteurella multocida* | Livestock and domestic pets | Animal scratches | Agricultural workers and veterinarians |
| Pertussis (whooping cough) | *Bordetella pertussis* | Humans | Inhalation of contaminated aerosols or contact with infected mouth or nose secretions | Health-care and child-care workers |
| Psittacosis | *Chlamydia psittaci* | Birds, rarely cats, dogs, goats or sheep | Inhalation of dust infected with bird faeces, or contact with eye or nasal secretions of infected animals. Rarely human to human | Veterinarians, pet shop workers, breeders |

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| Q fever | *Coxiella burnetii* | Sheep, goats | Inhalation of contaminated aerosols | Abattoir workers, researchers, veterinarians, farmers |
| Salmonellosis | *Salmonella* species | Domestic and wild animals, birds and reptiles | Faecal/oral transmission, ingestions of contaminated foods | Child-care, health-care, hospitality and agricultural workers |
| Shigellosis | *Shigella sonnei* | Humans | Faecal/oral transmission, ingestion of contaminated food, water or milk | Child-care and health-care workers, laboratory workers |
| Escherichia coli infection | *Escherichia coli* (enterohaemorrhagic, enteropathogenic, enterotoxigenic or enteroinvasive) | Soil, dusts, silage, domestic and wild animals, reptiles, birds and fish | Faecal/oral transmission, ingestion of contaminated food and water. Contact with infected animals | Healthcare, child-care, laboratory and agricultural workers |
| Spotted fevers (Queensland tick typhus and Flinders Island spotted fever) | *Rickettsia australis*, *R. honei* | Ticks and marsupials (suspected) | Tick bites | Agriculture, forestry and fisheries workers, veterinarians and horticulturalists |
| **Fungal** | | | | |
| Cryptococcal infections | *Cryptococcus neoformans var. neoformans**, *var. gattii*** | Bird droppings* *Eucalyptus* sp.** | Inhalation of fungal spores | *Forestry workers, horticulturalists, veterinarians and* agricultural workers |

**Table 16.1** Infectious diseases in Australian occupational environments *continued*

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| Dermatophytosis (tinea) | *Trichophyton tonsurans**, *T. verrucosum*** | Humans*, cattle** | Skin to skin, contact with contaminated objects or surfaces | Veterinarians, abattoir and agricultural workers |
| Histoplasmosis | *Histoplasma capsulatum* | Soil and bats | Inhalation of spores | Bat handlers and people who enter bat caves |
| **Protozoan** | | | | |
| Cryptosporidiosis | *Cryptosporidium parvum* | Humans, cattle and domestic animals | Faecal/oral transmission, ingestion contaminated food or water | Health-care, child-care and recreational industry workers, agricultural workers and veterinarians |
| Giardiasis | *Giardia lamblia* | Humans, animals and contaminated waters | Faecal/oral transmission, contacted with contaminated items or ingestion of contaminated water | Health-care and child-care workers, recreation industry, waste management, agricultural and forestry workers, veterinarians |
| Toxoplasmosis | *Toxoplasma gondii* | Felines; intermediate hosts include domestic and wild animals and birds | Ingestion of contaminated food, unpasteurised milk, contaminated soil; blood transfusion, organ transplant and maternal transmission | Laboratory, agricultural and abattoir workers, veterinarians, hunters and agricultural workers |

| Disease | Agent | Reservoir | Transmission | High-risk occupations |
|---|---|---|---|---|
| **Parasitic** | | | | |
| Echinococcosis (hydatid disease) | *Echinococcus granulosus* | Canine/domestic and wild animal cycle. Sheep are main intermediate host | Hand to mouth transfer of eggs from dog faeces | Farmers (particularly sheep) and hunters |

(*Sources:* NOHSC 1990; McCormack & Allworth 2002; Harries & Lear 2004; Heymann 2008; Russell et al. 2009; Heinsohn 2011; Jordan et al. 2011; McLeod et al. 2011; Morrell & Stratman 2011; Smith et al. 2011; Biosecurity Australia 2012; Victorian Department of Health 2012; Russell & Doggett 2012)

Workers in hospitals and in pathology and research laboratories may be at risk of certain serious infectious diseases such as tuberculosis, as well as blood-borne viral diseases such as the hepatitis viruses. Legionnaires' disease is a pneumonia caused by the *Legionella* species. Groups of workers at risk include those who are exposed to aerosols from cooling towers or potting mix, which potentially contain *Legionella* bacteria. Smokers and the immunocompromised are at higher risk of contracting this illness. Q fever is a zoonotic, flu-like illness caused by the organism *Coxiella burnetii*. Workers most at risk include those exposed to animal placental material or urine of infected animals, such as veterinarians, abattoir workers and others who handle livestock. Q fever is the most reported of six zoonoses that appear on the National Notifiable Disease Surveillance System. An effective vaccine is currently available, but must be given before exposure to be effective.

For further information, consult the *Control of Communicable Diseases Manual*, published by the American Public Health Association (Heymann 2008), or the following online reference sources:

- <http://ideas.health.vic.gov.au/bluebook.asp>
- <www.animalhealthaustralia.com.au>
- <http://medent.usyd.edu.au/arbovirus/index.html>
- <www.uq.edu.au/aid>
- <www.nc.cdc.gov/eid>
- <www.who.int/topics/infectious_diseases/en>
- <www.health.gov.au/internet/main/publishing.nsf/content/cda-cdna>.

## 16.3.3 RESPIRATORY DISEASE

Respiratory diseases associated with exposure to biological hazards can be divided into allergic and non-allergic diseases (Table 16.2). An allergic response can be defined as a respiratory disease that is triggered by activation of the adaptive (active) immune system by a foreign substance, while a non-allergic respiratory disease is caused by mechanical blockage, irritation or innate (passive) immune-system response.

### 16.3.3.1 Allergic responses

Materials of biological origin can cause allergic reactions in workers who come into contact with them. An allergic response occurs when the human body identifies a foreign material (antigen) and an immune response is stimulated. The body reacts to the presence of antigens by forming antibodies, which bind to the antigens and normally render them harmless. Sometimes the body overreacts to an antigen and this process results in inflammation and tissue damage—that is, an allergic reaction/hypersensivity disease (Tillman 2007). There are four types of allergic/hypersensitivity reaction:

- **Type 1:** Immediate hypersensitivity, characterised by a rapid reaction (15–30 minutes) mediated by immunoglobulin E (IgE). Diseases include asthma, eczema, conjunctivitis, allergic rhinitis and gastroenteritis (Bogaert et al. 2009).
- **Type 2:** Cytotoxic hypersensitivity occurs when antigens bind to cells and may affect a variety of organs and tissues. Type 2 is primarily mediated by IgM and IgG. Diseases include anaphylactic shock in response to pencillin, and rheumatic fever.

