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Aeromicrobiology

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5.1 INTRODUCTION

In the 1930s, F.C. Meier coined the term **aerobiology** to describe a project that involved the study of life in the air (Boehm and Leuschner, 1986). Since then, aerobiology has been defined by many as the study of the aerosolization, aerial transmission and deposition of biological materials. Others have defined it more specifically as the study of diseases that may be transmitted via the respiratory route (Dimmic and Akers, 1969). Despite the variations in definition, this evolving area is becoming increasingly important in many aspects of diverse fields including public health, environmental science, industrial and agricultural engineering, biological warfare and space exploration.

This chapter introduces the basics of aerobiology, including the nature of aerosols and the fundamentals of the **aeromicrobiological (AMB) pathway**. The remainder of the chapter focuses on a subset of the science that we shall term aeromicrobiology. **Aeromicrobiology**, as defined for the purpose of this text, involves various aspects of

intramural (indoor) and **extramural** (outdoor) aerobiology, as they relate to the airborne transmission of environmentally relevant microorganisms, including viruses, bacteria, fungi, yeasts and protozoans.

5.2 AEROSOLS

Particles suspended in air are called **aerosols**. These pose a threat to human health mainly through respiratory intake and deposition in nasal and bronchial airways. In addition, soil or dust particles can act as a “raft” for biological entities known as bioaerosols (Brooks *et al.*, 2004). Smaller aerosols travel further into the respiratory system and generally cause more health problems than larger particles. For this reason, the United States Environmental Protection Agency (USEPA) has divided airborne particulates into two size categories: **PM₁₀**, which refers to particles with diameters less than or equal to 10 μm (10,000 nm), and **PM_{2.5}**, which are particles less than or equal to 2.5 μm

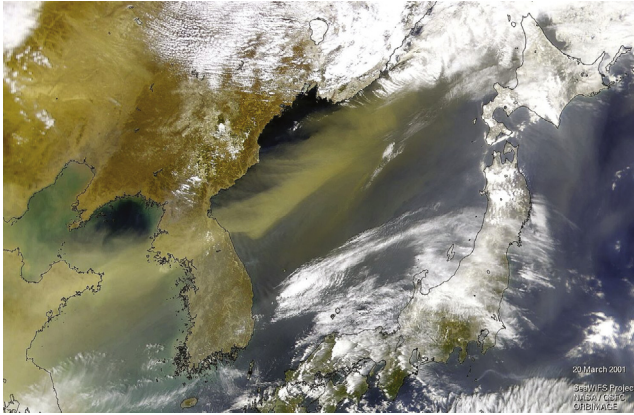


FIGURE 5.1 Mongolian dust over the Sea of Japan. Image provided by NASA.

(2500 nm) in diameter. For this classification, the diameter of aerosols is defined as the **aerodynamic diameter**:

$$d_{pa} = d_{ps}(\rho_p/\rho_w)^{1/2} \quad (\text{Eq. 5.1})$$

where:

- d_{pa} = aerodynamic particle diameter (μm)
- d_{ps} = Stokes' diameter (μm)
- ρ_p = particle density (g/cm^{-3})
- ρ_w = density of water (g/cm^{-3})

Atmospheric particulate concentration is expressed in micrograms of particles per cubic meter of air ($\mu\text{g}/\text{m}^3$). The USEPA established a **National Ambient Air Quality Standard (NAAQS)** for PM_{10} of $150 \mu\text{g}/\text{m}^3$ averaged over a 24-hour period, and $50 \mu\text{g}/\text{m}^3$ averaged annually. More recently, separate standards for $\text{PM}_{2.5}$ of $65 \mu\text{g}/\text{m}^3$ for 24 hours and $15 \mu\text{g}/\text{m}^3$ annually have been introduced.

Symptoms of particulate matter inhalation include: decreased pulmonary function; chronic coughs; bronchitis; and asthmatic attacks. The specific causal mechanisms are poorly understood. One well-documented episode occurred in London in 1952, when levels of smoke and sulfur dioxide aerosols, largely associated with coal combustion, reached elevated levels due to local weather conditions. Over a 10-day period, approximately 4000 deaths were attributed to cardiovascular and lung disorders brought on or aggravated by these aerosols.

Airborne particles can travel great distances. Intense dust storms during 1998 and 2001 in the Gobi desert of western China and Mongolia (Figure 5.1) elevated aerosol levels to concentrations near the health standard in western North America several thousand miles away.

Smaller particles tend to travel greater distances than large particles. Stokes' law (Eq. 5.2) is used to describe the fall of particles through a dispersion medium, such as air or water:

$$V = [D^2 \times (\rho_p - \rho_1) \times g] / 18\rho \quad (\text{Eq. 5.2})$$

Information Box 5.1 Influence of Particle Size on Velocity of Deposition of Particles in Air, Calculated Using Stokes' Law

Particle Diameter (mm)	Particle Type	Rate of Fall in Air (cm s^{-1})
1	Sand	7880
0.1	Silt	79
0.001	Clay	7.9×10^{-5}
0.002	Clostridial spore	0.016

From Pepper et al., 2006.

where:

- V = velocity of fall (cm/s^{-1})
- g = acceleration of gravity ($980 \text{ cm}/\text{s}^{-2}$)
- D = diameter of particle (cm)
- ρ_p = density of particle (density of quartz particles is $2.65 \text{ g}/\text{cm}^{-3}$)
- ρ_1 = density of dispersion medium (air has a density of about $0.001213 \text{ g}/\text{cm}^{-3}$; water has a density of about $1 \text{ g}/\text{cm}^{-3}$)
- ρ = viscosity of the dispersion medium (about 1.83×10^{-4} poise or $\text{g cm}^{-1}\text{s}^{-1}$ for air; 1.002×10^{-2} poise for water)

Using Stokes' law, we can calculate the rate of fall of particles in air (Information Box 5.1). Small particles are thus a greater concern than larger particles for several reasons. Small particles stay suspended longer and so they travel further and stay suspended longer. This results in an increased risk of exposure. Small particles also tend to move further into the respiratory system, exacerbating their effects on health. Stokes' law explains why we can expect viruses to persist as a bioaerosol longer than bacteria, which are much larger.

5.3 NATURE OF BIOAEROSOLS

Biological contaminants include whole entities such as bacterial and viral human pathogens. They also include airborne toxins, which can be parts or components of whole cells. In either case, biological airborne contaminants are known as bioaerosols, which can be ingested or inhaled by humans.

Bioaerosols vary considerably in size, and composition depends on a variety of factors including the type of microorganism or toxin, the types of particles they are associated with such as mist or dust, and the gases in which the bioaerosol is suspended. Bioaerosols in general range from 0.02 to 100 μm in diameter and are classified on the basis of their size. The smaller particles ($<0.1 \mu\text{m}$ in diameter) are considered to be in the **nuclei mode**, those ranging from 0.1 to 2 μm are in the **accumulation mode** and larger

Information Box 5.2 Possible Modes of Respiratory Transmission of Influenza A

Direct Contact

Transmission occurs when the transfer of microorganism results from direct physical contact between an infected or colonized individual and a susceptible host.

Indirect Contact

Transmission occurs by the passive transfer of microorganisms to a susceptible host via inanimate contaminated object or fomite.

Droplet

Transmission occurs via large droplets ($\geq 5 \mu\text{m}$ diameter) generated from the respiratory tract of the infected individual during coughing or sneezing, talking or during procedures such as suctioning or bronchoscopy. These droplets are propelled a distance of less than 1 m through the air, and are deposited on the nasal or oral mucosa of the new host or in their immediate environment. These large droplets do not remain suspended in the air and true aerosolization does not occur.

Airborne

Transmission occurs via the dissemination of microorganisms by aerosolization. Organisms are contained in droplet nuclei (airborne particles less than $5 \mu\text{m}$ that result from the evaporation of large droplets), or in dust particles containing skin cells and other debris that remain suspended in the air for long periods of time. Microorganisms are widely dispersed by air currents and inhaled by susceptible hosts.

See also Section 5.6.2 and Case Study 5.2.

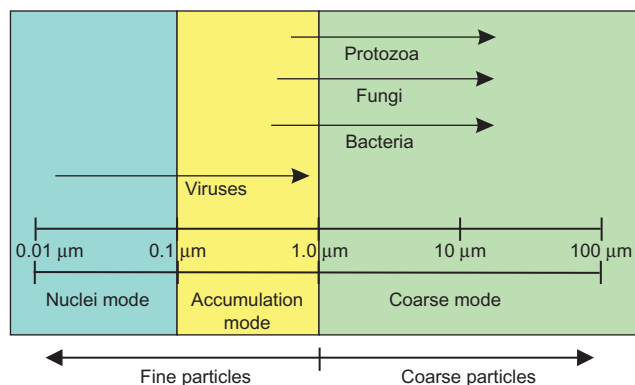


FIGURE 5.2 Diagrammatic representation of the relative sizes of bioaerosols. The depictions of the various kinds of organisms are indicative of their potential sizes when associated with airborne particles (rafts). The terminologies used to describe the various sizes of the bioaerosols are also indicated.

particles are considered to be in the **coarse mode** (Committee on Particulate Control Technology, 1980). As shown in **Figure 5.2**, particles in nuclei or accumulation mode are considered to be fine particles and those in coarse mode are considered coarse particles.