**Table 16.2** Respiratory diseases from exposure to non-infectious biological hazards

| Respiratory disease | Agent | High-risk occupations |
|---|---|---|
| *Non-allergic* | | |
| Non-allergic asthma, non-allergic rhinitis/ mucous membrane irritations (MMI), chronic bronchitis, chronic airflow obstruction, organic toxic dust syndrome (ODTS) | Fungi, bacteria actinomycetes, endotoxin, ß-(1,3)-D glucans, peptidoglycan, mycotoxin | Agriculture, forestry and fishing workers, waste treatment workers, composting and recycling workers, textile and food production workers, horticulturalists, metal workers and machinists, veterinarians, zookeepers, laboratory workers, construction workers, archaeologists and biofuel production workers |
| *Allergic* | | |
| Allergic asthma, allergic rhinitis, hypersensitivity pneumonitis (allergic alveolitis/farmer's lung) | Fungi, thermophilic mycelial bacteria, *Mycobacterium immunogenum* MVOCs, spores, allergens (dust mite, plant, insect and animal proteins, pollens etc), endotoxin, ß-(1,3)-glucans | Waste treatment, composting and recycling workers, biomedical researchers, enzyme production workers and lab animal tenders, healthcare workers, bakers, industrial (detergent and biopesticide manufacturing) workers, pet shop owners, agricultural, forestry and fishing workers, wood processors/furniture makers and horticulturalists |

(*Source:* adapted from Liebers, Raulf-Heimsoth & Brüning 2008)

- **Type 3:** Immune-complex hypersensitivity may occur three to 10 hours after exposure and involves exposure to soluble antigens that are not attached to the target organ, and mediated IgG, IgM and complement. Examples of immune-complex diseases include hypersensitivity pneumonitis (Bogaert et al. 2009).
- **Type 4:** Delayed hypersensitivity is associated with many infectious diseases including leprosy, toxoplasmosis and tuberculosis, as well as contact dermatitis expressed as papular lesions. Unlike Types 1 to 3, Type 4 is cell rather than antibody mediated. The response time is 48–72 hours (Bogaert et al. 2009).

The lungs are particularly susceptible to allergens because they are exposed to large quantities of airborne substances. They respond in a variety of ways depending on various extrinsic or intrinsic factors, including the antigen itself, genetics, whether the person smokes, exposure time and intensity (Macher 1998). The body's response to inhaled

allergens can range from mild rhinitis (hayfever) to the more severe allergic asthma and allergic alveolitis discussed below.

Allergic rhinitis, also called 'hayfever', is generally associated with a blocked or runny nose, sneezing and sometimes also itchy, sore eyes and throat (Bousquet et al. 2008). It is typically ascribed to pollen or fungal exposure (Douwes, Edvard & Thorne 2008). However, it has also been reported after inhalation of other bioaerosols, including insects and mites, grain dust, latex, α-amylase in flour, biological enzymes, fish and seafood proteins and wood dusts (Greiner et al. 2011). Cases have been reported in corn farming (Sung et al. 2012) and in the flower industry (Wiszniewska et al. 2011).

Extrinsic/allergic asthma is an airway-obstructive condition that may be referred to as atopic asthma or, in a workplace, occupational asthma. Symptoms of allergic asthma may occur outside of work hours, often at night, making it harder to link cause and effect and leading to a delay in recognising the workplace as the source of the allergen (Tillman 2007). A worker who has a history of asthma or eczema may be more likely than other workers to develop asthma in certain conditions. High-risk industries include:

- agriculture and forestry
- waste management
- wood production
- baking
- biotechnology
- production of biological enzymes used in detergents and food production (Douwes, Edvard & Thorne 2008)

An employer who is hiring for a position that requires high exposure to allergens (e.g. wood working, animal handling) may consider undertaking pre-employment screening to exclude workers with a history of hypersensitivity reactions to common environmental antigens. Diagnosis of occupational asthma is performed by a general practitioner or occupational physician (ASCC 2006).

Allergic alveolitis, otherwise known as hypersensitivity pneumonitis (HP), is a complex lung disease caused by an immune response to inhaled organic bioaerosols less than 5 μm in diameter which can reach the alveoli of the lungs (Selman, Pardo & King 2012). The disease presents as flu-like symptoms, shortness of breath or bronchitis-like illness, and is usually caused by microbial contamination of plant matter that is stored in a damp environment. Acute HP can present symptoms very similar to those of the non-immunogenic organic toxic dust syndrome (ODTS), and must be clinically distinguished from this more common disease, which occurs approximately 50 times more often than HP (Selman et al. 2012). It has been suggested that HP is acquired by a twofold mechanism, with antigen exposure as the inducing factor, and genetic susceptibility and/or environmental conditions as the promoting risk factors (Selman, Pardo & King 2012). The occurrence of HP in various industries has resulted in occupationally specific names, including farmer's lung, bagassosis, mushroom lung, suberosis, aspergillosis, malt worker's disease, maple bark disease, sequoiosis, wood pulp worker's disease, cheese washer's disease, greenhouse lung, tobacco worker's disease, wine grower's lung, peat moss lung, pigeon breeder's disease, chicken breeder's disease and shell lung (Bønløkke, Cormier & Sigsgaard 2010). A comprehensive list of HP diseases and their aetiological agents is available in Selman, Pardo & King (2012).

Farmer's lung is the best known of the HP variants, and is associated with inhalation of spores from thermophilic actinomycetes (Pepys et al. 1963), *Aspergillus fumigatus* (Kozakiewicz 1995), *Saccharopolyspora rectivirgula* (Blais Lecours et al. 2012) and other fungi that can thrive in wet hay and grass. The spores are often found in large numbers in farmyard dusts, and are released when hay or compost is disturbed. Two cases of mushroom worker's lung were reported at a large commercial farm that produces *Agaricus bisporus* mushrooms (Hoy et al. 2007).

The HP disease wood worker's lung presents as chronic respiratory effects and is caused by exposure to unspecified woods. There are also specific allergic responses to certain woods such as western red cedar, which is a well-known cause of asthma and rhinitis. The Australian natives *Eucalyptus hemiphloia* (white box) and *E. maculata* (spotted gum) both cause skin irritation, and various other woods can cause sensitisation dermatitis. Separate exposure standards exist for hardwood and softwood dusts, both of which are sensitisers. Wood dust is also one of most commonly reported causes of occupational asthma in the Surveillance of Australian Workplace-Based Respiratory Events (SABRE) (Hannaford-Turner et al. 2010).

Byssinosis, an HP disease associated with the cotton industry, has been a recognised respiratory disease for over 100 years (Pickering 1994), and early Australian studies are available in the literature (Barnes & Simpson 1973, 1976; Gun et al. 1983). Byssinosis is now rare in Australia as a result of dust-minimisation procedures in cotton processing mills (Tillman 2007). The aetiological agent of byssinosis is unknown, but the inflammatory potential of cotton dust can also be reduced by steaming or washing cotton before processing (Selman, Pardo & King 2012).