The composition of bioaerosols can be liquid or solid, or a mixture of the two, and should be thought of as microorganisms associated with airborne particles, or as airborne particles containing microorganisms. This is because it is rare to have microorganisms (or toxins) that are not associated with other airborne particles such as dust or water. This information is derived from particle size analysis experiments, which indicate that the average diameter of airborne bacterial particles is greater than $5 \mu\text{m}$ (Fengxiang *et al.*, 1992). By comparison, the average size of a soil-borne bacterium, 0.3 to $1 \mu\text{m}$, is less than one-fifth this size. Similar particle size analysis experiments show the same to be true for aerosolized microorganisms other than bacteria, including viruses.

5.4 AEROMICROBIOLOGICAL PATHWAY

The aeromicrobiological pathway describes: (1) the **launching** of bioaerosols into the air; (2) the subsequent **transport** via diffusion and dispersion of these particles; and finally (3) their **deposition**. An example of this pathway is that of liquid aerosols containing the influenza virus launched into the air through a cough, sneeze or even through talking. These virus-associated aerosols are dispersed by a cough or sneeze, transported through the air, inhaled, and deposited in the lungs of a nearby person, where they can initiate a new infection (**Figure 5.3**). Traditionally, the deposition of viable microorganisms and the resultant infection are given the most attention, but all three processes (launching, transport and deposition) are of equal importance in understanding the aerobiological pathway.

5.4.1 Launching

The process whereby particles become suspended within Earth's atmosphere is termed launching. Because



FIGURE 5.3 A cough or sneeze launches infectious microbes into the air. Anyone in the vicinity may inhale the microbes, resulting in a potential infection.

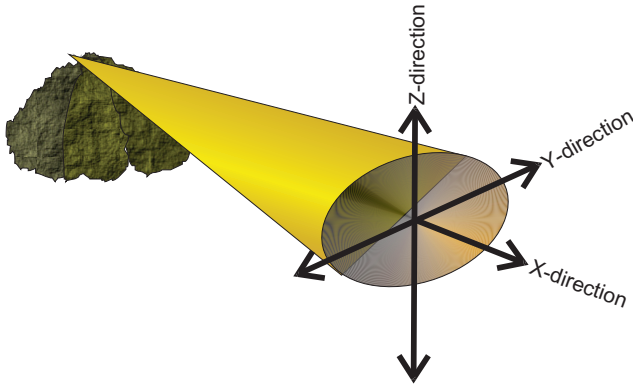


FIGURE 5.4 Schematic representation of the type of bioaerosol distribution expected from a point source, given three planes of diffusion: (1) the X-direction is the mean direction in which the wind is blowing; (2) the Y-direction is the lateral diffusion; and (3) the Z-direction is the vertical diffusion.

bioaerosols must be launched into the atmosphere to be transported, it is important to understand this process. The launching of bioaerosols is mainly from terrestrial and aquatic sources, with greater airborne concentrations or atmospheric loading being associated with terrestrial sources than with aquatic sources. A recent model estimated that the total global emission of bacteria containing particles to the atmosphere to be 7.6×10^{23} to 3.5×10^{24} (Burrow *et al.*, 2009). Some researchers speculate that there may even be atmospheric sources of bioaerosols in addition to terrestrial and aquatic ones. This phenomenon is related to the limited potential for microorganisms to reproduce while airborne. This, however, is an area of aeromicrobiology for which there is little available information.

Launching into the surface boundary layers can include, but is certainly not limited to, diverse mechanisms such as: air turbulence created by the movement of humans, animals and machines; the generation, storage, treatment and disposal of waste material; natural mechanical processes such as the action of water and wind on contaminated solid or liquid surfaces; and the release of fungal spores as a result of natural fungal life cycles.

Airborne particles can be launched from either point, linear or area sources. A **point source** is an isolated and well-defined site of launching such as a pile of biosolid material, before it is applied over a field. Point sources tend to display a general conical-type dispersion (Figure 5.4). Point sources can be further defined on the basis of the type of launching phenomenon: (1) **instantaneous point sources**, for example, a single event such as a sneeze; or (2) **continuous point sources**, from which launching occurs over extended periods of time, such as a biosolid pile.

In contrast to point sources, **linear sources** and **area sources** involve larger, less well-defined areas. When considered on the same size scale, linear and area sources display more particulate wave dispersion as opposed to the conical type of dispersion displayed by point sources.



FIGURE 5.5 A linear bioaerosol source using the example of the release of biological warfare agents. This is an illustration of an instantaneous linear bioaerosol release.

Linear and area sources can also be divided into instantaneous and continuous launching points of origin. For example, an instantaneous linear source might be a passing aircraft releasing a biological warfare agent (Figure 5.5). A continuous area source might be exemplified by release of bioaerosols from a large field that has received an application of biosolids or animal manures.

5.4.2 Transport

Transport or dispersion is the process by which kinetic energy provided by the movement of air is transferred to airborne particles, with resultant movement from one point to another. This “energy of motion” gained by airborne particles is considerable, and can result in dissemination of airborne microorganisms over long distances. Transport of bioaerosols can be defined in terms of time and distance. **Submicroscale transport** involves short periods of time, under 10 minutes, as well as relatively short distances, under 100 m. This type of transport is common within buildings or other confined spaces. **Microscale transport** ranges from 10 minutes to 1 hour, and from 100 m to 1 km, and is the most common type of transport phenomenon. **Mesoscale transport** refers to transport in terms of days and distances up to 100 km, and in **macroscale transport**, the time and distances are extended even further. Because most microorganisms have limited ability to survive when suspended in the atmosphere, the most common scales considered are the submicroscale and microscale. It should be noted, however, that some viruses, spores and spore-forming bacteria have been shown to enter into mesoscale and even macroscale transport.

As bioaerosols travel through time and space, different forces act upon them such as diffusion, inactivation and

ultimately deposition. **Diffusion** is the scattering and/or dissipation of bioaerosols in response to a concentration gradient as well as gravity, and is generally aided by airflow and atmospheric **turbulence**. The amount of turbulence associated with airflow, and thus the relative amount of diffusion that may occur in association with particulates such as bioaerosols, can be estimated using the method of Osbert Reynolds. Reynolds found that factors associated with mean **wind velocity**, the **kinetic viscosity** of the air and the relative dimension of the interfering structures could provide an indication of the amount of turbulence associated with linear airflow. Without turbulence, airborne particles from a point source would travel in a concentrated stream directly downwind. The **Reynolds equation** is written as follows:

$$\text{Reynolds number} = \frac{\text{velocity} \times \text{dimension}}{\text{viscosity}} \quad (\text{Eq. 5.3})$$

Consider, for instance, a situation in which there are relatively high winds (500 cm/sec) that are passing over a small bush (24 cm). Because the occurrence of frictional turbulence associated with an object depends on the wind velocity being high enough, and the object it is flowing over being large enough, we find that at normal air viscosity (0.14 cm²/sec) the Reynolds number (**Re**) becomes:

$$\text{Re} = \frac{500 \text{ cm/sec} \times 24 \text{ cm}}{0.14 \text{ cm}^2/\text{sec}} = 85,700 \quad (\text{Eq. 5.4})$$

The limiting value for the Reynolds equation is usually considered to be 2000, with values above this number indicating turbulent conditions. The higher this value, the higher the relative turbulence of the airflow, and the greater the microorganism-associated particle diffusion that occurs per unit time. In the preceding example, one would expect a great deal of turbulence around items such as a bush, which would increase the diffusion rates of passing bioaerosols.

When dealing with particulate transport over time and distance, **Taylor (1915)** indicated that diffusion during horizontal transport could be viewed as an increase in the standard spatial deviation of particles from the source over time. What does this mean? For an instantaneous point source under the influence of a mean wind direction, spread would be a standard spatial deviation from a linear axis (*x*) extending from the source (origin) in the mean direction of wind flow, with diffusion caused by turbulence occurring in the lateral (*y*) and vertical (*z*) axes (**Figure 5.4**). The standard deviation of particulate diffusion cannot be considered constant over a particular spatial orientation, but is instead dependent on the time taken to reach the particular distance. Mathematical models that attempt to estimate the transport of airborne particles use this basic premise as a foundation for predictions. To picture this concept, imagine standing at the door of a room,

where someone is holding a smoking candle. If there is no air current in the room the smoke will still eventually reach you at the door, but it will be very diffuse as it is also spreading in every other direction. However, if there is a fan behind the person holding the smoking candle and this fan is pointed at the door, then the smoke from the candle will be carried by this air current. It will travel the same distance as it did before, but it will travel faster, undergo less diffusion and as a result be more concentrated when it reaches you. This is the principle of time-dependent diffusion as indicated by Taylor's theory.

5.4.3 Deposition

The last step in the aeromicrobiology pathway is deposition. An airborne bioaerosol will eventually leave the turbulence of the suspending gas and will ultimately be deposited on a surface by one or a combination of interrelated mechanisms. These mechanisms are discussed in the following sections and include: gravitational settling; downward molecular diffusion; surface impaction; rain deposition; and electrostatic deposition. These processes are linked in many ways, and even though viewed separately, they all combine to create a constant, if not steady, deposition of particles.

5.4.3.1 Gravitational Settling

The main mechanism associated with deposition is the action of gravity on particles. The force of gravity acts upon all particles heavier than air, pulling them down and essentially providing spatial and temporal limitations to the spread of airborne particles. Steady-state gravitational deposition (**Figure 5.6**) in the absence of air movement can be described in very simplistic terms by **Stokes' law**, which takes into account gravitational pull, particle density, particle diameter and air viscosity (**Section 5.2**).

5.4.3.2 Downward Molecular Diffusion

Downward molecular diffusion, as indicated by the name, can be described as a randomly occurring process caused by natural air currents and eddies that promote and enhance the downward movement of airborne particulates (**Figure 5.7**). These random movements exist even in relatively still air and tend to be in the downward direction because of gravitational effects. As a result, measured rates of gravitational deposition tend to be greater than those predicted by the Stokes equation. The increase in the rate of deposition is due to the added effects of downward molecular diffusion. Molecular diffusion is also influenced by the force of the wind. Molecular diffusion-enhanced deposition rates tend to increase with increasing wind speed and turbulence.

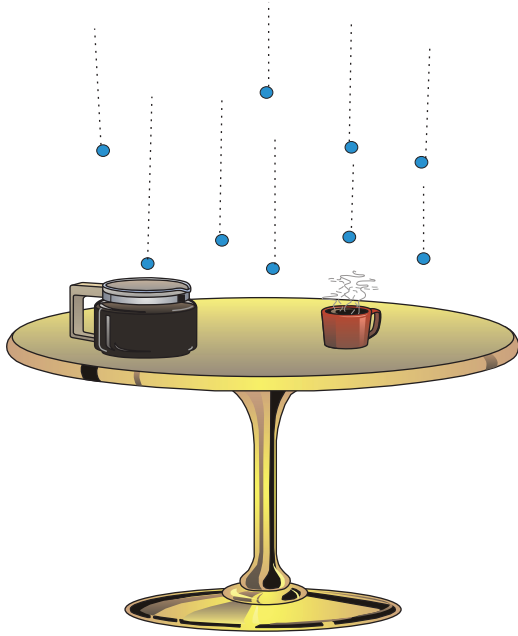


FIGURE 5.6 Schematic representation of gravitational settling, which is a function of Earth's gravitational pull, particle density, particle diameter and the viscosity of air. This figure does not take into account random air movement. Stokes' equation was developed to give an estimate of the terminal velocity achieved by particles as a function of gravitational settling.

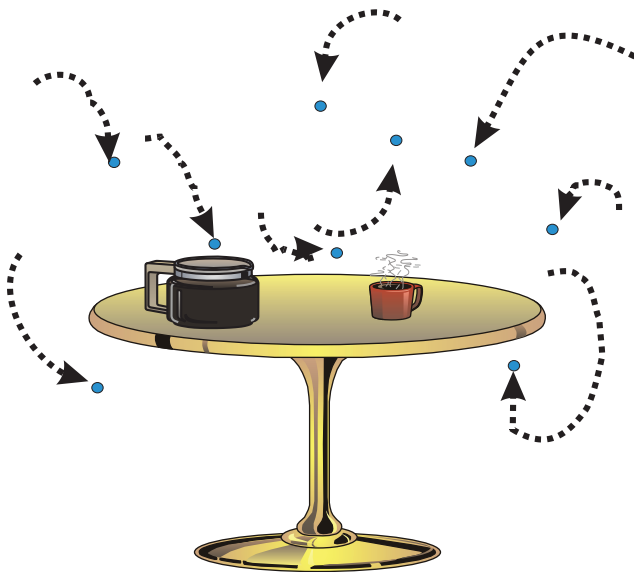


FIGURE 5.7 Schematic representation of downward molecular diffusion, a naturally occurring process caused by the air currents and eddies that promotes and enhance gravitational settling of airborne particles. Although molecular diffusion can occur in any direction, due to the effects of gravity the overall trend of the process results in net downward movement and deposition.

5.4.3.3 Surface Impaction

Surface impaction is the process by which particles make contact with surfaces, such as leaves, trees, walls and computers. With impaction there is an associated loss of kinetic energy. In nature, it is rare to find flat, smooth surfaces on which wind currents are unobstructed. Thus, surface impaction is a very critical factor influencing transport and deposition, especially for bioaerosols.

Impaction potential is the relative likelihood that an airborne object will collide with another object in its path. Impaction does not necessarily result in permanent deposition, however. Once a particle collides with an object, it has the potential to bounce. Bouncing off a surface causes the particle to reenter the air current at a lower rate, which can have one of two effects: (1) it can allow subsequent downward molecular diffusion and gravitational settling to occur, resulting in deposition on another nearby surface; or (2) it can allow the particle to escape the surface and once again reenter the air current. Studies have shown that impaction is influenced by the velocity and size of the particle, as well as the size and shape of the surface it is approaching.

5.4.3.4 Rain and Electrostatic Deposition

Rainfall and electrostatic charge also can affect deposition. Rainfall deposition occurs as a condensation reaction between two particles (raindrop and bioaerosol), which combine and create a bioaerosol with a greater mass, which settles faster. This can be described mathematically using the Stokes equation. In the example presented in [Information Box 5.1](#), a clostridial spore alone has a calculated terminal velocity of 0.016 cm/sec. The same spore (bioaerosol), if it condensed with another particle such as a water droplet, has a greater mass and thus a greater terminal velocity. For instance, if the clostridial spore were to condense with a water droplet that doubled the bioaerosol density from 1.3 to 2.6 g/cm³, the terminal velocity would be increased from 0.016 to 0.032 cm/sec. The overall efficiency of rain deposition also depends on the spread area of the particle plume. Larger, more diffuse plumes undergo stronger impaction than smaller, more concentrated plumes. Rain deposition is also affected by the intensity of the rainfall. The heavier the rainfall, the greater the overall rates and numbers of the condensation reactions, and the greater the subsequent increase in rain deposition.

Electrostatic deposition also condenses bioaerosols, but is based on electrovalent particle attraction. All particles tend to have some type of associated charge. Microorganisms typically have an overall negative charge associated with their surfaces at neutral pH. These negatively charged particles can associate with other positively charged airborne particles, resulting in electrostatic condensation. The major phenomenon occurring may be a

coagulation effect between particles (much like the condensation of the clostridial spore with the water droplet), which would increase the bioaerosol mass and enhance deposition. It might also be assumed that as an electromagnetically charged bioaerosol comes into close proximity with an electromagnetically charged surface, electroattractive or electrorepulsive influences may be present.

5.5 MICROBIAL SURVIVAL IN THE AIR

The atmosphere is an inhospitable climate for microorganisms mainly because of desiccation stress. This results in a limited time frame in which microbes can remain biologically active. Many microorganisms, however, have specific mechanisms that allow them to be somewhat resistant to the various environmental factors that promote loss of biological activity. Spore-forming bacteria, molds, fungi and cyst-forming protozoa all have specific mechanisms that protect them from harsh gaseous environments, increasing their ability to survive aerosolization. For organisms that have no such specific mechanisms, the survival in aerosols can often be measured in seconds. In contrast, organisms with these mechanisms can survive indefinitely.

As a result, viability is highly dependent on the environment, the amount of time the organism spends in the environment and the type of microorganism. In addition, microbes may be viable but nonculturable (Chapter 3), but for simplicity in this chapter we will use the term viable rather than the term culturable. Many environmental factors have been shown to influence the ability of microorganisms to survive. The most important of these are relative humidity and temperature. Oxygen content, specific ions, UV radiation, various pollutants and AOFs (air-associated factors) are also factors in the loss of biological activity. Each of these factors is discussed in the following sections.

The loss of biological activity can be termed **inactivation** and can generally be described using the following equation:

$$X_t = X_0 e^{-kt} \quad (\text{Eq. 5.5})$$

where:

- X_t represents the viable organisms at time t
- X_0 is the starting concentration
- k is the inactivation constant, which is dependent on the particular species of microorganisms as well as a variety of environmental conditions

5.5.1 Relative Humidity

The **relative humidity** or the relative water content of the air has been shown to be of major importance in the survival of airborne microorganisms. [Wells and Riley \(1937\)](#)

were among the first to show this phenomenon, indicating that as the relative humidity approaches 100%, the death rate of *Escherichia coli* increases. In general, it has been reported that most Gram-negative bacteria associated with aerosols tend to survive for longer periods at low to mid levels of relative humidities, with enhanced decay at relative humidities above 80% ([Brooks et al., 2004](#)). The opposite tends to be true for Gram-positive bacteria, which tend to remain viable longer in association with high relative humidities ([Theunissen et al., 1993](#)). Thus, the ability of a microorganism to remain viable in a bioaerosol is related to the organism's surface biochemistry. One mechanism that explains loss of viability in association with very low relative humidity is a structural change in the lipid bilayers of the cell membrane. As water is lost from the cell, the cell membrane bilayer changes from the typical crystalline structure to a gel phase. This structural phase transition affects cell surface protein configurations and ultimately results in inactivation of the cell ([Hurst et al., 1997](#)). In general, Gram-negative bacteria react unfavorably to desiccation, whereas Gram-positive cells are more tolerant of desiccation stress ([Mohr, 2001](#)).