Bird fancier's lung is one of the most common presentations of HP, and is caused by the inhalation of proteins present in bird droppings and feathers (Chan et al. 2012). It presents in acute, sub-acute and chronic forms. Symptoms include breathlessness, fever, anorexia, respiratory failure, pulmonary fibrosis and COPD in chronic cases (Morell et al. 2008; Chan et al. 2012).

Laboratory animal allergy (LAA) is an HP illness that usually presents as symptoms of 'hay fever'—rhinitis, sneezing, itchy eyes—and may progress to asthma in a certain percentage of workers. It has been estimated that approximately one-third of lab animal workers have an allergy to animal dander, and a further 11–44 per cent report work-related allergies (Bush & Stave 2003). Sensitisation typically occurs within the first three years of employment (Bush, Wood & Eggleston 1998). Better animal housing, including individually ventilated cages (IVCs) for smaller laboratory animals, may be improving conditions for modern workers and reducing the incidence of LAA in certain situations. Regular monitoring of staff at risk should be undertaken by an occupational physician to enable early recognition of symptoms (Bush, Wood & Eggleston 1998; Elliott et al. 2005).

#### 16.3.3.2 Non-allergic response

Certain bioaerosols can act as irritants, cause airway blockages or stimulate the innate immune system, even when they do not produce an allergic response in the worker. In some jobs where workers are exposed to bioaerosols, there may be a 'healthy worker effect' where those workers most prone to allergy (atopy) are more likely to leave the job. Of the remaining workers, some develop other, non-allergy-related health effects (Tillman 2007). One of the most common symptoms, mucous membrane irritation (MMI), may

appear consistently during the work shift. This usually takes the form of irritation and inflammation of the upper respiratory tract—conjunctivitis, sinusitis, rhinitis, pharyngitis, laryngitis and tracheitis. There may also be a chronic cough or bronchitis (Clapp et al. 1994; Rusca et al. 2008; Schlosser et al. 2009). Important non-allergenic diseases include non-allergic asthma, inhalation fever and organic toxic dust syndrome (OTDS).

Non-allergic asthma is frequently diagnosed in farmers or other workers with high bioaerosol exposure, at rates of 20–50 per cent. This type of asthma results in a marked reduction of lung function across the period of a work shift and is closely associated with grain and cotton dust, among other agents (Linaker & Smedley 2002). Although it is similar to allergic asthma in presentation, non-allergic asthma can result in a relatively quick decline in lung function, and is not accompanied by production of IgE antibodies, as is the case in allergic asthma (Linaker & Smedley 2002).

Inhalation fevers are flu-like illnesses caused by the inhalation of microbially contaminated aerosols from agricultural dust, air humidifiers or other aerosols containing *Legionella* bacteria. They appear after a short incubation period and are self-limiting. Some examples of inhalation fevers include:

- **Humidifier fever**, which can occur in buildings with humidification systems or air-conditioning. Symptoms appear within 4–12 hours of exposure, and the illness is self-limiting, with recovery in a matter of days.
- **Pontiac fever** results from exposure to *Legionella pneumophilis* and other *Legionella* species. This is a variant of legionellosis that is mild, self-limiting, and does not result in pneumonia (Tortora, Funke & Case 2011).

Organic toxic dust syndrome (ODTS), otherwise referred to as toxic alveolitis, can occur as a result of exposure to high levels of bioaerosols (Flannigan, Samson & Miller 2011). The symptoms of OTDS include fever, shivering, dry cough, chest tightness, dyspnoea, headache, muscular and joint pain, fatigue, nausea and general malaise. Symptoms typically disappear after 24 hours but may persist for up to seven days (Douwes, Edvard & Thorne 2008). Exposure to endotoxins and long periods of exposure are linked to an increased risk of ODTS. Fungal spores and fragments may also be a factor. Exposure to organic dusts from mouldy hay, silage and corn has been associated with the disease (Madelin & Madelin 1995). A European study of 3880 agricultural workers, veterinarians and biofuel workers observed an association between ODTS and elevated endotoxin exposures (Basinas et al. 2012), while clusters of OTDS cases have also been reported in grass-seed plants (Madsen et al. 2012) and wastewater processing facilities (Smit, Spaan & Heederik 2005).

### 16.3.4 CANCER

Exposure to bioaerosols has been implicated as a cause of cancer in a wide variety of work situations. The oncogenic viruses have clearly been shown to cause cancer in humans. Other biological sources of cancer are less well established, however. The aflatoxins, produced by the *Aspergillus* species, have been confirmed as human carcinogens. Other mycotoxins are also suspected of being carcinogenic (IARC 2002). Workers in industries such as farming and processing of peanuts or grains have been found to be at higher risk of

developing certain cancers. The International Agency for Research on Cancer has assigned an A1 rating (confirmed human carcinogen) for oak and beech wood dust, and an A2 (suspected human carcinogen) rating for dusts of birch, mahogany, teak and walnut. All other wood dusts are currently classified as A4 (ACGIH® 2012). Logging, sawmilling and carpentry are just some of the occupations that involve significant exposure to wood dust. Workers employed in meat processing, such as butchers, have a relatively high incidence of lung cancers and leukemia. It has been suggested that exposure to zoonotic oncogenic viruses may be responsible (Douwes et al. 2003; Douwes, Edvard & Thorne 2008).

## 16.4 REGULATIONS AND STANDARDS PERTAINING TO BIOLOGICAL HAZARDS

There are a number of areas in which biological hazards in Australia are either regulated or subject to Australian or International Standards.

### 16.4.1 SECURITY-SENSITIVE BIOLOGICAL AGENTS (SSBAS)

The Department of Health and Ageing administers the legislation relating to SSBAs. The *National Security Act 2007* and the associated *National Security Regulations 2008* describe a number of biological agents that are regulated owing to their potential for use as biological weapons. For details of the organisms and requirements of the regulations, see <www.health.gov.au/SSBA>.

### 16.4.2 GENETICALLY MANIPULATED ORGANISMS (GMOS)

The Office of the Gene Technology Regulator (OGTR) administers the legislation relating to genetically modified (GM) biological materials. The *Gene Technology Act 2000* and the *Gene Technology Regulations 2001* aim to 'protect the health and safety of people, and the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with genetically modified organisms (GMOs)' <www.comlaw.gov.au/Details/C2011C00539>. As well as regulating GM organisms, the OGTR also sets requirements for laboratory construction and procedures to be used when handling GM biological materials. For further information on OGTR requirements, see <www.ogtr.gov.au>.