Early studies by [Loosli et al. \(1943\)](#) showed that the influenza virus was also adversely affected by an increase in relative humidity. More recent work suggests that viruses possessing enveloped nucleocapsids (such as the influenza virus) have longer airborne survival when the relative humidity is below 50%, whereas viruses with naked nucleocapsids (such as the enteric viruses) are more stable at a relative humidity above 50% ([Mohr, 2001](#)). It should be noted that viruses with enveloped nucleocapsids tend to have better survival in aerosols than those without. Some viruses are also stable in the AMB pathway over large ranges of relative humidity, which makes them very successful airborne pathogens.

5.5.2 Temperature

Temperature is a major factor in the inactivation of microorganisms. In general, high temperatures promote inactivation, mainly associated with desiccation and protein denaturation, and lower temperatures promote longer survival times ([Mohr, 2001](#)). When temperatures approach freezing, however, some organisms lose viability because of the formation of ice crystals on their surfaces. The effects of temperature are closely linked with many other environmental factors, including relative humidity.

5.5.3 Radiation

The main sources of radiation damage to microorganisms including bacteria, viruses, fungi and protozoa are the shorter UV wavelengths and ionizing radiation such as

X-rays. The main target of UV irradiation damage is the nucleotides that make up DNA. Ionizing radiation or X-rays cause several types of DNA damage, including single strand breaks, double strand breaks and alterations in the structure of nucleic acid bases. UV radiation causes damage mainly in the form of intrastrand dimerization, with the DNA helix becoming distorted as thymidines are pulled toward one another (Freifelder, 1987). This in turn causes inhibition of biological activity such as replication of the genome, transcription and translation.

Several mechanisms have been shown to protect organisms from radiation damage. These include association of microbes with larger airborne particles, possession of pigments or carotenoids, high relative humidity and cloud cover, all of which tend to absorb or shield bioaerosols from radiation. Many types of organisms also have mechanisms for repair of the DNA damage caused by UV radiation. An example of an organism that has a radiation resistance mechanism is *Dienococcus radiodurans*. *D. radiodurans* is a soil bacterium that is considered the most highly radiation-resistant organism that has yet been isolated. An important component of its radiation resistance is the ability to enzymatically repair damage to chromosomal DNA. The repair mechanism used by these bacteria is so highly efficient that much of the metabolic energy of the cell is dedicated exclusively to this function.

5.5.4 Oxygen, OAF and Ions

Oxygen, open air factors (OAFs) and ions are environmental components of the atmosphere that are difficult to study at best. In general, it has been shown that these three factors combine to inactivate many species of airborne microbes. **Oxygen toxicity** is not related to the dimolecular form of oxygen (O₂), but is instead important in the inactivation of microorganisms when O₂ is converted to more reactive forms (Cox and Heckley, 1973). These include superoxide radicals, hydrogen peroxide and hydroxide radicals. These radicals arise naturally in the environment from the action of lightning, UV radiation, pollution, etc. Such reactive forms of oxygen cause damage to DNA by producing mutations, which can accumulate over time. The repair mechanisms described in the previous section are responsible for control of the damaging effects of reactive forms of oxygen.

Similarly, the **open air factor (OAF)** is a term coined to describe an environmental effect that cannot be replicated in laboratory experimental settings. It is closely linked to oxygen toxicity, and has come to be defined as a mixture of factors produced when ozone and hydrocarbons (generally related to ethylene) react. For example, high levels of hydrocarbons and ozone can cause increased inactivation rates for many organisms, probably because of damaging effects on enzymes and nucleic

acids (Donaldson and Ferris, 1975). Therefore, OAFs have been strongly linked to microbial survival in the air.

The formation of other ions, such as those containing chlorine, nitrogen or sulfur, occurs naturally as the result of many processes. These include the action of lightning, shearing of water and the action of various forms of radiation that displace electrons from gas molecules, creating a wide variety of anions and cations not related to the oxygen radicals. These ions have a wide range of biological activity. Positive ions cause only physical decay of microorganisms, e.g., inactivation of cell surface proteins, whereas negative ions exhibit both physical and biological effects such as internal damage to DNA.

5.6 EXTRAMURAL AEROMICROBIOLOGY

Extramural aeromicrobiology is the study of microorganisms associated with outdoor environments. In the extramural environment, the expanse of space and the presence of air turbulence are two controlling factors in the movement of bioaerosols. Environmental factors such as UV radiation, temperature and relative humidity modify the effects of bioaerosols by limiting the amount of time that aerosolized microorganisms will remain viable. This section provides an overview of extramural aeromicrobiology that includes: aerosolization of indigenous soil pathogens; influenza pandemics; the spread of agricultural pathogens; the spread of airborne pathogens associated with waste environments; and important airborne toxins.

5.6.1 Aerosolization of Indigenous Soil Pathogens

Geo-indigenous pathogens are those found in soils that are capable of metabolism, growth and reproduction (Pepper *et al.*, 2009). These are found in all soils and include both prokaryotic and eukaryotic organisms, many of which are spore formers. Such spores can potentially be aerosolized and cause human infections. *Bacillus anthracis* is a bacterial geo-indigenous pathogen that causes lethal disease in humans via pulmonary, gastrointestinal or cutaneous modes of infection (Gentry and Pepper, 2002). The organism is found worldwide and, because it is a spore former, can remain viable in soil for years.

Studies have shown the potential for anthrax to be disseminated by aerosols. Turnbull *et al.* (1998) found airborne concentrations of anthrax spores as high as 2.1×10^{-2} CFU L⁻¹ of air, and airborne movement as far as 18 m from a contaminated carcass in Etosha National Park, Namibia. However, the majority of samples taken were negative, and the number of spores collected in positive samples was very low, making airborne contraction of disease at a distance from the carcass unlikely. A more

serious outbreak in humans resulting from a *B. anthracis* aerosol is described in [Case Study 5.1](#).

Important fungal geo-indigenous pathogens include *Coccidioides immitis* and *Histoplasma capsulatum*. *Coccidioides immitis* is a soil-borne fungi that causes a respiratory illness known as Valley Fever. It preferentially

grows in the soils of semiarid regions of the Southwest United States, including California, Arizona, New Mexico and Texas ([Baptista-Rosas et al., 2007](#)). Symptoms can be mild to fatal. *Histoplasma capsulatum*, another fungus causing respiratory infections, is found worldwide in soils, but, in the United States, it is endemic to southeastern and midwestern states ([Deepe and Gibbons, 2008](#)). Histoplasmosis can be asymptomatic or mild, but the infections can be very serious or even fatal for immunocompromised individuals.

Case Study 5.1 Anthrax

In 1979, an anthrax outbreak occurred in Sverdlovsk, in the then U.S.S.R., due to the accidental release of a bioaerosol from a military microbiological facility ([Meselson et al., 1994](#)). At least 66 people died as a result of the release. Human anthrax cases extended 4 km along an axis to the south of the military facility and livestock cases extended up to 50 km in the same direction. The geographic distribution of human and animal cases was consistent with meteorological patterns existing when the accidental release was believed to have occurred. There has been no indication that human anthrax cases have occurred in Sverdlovsk since 1979.

Case Study 5.2 The Spanish Influenza Pandemic of 1918

This pandemic affected approximately one-third of the world population at that time, with 3–6% dying ([Barry, 2004](#)). The pandemic lasted from January 1918 to December 1920, the responsible virus being H1N1. This was the first outbreak resulting from H1N1, with the second epidemic occurring in 2009. Although the pandemic did not originate in Spain, the term “Spanish flu” was coined due to the severity of the infections in Spain. It is believed that the pandemic began in Haskell County, Kansas, before spreading rapidly to Europe. Estimates of the total number of deaths range from 50 to 100 million worldwide with 500,000 to 675,000 deaths in the U.S. A. ([Barry, 2004](#)).

5.6.2 Influenza Pandemics

Influenza pandemic is the term given to an epidemic of an influenza virus that occurs on a worldwide scale with a resultant infection of a large proportion of the human population. Known colloquially as the “flu,” influenza is an infectious disease of birds and mammals caused by an RNA virus of the family *Orthomyxoviridae*. Influenza can cause the common flu symptoms of muscle ache, headache, coughing, weakness and fatigue, or pneumonia which can be fatal.

Avian influenza refers to a large group of influenza viruses that primarily affect birds, but have the potential to adapt and infect humans. An influenza pandemic occurs when an avian influenza virus adapts into a strain that is contagious among humans and that has not previously circulated within humans. Such adaptations can be devastating, as illustrated in [Table 5.1](#).

Influenza virus transmission among humans can occur via four mechanisms: by direct contact with infected individuals; by indirect contact with contaminated objects of fomites; by inhalation of droplets that contain the virus; or by inhalation of aerosolized virus. Interestingly, despite 70 years of research since the influenza A virus was discovered, there is still debate about the modes of influenza transmission, specifically whether influenza is mainly transmitted via true bioaerosols, or by droplets, or by direct or indirect contact ([Brankston et al., 2007](#)).