### 16.4.3 DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY (DAFF) BIOSECURITY

DAFF Biosecurity regulates the importation of biological materials from overseas. It also authorises and inspects Quarantine Approved Premises (QAP) within Australia. QAP may be used to hold or work on particular imported biological materials, including plants, animals or other products. DAFF Biosecurity (previously AQIS) currently operates under the *Quarantine Act 1908*, but this is to be updated, with a new *Biosecurity Act* being developed. For more information, refer to www.daff.gov.au/aqis/import/general-info/qap or http://biosecurity.govspace.gov.au.

### 16.4.4 AUSTRALIAN STANDARD/NEW ZEALAND STANDARD 2243.3: SAFETY IN LABORATORIES

This Standard is relevant to the operation of biological laboratories, including those used for research, teaching and pathology among others—particularly labs that are not regulated by other means (e.g. by the OGTR). AS/NZ 2243.3 sets out facility construction and practices requirements for laboratory, animal, plant and invertebrate containment facilities. Facilities are divided into four levels of containment depending on the work to be conducted in them (Table 16.3). The majority of biological laboratories in Australia are classified as physical containment (PC) level 2. This Standard also classifies microorganisms into risk groups (RG) according to their pathogenicity (Table 16.4).

**Table 16.3** AS/NZ2243.3 Definition of physical containment levels for laboratories

| PC level | Work undertaken |
|---|---|
| PC1 | Low hazard, Risk Group (RG) 1 microorganisms, e.g. undergraduate teaching laboratory |
| PC2 | Research or diagnostic laboratory, RG2 microorganisms |
| PC3 | Research or diagnostic laboratory, RG3 microorganisms |
| PC4 | High-risk specialist work with RG4 microorganisms |

**Table 16.4** AS/NZ2243.3 Definition of microorganism risk groups

| RG level | Work undertaken |
|---|---|
| RG1 | Unlikely to cause human or animal disease |
| RG2 | May infect and individual worker, but treatment is likely to be available and spread to the wider community is unlikely |
| RG3 | May pose a significant risk to an individual worker, but further spread to the community is most likely able to be controlled |
| RG4 | May cause life-threatening human or animal disease and may spread in the community or environment. Treatment may not be available |

Risk groupings of organisms relate directly to the containment levels of the associated laboratories. In other words, RG1 organisms should be handled in a PC1 laboratory, and so on. AS 2243.3 gives other useful information for laboratories, such as standards for spill clean-up, transportation of biological materials, use of specialist equipment (centrifuges, biological safety cabinets, etc.), useful disinfectants for various organisms and waste disposal. Australian Standards are available from the SAI Global website, at <www.saiglobal.com>.

### 16.4.5 NOTIFIABLE DISEASE SURVEILLANCE

In Australia, there are a number of notifiable diseases for which reporting is mandatory. These diseases are listed in the *National Health Security Act (National Notifiable Disease List) Instrument 2008*, and include potential occupationally acquired diseases such as Q fever, leptospirosis and malaria. A full list of the conditions is available at <www.comlaw.gov.au/Details/F2008L00800>, and more information on legislation applying to communicable diseases is available through the Health Protection and Surveillance branch of the Department of Health and Ageing <www.health.gov.au/cda>.

## 16.5 OCCUPATIONAL EXPOSURE LIMITS (OELS)

There are a limited number of OELs for hazards of biological origin. These include:

- nuisance dusts, wood, cotton, cellulose and grain dusts (ACGIH 2012; Health and Safety Executive [HSE] 2011; SWA 2012)
- endotoxins (Dutch Expert Committee on Occupational Safety 2010)
- volatile compounds such as ammonia and hydrogen sulfide, which can be produced by biological organisms (SWA 2012)
- subtilisins, enzymes obtained from *Bacillus subtilis* (ACGIH® 2012).

Researchers have also put forward recommended limits for biological aerosols in specific environments such as total dust and ammonia in animal-handling areas (Donham et al. 1995), grain dust and flour dust (Nielsen et al. 2012). Macher (1998) provides a comprehensive discussion of the issues associated with the development of OEL for biological hazards. In brief, these are:

- Microorganisms in a sample may be viable—that is, living—but for a variety of reasons may not be grown in culture.
- Sampling times may be short because sampling media can easily be overloaded in heavily contaminated environments.
- Sampling may not be consistently repeatable: results can vary widely, even among repeat samples.
- A single sampling method may not allow all biological components to be collected and evaluated.

There are also difficulties in determining a safe level of exposure (no-adverse-effect level), although there has been some progress in relation to fungal spores. For the majority of biological agents, their role in the initiation and development of occupationally acquired illnesses is still poorly understood. Also, other factors aside from dose, such as gene–environment interactions, also play a role in the induction and severity of disease (Kleeberger & Peden 2005; Smoak, Magill & Sharp 2006).

## 16.6 CONTROL

Controls in the context of biological hazards follow the standard hierarchy of controls model, beginning with elimination as the best form of control. A risk assessment at the

start of any project should consider whether biological hazards are present and their likely effect on the workers. The risk assessment should then consider possible controls, beginning at the top of the hierarchy of controls and working down. Administrative controls and personal protective equipment (PPE) are considered weak controls, and should not be used alone. The AIHA has produced a comprehensive guide for control of exposure to biological hazards during a pandemic. This guide is a recommended resource for hygienists dealing with communicable disease outbreaks (Senthilselvan et al. 2009). Table 16.5 provides examples of the different types of controls that may be applied in a variety of industries.

**Table 16.5** Potential controls for biological hazards

| Hierarchy of control | Examples |
|---|---|
| Elimination | Undertaking in vitro experiments instead of using an animal model |
| Substitution | Substituting high-dust animal feed with a low-dust variety<br>Substituting an organism in a lower risk group for one in a higher risk group |
| Engineering | Use of biological safety cabinets when handling hazardous microorganisms<br>Vaccination of waste management workers for potential hazards<br>Use of fully enclosed and air-conditioned tractor cabs to protect the operator from outside dust and exhausts<br>Installation of mechanical ventilation in confined animal feeding operations and dairy parlours<br>Use of Australian Standards approved sharps containers to dispose of needles or other sharp items<br>Using single-use equipment to remove the risk of transferring an infectious disease from one patient or client to another, such as in medical centres or beauty salons<br>Maintenance of negative air pressure in mould-affected areas or other spaces where biological hazards may be handled or naturally present |
| Administrative | Use of a documented safe work procedure<br>Use of a documented biological waste segregation and disposal system, e.g. in a hospital, laboratory or home health-care setting<br>Scheduling work to limit exposure during high-dust activities<br>Implementation of an induction or training process |
| PPE | Safety glasses, goggles or face shield<br>Lab coat, gown or overalls<br>Gloves—of latex, vinyl or other materials<br>Half-face respirator with a P2 filter |

## 16.7 SAMPLING AND ANALYSIS OF BIOLOGICAL HAZARDS

It is essential that the hygienist consult with a microbiology laboratory, microbiologist or communicable disease expert before undertaking monitoring for biological hazards. Unlike sampling and analytical methods for the investigation of food- and water-borne infectious disease outbreaks, the sampling and analysis of biological hazards in occupational environments, especially bioaerosols, is confounded by the complexity of the aerosols and their environmental matrices. Knowledge of the aetiological pathways of bioaerosol hazards such as microbial cell-wall components is limited, and there limited data relating to no-adverse-effect level unavailable for many biological agents. These issues hinder the development of standard methods for sampling and analysis, as well as the development of regulatory and advisory standards for exposure. A comprehensive discussion of all the available sampling and analytical methods is beyond the scope of this chapter, and readers are encourage to consult key texts such as *Bioaerosols: Assessment and control* (Macher 1998), *Bioaerosols Handbook* (Cox & Wathes 1995), *Aerosol Sampling: Science, standards, instrumentation and application* (Vincent 2007) and the *Field Guide for Determination of Biological Contaminants in Environmental Samples* (Hung, Miller & Dillon 2005).

The main factor influencing the selection of methods for sampling and analysis of biological agents will be the purpose of the investigation. Reasons for monitoring of biological agents vary, and include:

- regulatory and compliance investigations; comparison against legislated standards
- complaint investigations; source identification, staff reassurance, exposure documentation
- epidemiological and research studies; identification of no-adverse-effect levels; research for exposure standards
- remediation assessments; determining the adequacy of remediation work
- assessment of engineering controls; determining whether controls are working effectively.

There is no one perfect method for the investigation of biological hazards, and researchers frequently use combinations of traditional and novel sampling and analytical methods from a multitude of scientific disciplines, including occupational hygiene, microbiology, food technology, veterinary and environmental science, and engineering, to name a few. Figure 16.2 indicates the multitude and complexity of monitoring methodologies available.

The most important consideration when designing a sampling strategy is how the data will be analysed so that results are meaningful, especially if there is no OEL for the agent of interest. In Australia, the most common practice is to collect outdoor samples as a background reference for identifying the potential indoor proliferation of fungi or bacteria and changes in species. Another popular approach is to monitor two or more indoor locations (the location of the reported case and a comparison location). Other key points to consider when developing a sampling plan include:

- the nature and potential concentration of the biological agent, to prevent over- or underestimation of exposure owing to poor sampling methodology
- the size distribution and/or environmental matrix of the biological agent, to ensure selection of suitable sampling devices and media

- the sampling duration (time, continuous, periodic, random or worst-case), equipment placement (personal or area), collection method (active or passive) and sample type (aerosol, bulk or surface)
- the cost, suitability and availability of sampling equipment
- the cost and availability of accredited analytical facilities
- any potential constraints that analytical methods may place on sample collection—such as transportation requirements and minimum detection limits
- the technical expertise required of field and laboratory personnel
- any potential sources of cross-contamination.

## 16.7.1 SAMPLE COLLECTION

Sampling methods may include the collection of aerosols, surface (swabs, RODAC plates, tapes, etc.), bulk (food, wash water, etc.) or biological samples (blood, urine and nasal secretions, etc.), as shown in Figure 16.2. Australian and international standardised methods for the sampling of bulk materials such as water and food are available from SAI Global. Techniques for swab and bulk sampling have been well defined within the food industry, and Standard methods such as AS 5013.11.2–2006 can be downloaded from SAI Global. Alternatively, the CDC provides free guidelines on its emergency response website, <www.cdc.gov/niosh/topics/emres>. For more information on biological sampling of human tissues and secretions, please refer to Chapter 10, on biological monitoring.

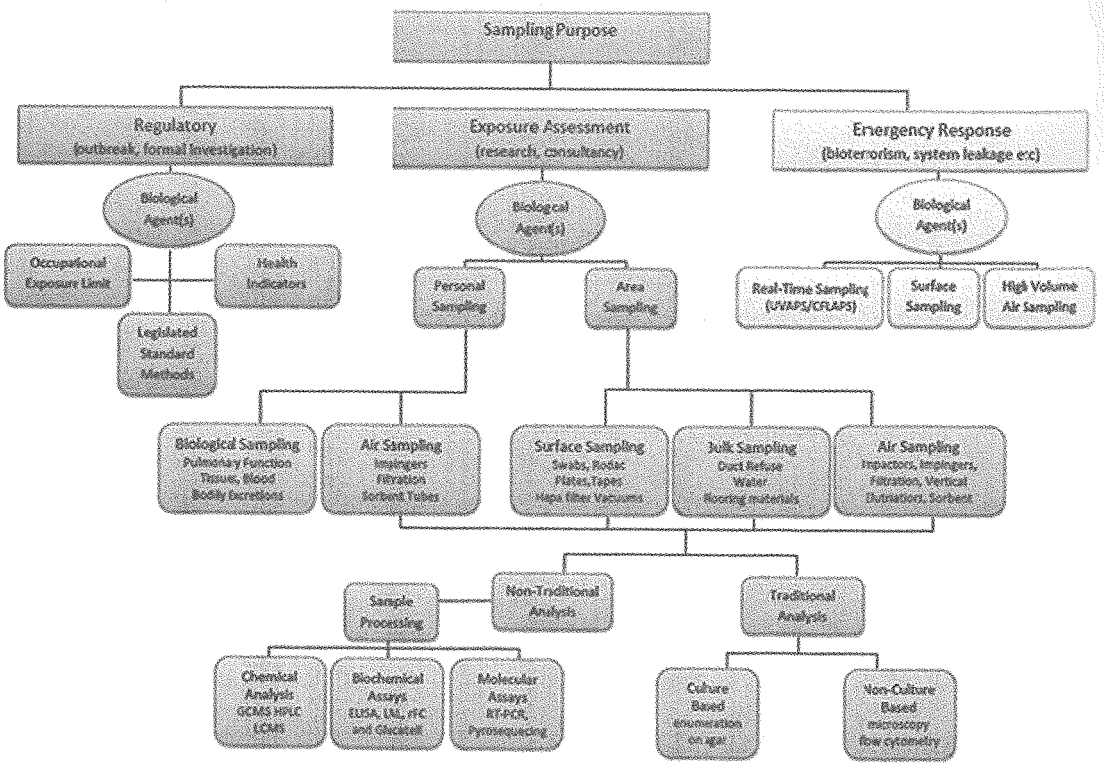



**Figure 16.2** Overview of selected sampling and analytical methods for assessing biological hazards

Bioaerosol sampling is one of the least standardised of all the occupational hygiene methodologies. To address this issue, hygienists should utilise only standardised (AS, ISO, NIOSH, etc.) methods when undertaking compliance sampling or epidemiological surveys to ensure that results are comparable with other research. The technology for bioaerosol research has developed rapidly over the past couple of decades. Bioaerosol researchers are continuously inventing and refining sampling devices incorporating various air–liquid interfaces, sampling media and physical approaches to improve collection efficiency and sensitivity. Researchers have also been modifying 'traditional' occupational hygiene bioaerosol measurement methods by incorporating alternative collection materials in standard devices. A recent example is the combination of filter and agar with an Andersen impactor by King and McFarland (2012) so they could undertake both culture and non-culture based analyses. Willeke and Macher (1998) identify three key performance principles that should be considered when reviewing and selecting sampling methodologies. These are inlet sampling efficiency (ability to remove atmospheric particulates); particle removal efficiency (ability of media to effectively retain aerosol); and biological recovery efficiency, or ensuring that collected particles maintain the chemical, physical and biological integrity of the environmental aerosols. Table 16.6 presents some of the more common commercially available sampling devices.