TABLE 5.1 History of Major Influenza Pandemics

Name of Pandemic	Period	Deaths	Influenza Subtype
Asiatic (Russian) flu	1889–1890	1 million	Unknown
Spanish flu	1918–1920	Up to 50 million	H1N1
Asian flu	1957–1958	2 million	H2N2
Hong Kong flu	1968–1969	1 million	H3N2
Swine flu	2009–2010	≈ 18,000	H1N1

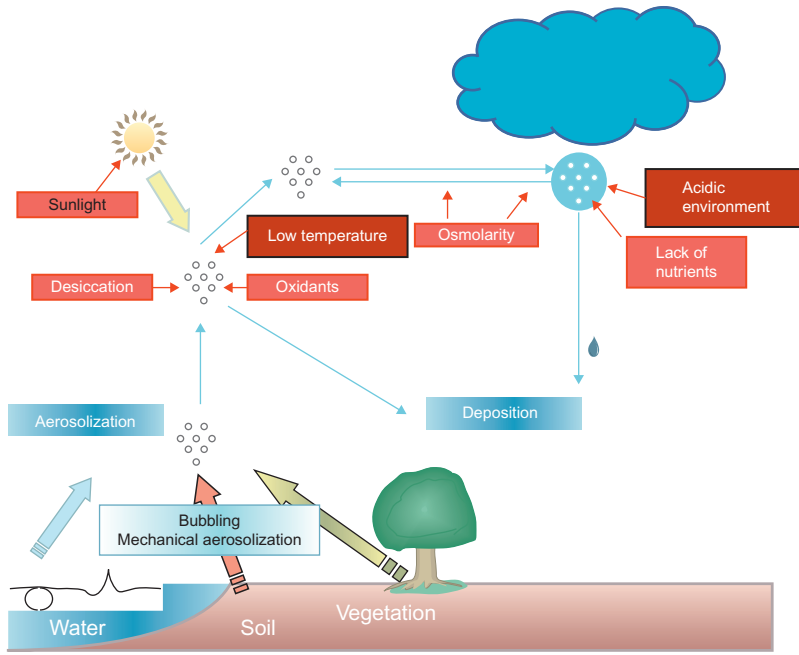


FIGURE 5.8 Cloud environmental factors that can adversely influence microbes.

5.6.3 Microbiology in the Clouds

Recent studies have suggested that microbes can potentially affect meteorological processes. In particular, some microorganisms, called ice nucleators, efficiently catalyze ice formation and may play a role in the formation and precipitation within clouds (Chistner, 2012). Based on recent studies, 95% of ice nuclei are biological particles and at least 40% originate from bacteria. Microorganisms are present in both clouds and fog. The abundance of culturable bacteria and fungi in clouds varies with the season, with greater numbers occurring in the summer and fall. While only 1% of the bacteria and 50% of the fungi in clouds are culturable, studies suggest that the majority are metabolically active (Delort *et al.*, 2010). Bacterial numbers range from 10^3 to 10^4 /ml compared to fungal numbers of 10^2 to 10^4 /ml. The cloud environment is a harsh environment with UV light irradiation, desiccation, low temperatures and other factors potentially adversely affecting microbes (Figure 5.8). Microorganisms may modify this environment by metabolizing organic compounds, and also by playing a role in cloud chemistry and physics, but much additional research is needed because the cloud environment is difficult to study.

5.6.4 Agriculture

Numerous plant pathogens are spread by the aeromicrobiological pathway (Information Box 5.3). Contamination of crops and animals via bioaerosols has a large worldwide economic impact. Rice and wheat are two of the major

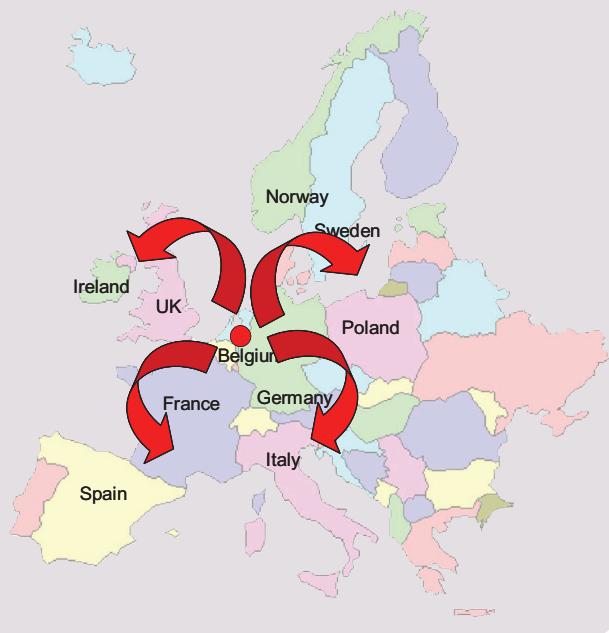
staple crops that are paramount to world food security. Major pathogens of such crops are the **wheat rust fungi**. These spore-forming fungi cause some of the most devastating diseases of wheat and other grains. In 1993, one type of wheat rust (leaf rust) was responsible for the loss of over 40 million bushels of wheat in Kansas and Nebraska alone. Even with selective breeding for resistance in wheat plants, leaf rust continues to have major economic impacts. The high concentration of wheat in areas ranging from northern Texas to Minnesota and up into the Dakotas makes this whole region highly susceptible to rust epidemics.

Spores of wheat rust are capable of spreading hundreds if not thousands of kilometers through the atmosphere (Ingold, 1971). The airborne spread of rust disease has been shown to follow a predictable trend, which starts during the fall with the planting of winter wheat in the southern plains. Any rust-infected plant produces thousands of spores, which are released into the air (Figure 5.9) by either natural atmospheric disturbance or mechanical disturbance during the harvesting process. Once airborne, these spores are capable of long-distance dispersal, which can cause downwind deposition onto other susceptible wheat plants. The generation time of new spores is measured in weeks, after which new spores are again released from vegetative fungi into the AMB pathway. For example, during the harvest of winter wheat in Texas, the prevailing wind currents are from south to north, which can allow rust epidemics to spread into the maturing crops farther north in Kansas and up into the young crops in the Dakotas (Figure 5.10). This epidemic spread of wheat rust and the resulting economic destruction produced are

Information Box 5.3 Examples of Airborne Plant Pathogens

Fungal Plant Disease	Pathogen
Dutch Elm disease	<i>Ceratocystis ulmi</i>
Potato late blight	<i>Phytophthora infestans</i>
Leaf rust	<i>Puccinia recondite</i>
Loose smut of wheat	<i>Ustilago tritici</i>
Downy mildew	<i>Pseudoperonospora humuli</i>
Maize rust	<i>Puccinia sorghi</i>
Powdery mildew of barley	<i>Erysiphe graminis</i>
Southern corn leaf blight	<i>Helminthosporium maydis</i>

The figure shows the airborne spread of late blight of potato that caused the 1845 epidemic known as the Irish potato famine. *Phytophthora infestans* spread from Belgium (mid-June) throughout Europe by mid-October. Famine related deaths are estimated from 750,000 to 1,000,000. Economic devastation from this famine caused the population of Ireland to decrease from approximately 8 million to 4 million from 1840 to 1911.



indicative of the impact that airborne microbial pathogens can have on agriculture.

A factor that complicates the control of such diseases is that chemical treatment for the control of pathogens is viewed as undesirable. This is because many pesticides have long half-lives and their residence in an ecosystem can be extremely harmful. Therefore, instead of using wheat rust fungicides, attempts are being made to breed strains of wheat that are more resistant to the fungi. Another method used for controlling phytopathogenic (plant pathogenic) fungi is spore monitoring as a disease control strategy. In this approach, the life cycle of the fungi, especially the release of spores, is monitored, and



FIGURE 5.9 Field of wheat highly infected by phytopathogenic wheat rust. The field is being harvested by a hay machine, which is releasing a cloud of rust spores into the aeromicrobiological pathway. These spores can spread thousands of miles and infect other crops downwind, causing catastrophic losses to wheat crops.

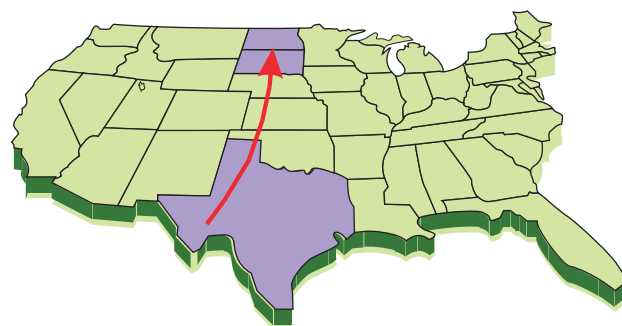


FIGURE 5.10 The arrow indicates the northern path of wheat rust infections as spread by the aeromicrobiological pathway. The wheat rust infection begins in the winter harvest in Texas and spreads northward with the prevailing wind currents. The epidemic spread of these phytopathogens infects maturing crops in Kansas and then moves up into the young crops in the Dakotas.

fungicide application is timed to coincide with spore release. This approach minimizes use of harmful chemicals. Thus, efficient aeromicrobiology pathway sampling, monitoring, detection and modeling have the ability to aid in the control of airborne pathogens.