**Table 16.6** Sampling devices

| Device | Media | Method | Aerosol | Comments |
|---|---|---|---|---|
| *Impactors* | | | | |
| Single-stage | Agar | Area | Viable microorganisms or spores (agar, tape and gelatin filters) | Relatively inexpensive, and equipment and analytical facilities readily available for viable microorganism analysis in Australia |
| Cascade | Agar or filters | Area and personal | | Slit impactors can give real-time indication of temporal variation in bioaerosols |
| Slit | Agar or tape strip | | Allergens, dusts, microbial cell-wall components (filters) | Collection and impaction on agar and gelatin filters can stress microorganisms, making them viable but non-culturable; selective for more resilient organisms. May require short sampling periods of 1–3 minutes in heavily contaminated atmospheres. Cascade samplers enable size-selective measurement of biological agents, and both personal and area samplers are commercially available. Gelatin filters have |

| Device | Media | Method | Aerosol | Comments |
|---|---|---|---|---|
| | | | | short sampling periods of 30 minutes, after which filters become brittle. Microorganisms may become viable and non-culturable through sampling process. Use of different filter media alters recovery of cell-wall components like endotoxin |
| *Impingers* | | | | |
| AS PCR | | Area | Viable and countable microorganisms | Samples can undergo a wide variety of culture- and non-culture-based analyses. |
| Rotating cup | | Area | Cell-wall components, MVOCs and toxins | Subject to inlet sampling losses |
| AGI–30 | | Area | | Require short sampling periods of less than 30 minutes owing to sample media evaporation |
| Biosampler | | Area | | |
| Midget | Liquid | Personal | | Glass presents personal injury risk with midget impingers. Bulky and cumbersome to wear |
| Filtration IOM | Filters | Area Personal | Dust, microbial and cell-wall components, spores, MVOC, toxins and allergens | Can be adapted for full work-shift sampling Best suited for microbial indicators such as cell wall components (endotoxin, muramic acid, etc.) or direct counts) owing to sampling stress such as desiccation on viable organisms |
| Button | | | | Gelatin filters for viable microorganisms have short sampling periods, maximum 30 minutes |
| Cyclone Versa Trap Microspore cassettes | | | | Particle removal efficiency and biological recovery efficiency will vary widely for various filter materials |
| *Other* | | | | |
| Vertical elutriators | Liquid | Area | Organic dust (<15 μm diameter) and endotoxin | Vertical elutriators are standard method in cotton and textile industry in relation to byssinosis |

| Device | Media | Method | Aerosol | Comments |
|---|---|---|---|---|
| Electrostatic precipitators | Liquid | Area | Vegetative cells | Experimental technology |
| Wetted wall cylones | Liquid | Area | Vegetative cells | Experimental technology, may not be efficient for hydrophobic bacteria or fungal spores. Subject to impaction and rehydration stresses |
| Settling plates | Agar | Area | Vegative cells and spores | Unsuitable for spore sampling. Highly subject to environmental conditions, and low repeatability |
| HEPA vacuum | Filter sock | Surface | Viable cells, dust, cell-wall components, toxins, etc. | Not representative of airborne exposures |

(*Sources:* Jensen et al. 1992; Madelin & Madelin 1995; Cage et al. 1996; Görner et al. 2006; Engelhart et al. 2007; Spurgeon 2007; Yao & Mainelis 2007; Yamamoto et al. 2011)

### 16.7.1.1 Impactors

Impactors are among the most commonly used bioaerosol sampling devices for indoor air-quality assessments, clean-room testing and industrial operations. They range from slit to single-stage or multistage (cascade) impactors, which enable size-selective sampling of viable and culturable microorganisms in different aerosol fractions. The single and cascade impactors work by directing an air stream on to a plate containing agar, which is subsequently incubated and the cultivated microorganisms counted, or on to a filter which can be analysed by a variety of methods including direct microscopy analysis (counts) and the extraction and biochemical/chemical analysis of cell-wall constituents. Particles in the airstream collect on the agar based on their velocity, with heavier/large particles with greater momentum collected on the top stages and smaller particles on the lower stages (Figure 16.3). Slit impactors direct the air stream through a rectangular slit on to agar,

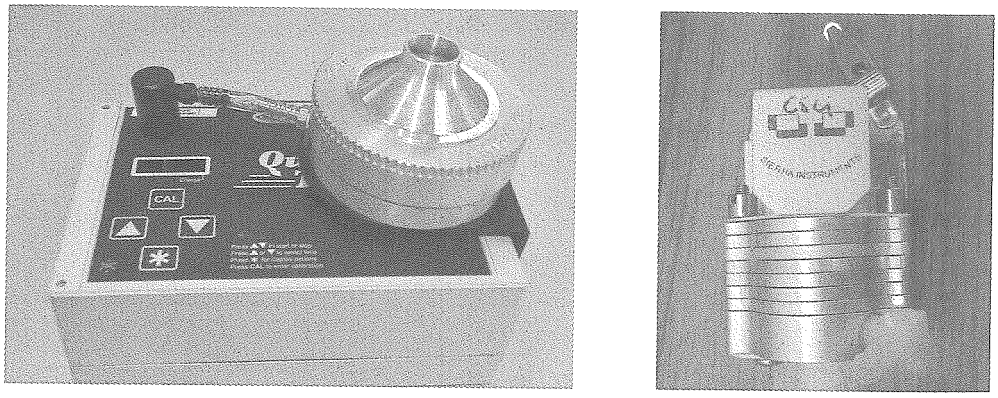
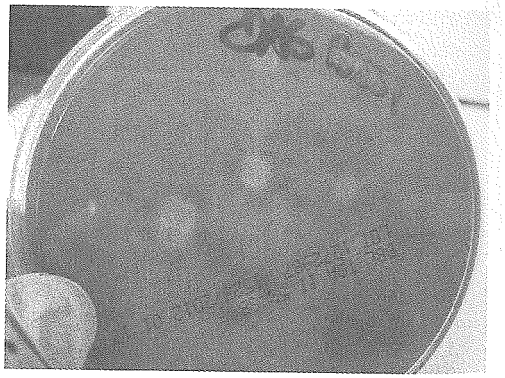