The airborne spread of pathogenic microorganisms is also highly important in the animal husbandry industry (Information Box 5.4). The occurrence of foot-and-mouth disease is an example of the importance of bioaerosols in the spread of airborne disease (Case Study 5.3). It has long been thought that bioaerosol spread is linked primarily to respiratory pathogens, but there is growing evidence that gastrointestinal pathogens are also important in airborne transmission of disease among animals. One example of bioaerosol spread of a gastrointestinal pathogen is

Information Box 5.4 Examples of Airborne Animal Pathogens

Animal Disease	Pathogen
Bacterial diseases	
Tuberculosis	<i>Mycobacterium bovis</i>
Brucellosis	<i>Brucella</i> spp.
Fungal diseases	
Aspergillosis	<i>Aspergillus</i> spp.
Coccidioidomycosis	<i>Coccidioides immitis</i>
Viral diseases	
Influenza	Influenza virus
Rabies	Rhabdoviridae
Foot-and-mouth disease	Aphthovirus

Case Study 5.3 The United Kingdom Foot and Mouth Crisis 2001

Outbreaks of foot and mouth disease have occurred worldwide including multiple occurrences within the U.S.A. However, one of the more devastating outbreaks occurred in the U.K. in the spring and summer of 2001. Foot and mouth viruses are from the *Aphthovirus* genus of the family Picornaviridae, and are single-stranded RNA viruses. The 2001 U.K. outbreak was due to a type O pan-Asia strain that was thought to have arisen from infected meat illegally imported into the U.K. Transmission of the virus can occur via direct contact or via bioaerosols (Grubman and Baxt, 2004). Overall, 2000 cases were reported throughout Britain, resulting in the culling of 7 million sheep and cattle, costing the U.K. \cong \$16 million.

transmission of *Salmonella typhimurium* among calves that are housed individually in small pens (Hinton *et al.*, 1983). The potential for bioaerosol spread of this pathogen was recognized because the initial symptoms resembled those of pneumonia and appeared randomly within these animals, two factors that are not characteristic of oral transmission. Oral transmission generally occurs sequentially from one pen to the next, whereas aerial transmission can carry organisms past nearby pens, infecting calves randomly. Furthermore, Wathes *et al.* (1988) showed that *S. typhimurium* could survive for long periods in an airborne state, and calves and mice exposed to aerosolized *S. typhimurium* developed symptoms, proving that gastrointestinal pathogens could be spread by aerosolization. Finally, Baskerville *et al.* (1992) showed that aerosolized *Salmonella enteritidis* could infect laying hens. These hens showed clinical symptoms and were shedding the test strain of salmonellae in their feces



FIGURE 5.11 Application of secondary treated wastewater onto agricultural lands. This method is highly efficient at conserving water and has been shown to improve the fertility of soils. Due to the presence of pathogens in wastewater, and the nature of these land application systems, there are high concentrations of bioaerosols generated. Currently, however, there is little epidemiological and microbial risk assessment information available to determine if there may be health concerns for populations living in the vicinity of such operations, though there is a growing base of information on the concentration and types of pathogens found in these bioaerosols.

within a few days. Thus, the aeromicrobiology pathway can be important even in the spread of diseases for which pathogens are not normally considered airborne.

5.6.5 Waste Disposal

Waste disposal is a multibillion dollar industry in the United States. However, there are many hazards inherent in the treatment and disposal of wastewater (Figure 5.11), animal manures and biosolid material. Figures 5.12–5.14 illustrate the potential for bioaerosol production via various methods of land application of biosolids and also loading operations. Major hazards associated with waste effluents are pathogenic microorganisms including bacteria, viruses, protozoa and helminths. Wastewater treatment plants utilize activated sludge and trickling filter systems, and all of these treatment processes potentially create relatively large amounts of aerosols, which have been shown to include pathogenic microorganisms. Other aspects of the treatment process such as composting and land disposal are also associated with the generation of aerosols containing pathogenic microorganisms.

One of the primary methods for the disposal of biosolids and manure is agricultural land application. The major concern associated with the aerosolization process in relation to waste disposal operations is the exposure of waste disposal workers to pathogenic microorganisms (**occupational risk**). In addition, nearby population centers are also potential



FIGURE 5.12 Land application of liquid biosolids via a spray application, and collection of air samples via biosamplers.



FIGURE 5.13 Land application of liquid biosolids via a sprinkler system.



FIGURE 5.14 Land application of cake biosolids via a slinger in Solano County, California.

exposure risks (**community risk**). The potential for aerosolization of pathogens from land application of biosolids has become a nationally debated issue. A major national study on aerosolization from land application in the United States was conducted by Brooks *et al.* (2005a,b). This study showed that occupational risks of infection from bioaerosols was greater than for offsite communities, where risks were minimal (Brooks *et al.*, 2012) (Case Study 5.4). Baertsch *et al.* (2007) used DNA-based microbial source tracking to measure aerosolization during land application.

5.6.6 Important Airborne Toxins

Microbial toxins can also be airborne. For example, a toxin from *Clostridium botulinum* (botulinum A toxin) is a potential biological warfare agent (Amon *et al.*, 2001). Botulinum toxin is a neurotoxin that is normally associated with ingestion of contaminated food. However, the lethal dose is so small that aerosolization can also be a means of dissemination. The lethal dose for botulinum toxin by inhalation is 0.3 μg , with death occurring 12 hours after exposure. Death is due to asphyxiation caused by the paralysis of respiratory muscles. Another toxin produced by bacteria is staphylococcal enterotoxin. On occasion, this toxin can be fatal with the lethal dose estimated to be 25 μg by inhalation. The symptoms include cramping, vomiting and diarrhea, which occur within 1 hour of exposure by aerosolization.

An important airborne toxin is lipopolysaccharide (LPS) (Hurst *et al.*, 1997). Lipopolysaccharide is derived from the outer membrane of Gram-negative bacteria. It is also referred to as **endotoxin** and is a highly antigenic biological agent that, when associated with airborne particles such as dust, is often associated with acute respiratory symptoms such as chest tightness, coughing, shortness of breath, fever and wheezing. Due to the ubiquity of Gram-negative bacteria, especially in soil, LPS is considered by some to be the most important aerobiological allergen. LPS (Figure 5.15) has three major components: a lipid A moiety, which is a disaccharide of phosphorylated glucosamines with associated fatty acids; a core polysaccharide; and an O-side chain. The lipid A moiety and the core polysaccharide are similar among Gram-negative bacteria, but the O-side chain varies among species and even strains. It is the O-side chain that is responsible for the hyperallergic reaction. There are many sources associated with the production of high levels of LPS, such as cotton mills, haystacks, sewage treatment plants, solid waste handling facilities, swine confinement buildings, poultry houses, and even homes and office buildings. LPS is liberated when Gram-negative bacteria in these environments are lysed but can also be released when they are actively growing.

In soils, bacterial concentrations routinely exceed 10^8 per gram and soil particles containing sorbed microbes can

Case Study 5.4 Occupational and Community Risks of Infection from Bioaerosols Generated During Land Application of Biosolids

In 2002, the National Research Council (NRC) issued a report titled, *Biosolids Applied to Land: Advancing Standards and Practices*. One of the recommendations of this report was the need to document and evaluate the risk of infections from bioaerosols generated during land application. In response to this the University of Arizona conducted a national study to evaluate occupational and community risks from such bioaerosols. Overall, more than 1000 aerosol samples were collected and analyzed for bacterial and viral pathogens (Tanner *et al.*, 2005; Brooks *et al.*, 2005b).

The study was undertaken in two parts. First, the emission rate of pathogens generated during loading of biosolids from trucks into spreaders, and also during land application of biosolids, was evaluated. This was assumed to be direct exposure to biosolid workers on-site, that is, occupational risk, since there was no pathogen transport required to for exposure. For community risk, fate and transport of pathogens was taken into account, since residents live off-site, allowing for natural attenuation of pathogens due to environmental factors such as desiccation and ultraviolet light.

Based on exposure data gathered on-site during land application, occupational risk of infection from Coxsackie virus A21 was determined using a one-hit exponential model: $P_i = 1 - \exp(-rN)$, where:

P_i = the probability of infection per work day,

r = parameter defining the probability of an organism initiating infection = 0.0253 for Coxsackie A21, and

N = number of pathogens inhaled per day

The annual risk of infection can be calculated from the daily risk using

$$P_{\text{year}} = 1 - (1 - P_i)^d$$

where d = number of days exposed per year.

Occupational Risk of Infection

Annual risk of infection for Coxsackie virus A21 during loading operations was 2.1×10^{-2} .

This risk suggests that approximately 1 worker per 50 is likely to be infected with Coxsackie virus A11 working on-site over the course of 1 year.

Community Risk of Infection

Risk was calculated for a distance of 30 m from a land application site assuming 6 days of land application annually, and 8 h exposure duration.

Annual risk of infection for Coxsackie virus A21 during loading was 3.8×10^{-5} .

Annual risk of infection for Coxsackie virus A21 during land application was 2.1×10^{-5} .

These data imply that community risks of infection are minimal. As a comparison, for drinking water a 1:10,000 risk of infection per year is considered acceptable (Haas *et al.*, 2014).

be aerosolized, and hence act as a source of endotoxin. Farming operations such as driving a tractor across a field have been shown to result in endotoxin levels of 469 **endotoxin units (EU)** per cubic meter as measured by the *Limulus* amebocyte assay. These values are comparable to those found during land application of biosolids operations (Table 5.2) (Brooks *et al.*, 2006). Daily exposures of as little as 10 EU/m^{-3} from cotton dust can cause asthma and chronic bronchitis. However, dose response is dependent on the source of the material, the duration of exposure and the frequency of exposures (Brooks *et al.*, 2004). The data in Table 5.2 illustrate that endotoxin aerosolization can occur during both wastewater treatment and land application of biosolids. However, the data also show that endotoxin of soil origin resulting from dust generated during tractor operations results in similar amounts of aerosolized endotoxin (see also Section 26.3.2).

5.7 INTRAMURAL AEROMICROBIOLOGY

The home and workplace are environments in which airborne microorganisms create major public health concerns.

In comparison with the extramural environment, intramural environments have limited circulation of external air and much less UV radiation exposure. Indoor environments also have controlled temperature and relative humidity, which are generally in the ranges that allow extended microbial survival. Thus, these conditions are suitable for the accumulation and survival of microorganisms within many enclosed environments, including office buildings, hospitals, laboratories and even spacecraft. In this section, we will consider these three diverse areas as examples of current topics related to intramural aeromicrobiology. Again, it should be noted that this section does not cover all aspects of intramural aeromicrobiology, but instead attempts to show the wide diversity of the science.

5.7.1 Buildings

Many factors can influence bioaerosols and therefore how “healthy” or how “sick” a building is. These include: the presence and/or efficiency of air filtering devices, the design and operation of the air circulation systems, the health and hygiene of the occupants, the amount of clean outdoor air

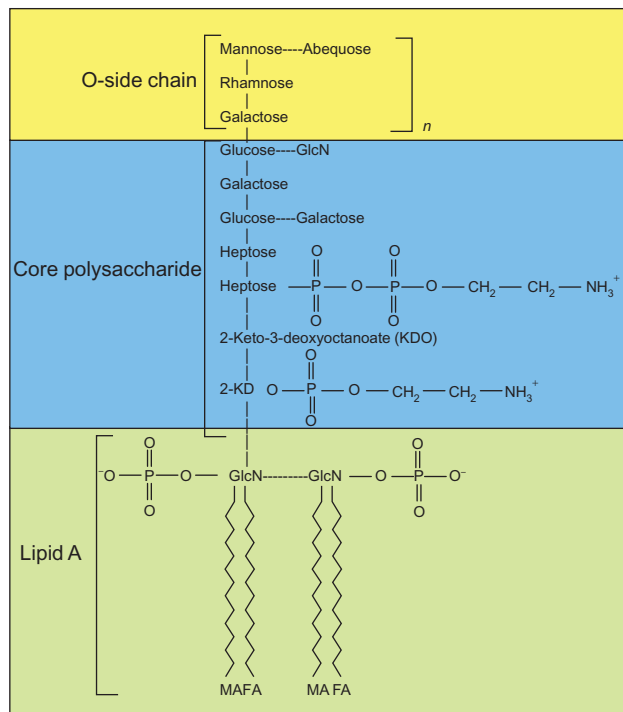


FIGURE 5.15 Schematic structural representation of the lipopolysaccharide from *Salmonella typhimurium*. The number of repeating units (n) in the side chain varies from 10 to 40. The sugars found in the side chain vary among bacterial species, whereas the composition of the core polysaccharide is usually the same. There is a molecule of beta-hydroxymyristic acid (MA), which is a 14-carbon fatty acid attached to each N-acetylglucosamine (GlcN) residue. Other fatty acids (FA) are attached to these residues as well.

circulated through the building, the type of lighting used, the ambient temperature in the building, and the relative humidity (Information Box 5.5).

Some pathogens are uniquely adapted for survival and transmission in the intramural environment. One good example of such an organism is *Legionella pneumophila*, the causative agent of both Legionnaires' disease and Pontiac fever. **Legionnaires' disease** or legionellosis is a pneumonia that causes disease in up to 5% of those exposed. Of those who contract the disease, up to 39% die from the infection. Pontiac fever is associated with flu-like symptoms and affects up to 100% of those exposed, although it is generally not associated with mortality. The causative agent of both diseases is a poorly staining, Gram-negative bacillus called *L. pneumophila*. This organism is named in association with the first highly characterized outbreak of the disease, which occurred in 1976 at an American Legion convention in Philadelphia.

Legionella spp. are ubiquitous in the environment. They are found in association with lakes, ponds, compost and streams, and have even been found in deep terrestrial subsurface environments. In addition to natural reservoirs, there are many human-made systems within which

legionellae can find a niche. These include cooling towers, evaporative condensers, plumbing systems, whirlpools, shower heads and hot-water faucets (Bollin *et al.*, 1985). In the case of the American Legion convention, the reservoir for the organism that caused the outbreak was a poorly maintained cooling tower, which provided optimal conditions for *Legionella* proliferation. Because of the poor design of the air circulation system at the convention, this proliferation led to the subsequent aerosolization and spread of the organisms throughout the building.

What conditions promote the proliferation of *Legionella* spp.? Stagnant water and temperatures in the range of 35–46°C are factors that can lead to the rapid multiplication of background levels of *Legionella* spp. Another interesting aspect of the ecology of *Legionella* is that they can grow intracellularly within cyanobacteria and protozoa. How can growth and spread of *Legionella* spp. be avoided? Several strategies can be used. In the maintenance of hot-water plumbing systems, operating temperatures should be greater than 50°C. All potential places where water can stagnate in water pipes should be avoided. For cooling towers, the recommendations involve the installation of ozonization units, dry convective heat exchange designs and the avoidance of any design that could potentially mix the wet system with the supply air. Biocidal agents such as chlorine or copper can also be effective when used regularly at low levels.

5.7.2 Hospitals and Laboratories

Hospitals and microbiology laboratories are the two indoor environments with perhaps the greatest potential for the aerosolization of pathogenic microorganisms. Hospitals, because they are centers for the treatment of patients with diseases, have a high percentage of individuals, including patients and staff, who are active carriers of infectious, airborne pathogens. Of particular concern are neonatal wards, surgical transplant wards and surgical theaters, all critical areas where the control of nosocomial infection is imperative. Illustrating this point is a study by Portner *et al.* (1965) that evaluated airborne microbial concentrations in surgical theaters, industrial clean rooms, typical industrial manufacturing areas and a horizontal laminar flow clean room designed for the space industry. The surgical theater had by far the highest counts of pathogenic airborne microbial contaminants, followed by the industrial manufacturing area, the industrial clean room and finally the laminar flow room, which had the lowest counts of airborne microbes.

Because microbiology laboratories often handle pathogens, procedures have been developed and refined to protect laboratory workers. However, even under the strictest of conditions, aerosolization events may occur. In 1988, for

TABLE 5.2 Aerosolized Endotoxin Concentrations Detected Downwind of Biosolids Operations, a Wastewater Treatment Plant Aeration Basin, and a Tractor Operation

Sample Type	# of Samples Collected	Distance From Site (m)	Aerosolized Endotoxin (EU m ^a)			
			Avg	Median	Minimum	Maximum
Controls						
Background	12	NA	2.6	2.49	2.33	3.84
Biosolids operations						
Loading	39	2–50	343.7	91.5	5.6	1807.6
Slinging	24	10–200	33.5	6.3	4.9	14.29
Biosolids pile	6	2	103	85.4	48.9	207.1
Total operation	33	10–200	133.9	55.6	5.6	623.6
Wastewater treatment plant						
Aeration basin	6	2	627.3	639	294.4	891.1
Nonbiosolids field						
Tractor	6	2	469.8	490.9	284.4	659.1

^aEU m = Endotoxin units per m³.

Information Box 5.5 Molds in Buildings

In moist environments within buildings mold and bacteria can proliferate rapidly within days and become established as colonies on solid surfaces, subsequently releasing toxins and/or allergens into the air. The most common indoor molds are *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria*. Molds can cause both allergic reactions and chemical toxicogenic responses from direct exposure to spores, cell wall components and mycotoxins. Molds and endotoxins can also be found within tobacco smoke (Pauly and Paszkiewicz, 2011).

instance, eight employees in a clinical microbiological laboratory developed acute brucellosis (Staszkievicz *et al.*, 1991). A survey of the laboratory and the personnel showed that a cryogenically stored clinical isolate of *Brucella* sp. had been thawed and subcultured without the use of a biosafety cabinet. Other than this, the laboratory worker claimed to have used good technique. This example demonstrates the ease with which a bioaerosol can spread within areas where pathogens are handled for research and clinical purposes, and indicates the importance of bioaerosol control methodologies. The following sections describe how bioaerosol formation and spread is actually controlled in the laboratory.