**Figure 16.3** Commercial impactors
(a) SKC BioStage single stage impactor; (b) Marple cascade impactor

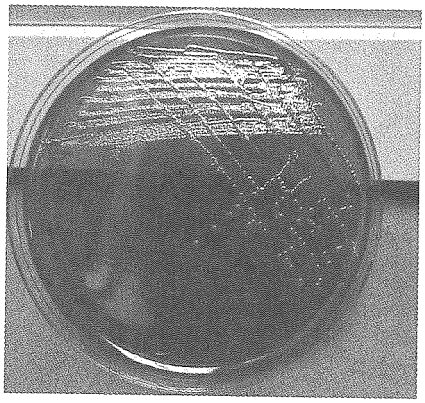
which undergoes incubation for culturable organisms (slit-to-agar sampler), or on to glass slides or moving tapes for microscopic analysis of pollen and spore traps (Willeke & Macher 1998). Commercially available devices include the BioSlide direct-to-slide sampler and the Hirst volumetric spore sampler from Burkard Scientific, which uses tape.

Single-stage and cascade impactors are loaded with petri dishes filled with general-purpose, enriched, selective or differential agars. The most commonly used agars for bioaerosol sampling include non-selective nutrient or tryptic soy agar (TSA) for bacteria (Figure 16.4a) and malt extract agar (MEA) for fungi. Other popular choices include selective agars such as Endo, eosin methylene blue (EMB) and MacConkey agars, which favour growth of Gram-negative bacteria through the addition of bile salts, fuschin and crystal violet dyes, or the addition of salt to TSA to select for Gram-positive bacteria. Differential media such as blood agar can be used to help presumptively identify bacteria through their growth morphology and ability to lyse red blood cells (Figure 16.4b). A number of agars can be both differential and selective. Examples include EMB agar, in which *E. coli* develop a gold sheen (Figure 16.4c), and chromagars, which are often used to select for and presumptively identify pathogenic organisms. Figure 16.4d shows presumptive *Salmonella* colonies growing on chromagar (mauve colonies). Chromagars are very

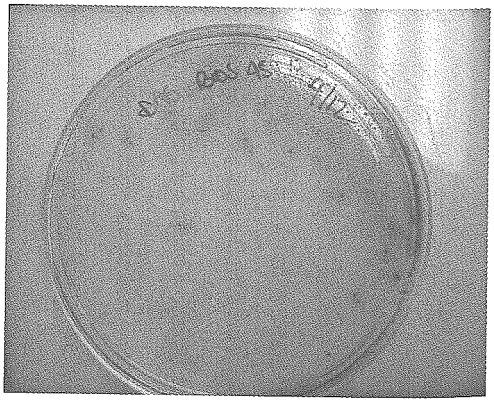
(a) *Bacillus* spp. on TSA



(b) Environmental bacteria on blood agar



(c) *E. coli* on EMB agar



(d) Presumptive *Salmonella* spp. on chromogenic agar

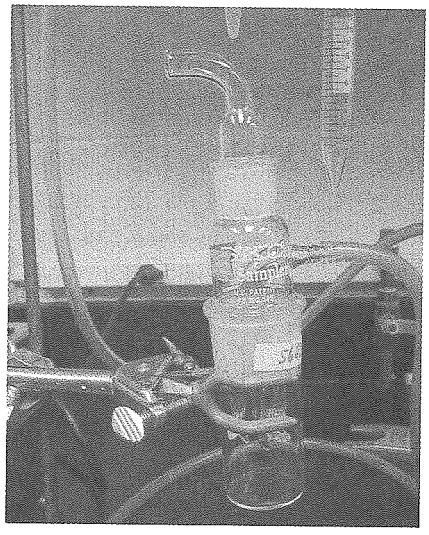
**Figure 16.4** General, selective and differential agars

efficient for rapid analysis, but they are very expensive in comparison to other agars, and environmental samples are prone to false positive results.

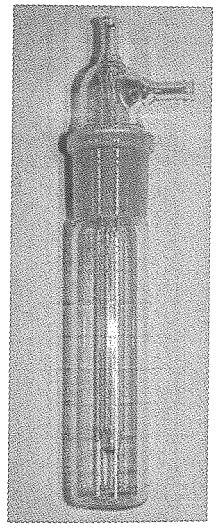
The advantage of using impactors such as the Andersen microbial sampler, SAS microbial air sampler or SKC BioSampler are that they are readily available, easy to operate and calibrate, and the instruments, media and sample analysis all are relatively inexpensive compared with other methods, such as endotoxin or MVOC analysis. The disadvantages of impactors are that results may not be truly representative of the microbial community because the impaction of microorganisms on to solid surfaces can render them viable but non-culturable, and various species may go undetected. Sampling times need to be very short (30 seconds to two minutes) in heavily contaminated environments because the agar plates quickly become overloaded, making it difficult if not impossible to determine what organisms are present. The majority of impactors are unsuitable for personal sampling. Bioaerosol loads may also be underestimated because they are based on plate counts of individual colonies, which may grow from an aggregate of cells or spores, rather than the individual cells, which can be viewed under a microscope during direct counts.

### 16.7.1.2 Impingers

Impingers can be used to sample a variety of biological aerosols, including viable and countable bacteria, fungi, viruses, pollens and cell-wall components, to name a few. They collect bioaerosols by drawing an air stream through a thin glass tube and aspirating into a liquid medium which separates out large particles. The liquid medium can be plated directly on to agar, examined by microscope and analysed either biochemically or using molecular techniques. The action of the air stream impinging into the liquid aids in the separation of aggregates, which, when plated on agar, can provide a more representative count than impactors can. Most impingers, including the SKC BioSampler and the AGI-30, are designed for area sampling and have large, cumbersome pumps (Figure 16.5a). Midget impingers (Figure 16.5b) designed for personal sampling have been around since the



(a) SKC BioSampler



(b) Midget impinger

**Figure 16.5** Commercially available impingers

1930s. However, they do represent a personal sampling hazard due to their glass construction. Multistage impingers are also available, but their glass construction makes it difficult to accurately predict the mass median diameters collected by each stage (Vincent 2007).

Common media used in impingers include distilled water, nutrient broths and Tween 20 or phosphate buffers. Use of phosphate buffers instead of distilled water increases collection of viable bioaerosols (Rule et al. 2009). Limitations of impingers include short sampling time owing to rapid evaporation of media (maximum sampling 30 minutes); glass construction, which makes them less robust than other samplers; the fact that hydrophobic particles will bounce and escape the system (Hung, Miller & Dillon 2005); and the effects of moderate changes in flow rate and liquid quantity on sample collection (Dart & Thornburg 2008). Impingers are also subject to inlet and internal losses as well as re-aerosolisation of organisms at lower concentrations (Kesavan, Schepers & McFarland 2010; Riemenschneider et al. 2010; Han & Mainelis 2012). ViaTrap mineral oil can be used as a collection liquid to reduce evaporation, allowing for eight-hour sampling periods, but such oils do not plate well and are better suited for microscopic analysis.

#### 16.7.1.3 Filtration

Biological aerosols can be collected using the same methods as for particulate sampling: AS 2985 and AS 3640 (Standards Australia 2009). These methods are better suited to the collection of non-viable bioaerosol samples such as pollen, allergens, spores, endotoxins and DNA/RNA. This is because viable cells can be sub-lethally injured during sampling, making them viable but non-culturable and causing both underestimation of bacterial loadings and sampling bias for hardier organisms such as the endospore-forming *Bacillus* species. Choice of collection media is also critical. Gelatin filters have been recommended for collection of viable microorganisms, but they become brittle and fragile during sampling and have a maximum sampling period of 30 minutes. Filter materials must be tested for background endotoxin and genetic materials, as well as for recovery efficiency of various bioaerosols such as endotoxin or β-D-glucans. Figure 16.6 displays different sampling heads that have been used for bioaerosol sampling. Metal sampling heads are ideal because they can be heat treated to remove background contamination. This is

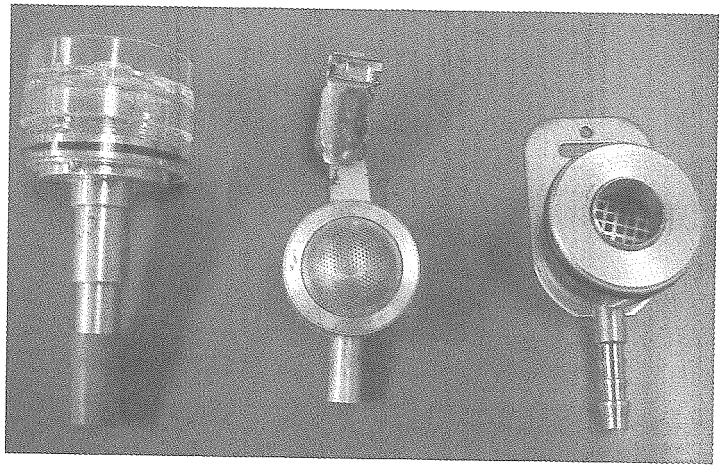


**Figure 16.6**
Filtration devices

important for endotoxin and genomic DNA, which may not be fully removed from devices using standard methods such as autoclaving and/or alcohol rinsing.

Single-use filter sampling devices are commercially available for collection of fungal spores, and reduce the risk of sample cross-contamination.

#### 16.7.1.4 Other samplers

Other commercially available samplers included wetted-wall, centrifugal and electrostatic samplers. Wetted-wall cyclones operate on the same principle as particle-sampling cyclones except that the internal surfaces are coated with suitable liquid to wash particles into the collection chamber (Vincent 2007). The hand-held, battery-operated Reuter Centrifugal Sampler (RCS) has been commercially available for years, and has been adopted in Canada as a reference sampling method for fungal contamination assessments (Vincent 2007). The operating principle is a centrifugal fan (impeller) which draws air into the sampling head and directs the stream on to an agar strip fitted to the internal walls. The strip is then incubated and the number of colonies counted to estimate the bioaerosol loading (Semple 2010).

BioTrak (BioTrak Research) and BioLaz (Particle Measuring Systems) are two commercially available real-time monitors that operate by measuring the fluorescence emitted by bioaerosols. Real-time monitors can be linked into existing alarm systems as engineering controls for biohazard containment or clean-room efficiency. More information on real-time monitors is available on the manufacturers' websites.

### 16.7.2 *SAMPLE STORAGE AND TRANSPORTATION*

Careful consideration must be given to the transport, extraction and storage of bioaerosol samples. Viable samples are susceptible to temperature fluctuations and storage times, while freeze/thaw cycling of endotoxin and genomic material causes sample losses. Hygienists should seek advice from their analytical laboratory on the optimal conditions for storage and transport of samples.

### 16.7.3 *SAMPLE ANALYSIS*

The selection of analytical methods will be primarily driven by compliance sampling requirements, budget, and access to analytical services. A good starting point is to search the AIOH website for consultants, or contact local government and Commonwealth laboratories, NATA and local universities. Alternatively, consulting peer-reviewed Australian research papers on the bioaerosol of interest may identify specialists and facilities that could be contacted for further advice. Table 16.7 outlines the various methodologies that can be used for analysis of bioaerosols.

## 16.8 EMERGING DISEASE ISSUES IN AUSTRALIA

The recent swine flu pandemics and occupational deaths from Hendra virus highlight the importance of hygienists remaining current on emerging biological health issues that may affect Australian workers. Sites such as ProMED-mail <www.promedmail.org> and

**Table 16.7** Analytical methodologies for biological agents

| Category | Methods | Endpoint | Comments |
|---|---|---|---|
| Culture-based | Plate counts, most probable number, membrane filtration, turbidity | Viable bacteria, fungi and viruses | Most common method in Australia at present. Accredited labs readily accessible in all states and territories. Most inexpensive method. Australian Standard methods available |
| Countable | Microscopic counts, flow cytometry | Total bacteria, fungi, spores and pollen | Accredited labs readily available for microscopy work. Flow cytometry primarily a research method |
| Chemical | GC/MS & GC/MSMS, LC/MSMS, HPLC, TLC, MALDI-TOF | MVOCs, lipopolysaccharide (Gram negative), muramic acid (Gram positive) and ergosterol (fungi) and 16s rDNA | Limited number of labs in Australia perform these analyses, which are mainly used for epidemiological and exposure research at present. More information is available in Hung, Miller & Dillon (2005) or Macher (2001) |
| Molecular | Real-time PCR, Western blot, 454-pyrosequencing, ribotyping, Illumina, Ion Torrent, Starlight, PacBio | RNA and DNA of viruses, fungi, bacteria, cytokines and allergens | Expensive, and laboratories offering these services for bioaerosol samples may not be readily available in Australia. Analysis of next-generation seqeuncing data (pyrosequencing, Illumina) can be complex and requires specialised bioinformatics software |
| Biochemical | Bacteria and fungi ID kits (API, Enterotube, etc.) | Bacteria and fungi | Commercial labs offer bacteria/fungi identification and ELISA analyses |
| | ELISA | Proteins/allergens, fungal extracellular polysaccharides, endotoxin | |
| | LAL & rFC assay | β-D-glucans | Endotoxin and β-D-glucans analysis of air samples not readily accessible in Australia, and mainly used for research at present. Kits are available, but a large number of variables must be addressed when using. Refer to BS EN 14031:2003 or ISO 29701:2010 for more information |

the Australian government outbreak website <www.outbreak.gov.au> provide a valuable resource for H&S practitioners, who should review them regularly to stay abreast of emerging biological hazards.

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