5.8 BIOAEROSOL CONTROL

The control of airborne microorganisms can be handled in a variety of ways. Launching, transport and deposition are all points at which the airborne spread of pathogens can be controlled. The mechanisms used to control bioaerosols include ventilation, filtration, UV treatment, biocidal agents and physical isolation. These are discussed in the following sections.

5.8.1 Ventilation

Ventilation is the method most commonly used to prevent the accumulation of airborne particles. This mechanism involves creating a flow of air through areas where airborne contamination occurs. This can mean simply opening a window and allowing outside air to circulate inward, or use of air-conditioning and heating units that pump outside air into a room. Ventilation is considered one of the least effective methods for controlling airborne pathogens, but is still very important. Ventilation relies on mixing of intramural air with extramural air to reduce the concentration of airborne particles. However, in some cases the addition of extramural air can actually increase airborne particles. For example, one study showed that hospitals in Delhi, India, that relied on ventilation alone contained airborne fungal loads that were higher inside the hospital than those outside. This indicates that

ventilation alone may not be sufficient to significantly reduce circulating bioaerosols. Thus, for most public buildings, especially hospitals, other forms of bioaerosol control need to be implemented.

5.8.2 Filtration

Unidirectional airflow filtration is a relatively simple and yet effective method for control of airborne contamination. Some filters, for example, **high-efficiency particulate air (HEPA)** filters are reported to remove virtually all infectious particles. These types of filters are commonly used in biological safety hoods. However, because of their high cost, they are not often used in building filtration systems. Instead, other filtration systems that rely on baghouse filtration (a baghouse works on the same principle as a vacuum cleaner bag) are used. Typically, air filters (baghouse, HEPA, etc.) are rated using the **dust-spot percentage**, which is an index of the size of the particles efficiently removed by the filter, with higher percentages representing greater filtration efficiencies. The typical rating for the filters used in most buildings is 30 to 50%. Studies have shown that a 97% dust-spot rating is required to effectively remove virus particles from the air. Other factors that influence filtration efficiency are related to the type of circulation system and how well it mobilizes air within the building, the type of baghouse system used and the filter material chosen (nylon wound, spun fiberglass, etc.) as well as the filter's nominal porosity (1 μm –5 μm). All these factors combine to influence the efficiency of the air filtration and removal of particles including bioaerosols. In spite of the high level of efficiency that can be achieved with filtration, many systems still cannot stop the circulation of airborne microorganisms, especially viruses, and added treatments may be required to ensure that air is safe to breathe.

5.8.3 Biocidal Control

Biocidal control represents an added treatment that can be used to eradicate all airborne microorganisms, ensuring they are no longer viable and capable of causing infection. Many eradication methods are available, for example, superheating, superdehydration, ozonation and UV irradiation. The most commonly used of these methods is **UVGI** or **ultraviolet germicidal radiation**. UVGI has been shown to be able to control many types of pathogens, although some microbes show various levels of resistance. The control of contagion using UV irradiation was tested in a tuberculosis (TB) ward of a hospital. Contaminated air was removed from the TB ward through a split ventilation duct and channeled into two animal holding pens that contained guinea pigs. One pen received air that had been treated with UV irradiation; the other received untreated

air. The guinea pigs in the untreated-air compartment developed TB, but none of the animals in the UV-treated compartment became infected. The **American Hospital Association (1974)** indicated that, properly utilized, UV radiation can kill nearly all infectious agents, although the effect is highly dependent on the UV intensity and exposure time. Thus, major factors that affect survival (temperature, relative humidity, UV radiation, ozone) in the extramural environment can be used to control the spread of contagion in the intramural environment.

5.8.4 Isolation

Isolation is the enclosure of an environment through the use of positive or negative pressurized air gradients and airtight seals. Negative pressure exists when cumulative airflow travels into the isolated region. Examples of this are the isolation chambers of the tuberculosis wards in hospitals used to protect others outside the TB wards from the infectious agent generated within these negative-pressure areas. This type of system is designed to protect other people in the hospital from the pathogens (*Mycobacterium tuberculosis*) present inside the isolation area. Air from these rooms is exhausted into the atmosphere after passing through a HEPA filter and biocidal control chamber.

Positive-pressure isolation chambers work on the opposite principle by forcing air out of the room, thus protecting the occupants of the room from outside contamination. One can reason that the TB ward is a negative-pressure isolation room, while the rest of the hospital, or at least the nearby anterooms, are under positive-pressure isolation. Other examples are the hospitals critical care wards for immunosuppressed patients such as organ transplant, human immunodeficiency virus (HIV)-infected and chemotherapy patients. These areas are protected from exposure to any type of pathogen or opportunistic pathogens. The air circulating into these critical care wards is filtered using HEPA filters, generating purified air essentially free of infectious agents.

5.9 BIOSAFETY IN THE LABORATORY

Many microbiological laboratories work specifically with pathogenic microorganisms, some of which are highly dangerous, especially in association with the aeromicrobiology pathway. Also, many types of equipment, such as centrifuges and vortexes (instruments for mixing of microbial suspensions) that are commonly used in microbiological laboratories can promote the aerosolization of microorganisms. Thus, laboratories and specialized equipment used in these laboratories (e.g., biosafety cabinets) are designed to control the spread of airborne microorganisms. There are essentially four levels of control designed



FIGURE 5.16 Biosafety cabinet Class II. From Telstar Life Science Solutions, photo courtesy J. Bliznick.

into laboratories, depending on the type of research being conducted. These levels of control are termed **biosafety levels 1–4**, with 1 being the lowest level of control and 4 the highest level of control. Within these laboratories, biosafety cabinets are essentially isolation chambers that provide safe environments for the manipulation of pathogenic microorganisms. In this section we will discuss biosafety cabinets and biosafety suits, followed by a short discussion of the actual biosafety levels imposed to achieve specific levels of control.

5.9.1 Biological Safety Cabinets

Biological safety cabinets (BSC) are among the most effective and commonly used biological containment devices in laboratories that work with infectious agents (US Department of Health and Human Services: CDC-NIH, 1993). There are two basic types of biosafety cabinets currently available (Class II and Class III), each of which has specific characteristics and applications that dictate the type of microorganism it is equipped to contain. Properly maintained biosafety cabinets provide safe environments for working with microorganisms. Class II biosafety cabinets are characterized by having considerable

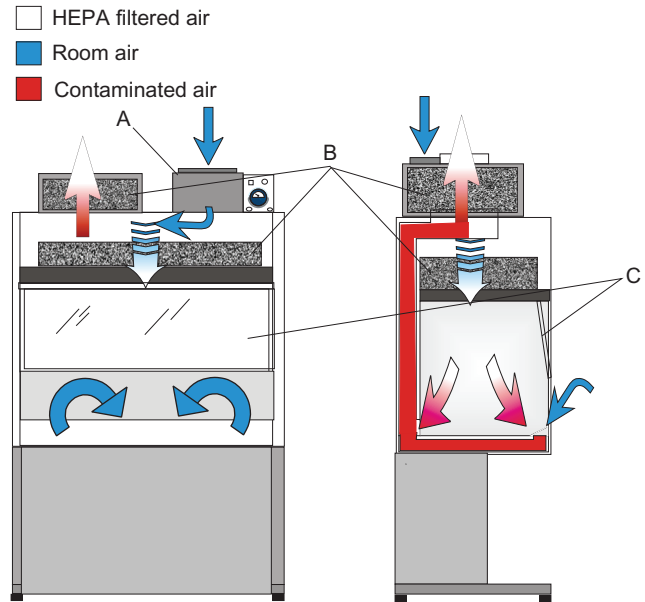


FIGURE 5.17 Schematic representation of the airflow paths within a typical Class II biosafety cabinet. Room air is drawn in from the top and from the front as indicated by the blue arrows. The nonpurified atmospheric air (blue) entering from the top of the cabinet is drawn in by an air pump (A) and then is purified by a HEPA filter (B) as it enters the workspace. Nonpurified air from the room (blue) entering from the front of the cabinet passes into the front grill and passes up through the top of the cabinet where it also passes through a HEPA filter before entering the workspace (C). This prevents the experiments in the workspace from being contaminated by airborne room contaminants. As the purified room air is exposed to the work environment and becomes contaminated (red) it is passed through yet another HEPA filter before being exhausted to the atmosphere. This pattern of airflow and purification ensures that the worker and the atmosphere are not exposed to the biohazards contained within the biosafety cabinet.

negative-pressure airflow that provides protection from infectious bioaerosols generated within the cabinet (Figures 5.16 and 5.17), and Class III biosafety cabinets are characterized by total containment (Figure 5.18). Class I cabinets are also in existence, but they are no longer produced and are being replaced by Class II cabinets for all applications.

Class II biosafety cabinets, of which there are several types, are suitable for most work with moderate-risk pathogens (Table 5.3). Class II biosafety cabinets operate by drawing airflow past the worker and down through the front grill. This air is then passed upward through conduits and downward to the work area after passing through a HEPA filter. Room air is also drawn into the cabinet through the top of the unit, where it joins the circulating air and passes through the HEPA filter and into the work area. About 70% of the air circulating in the work area is then removed by passing it through the rear grill of the cabinet, where it is discharged into the exhaust system. The remaining 30% is passed through the front grill, essentially recirculating in the cabinet (Figure 5.17).

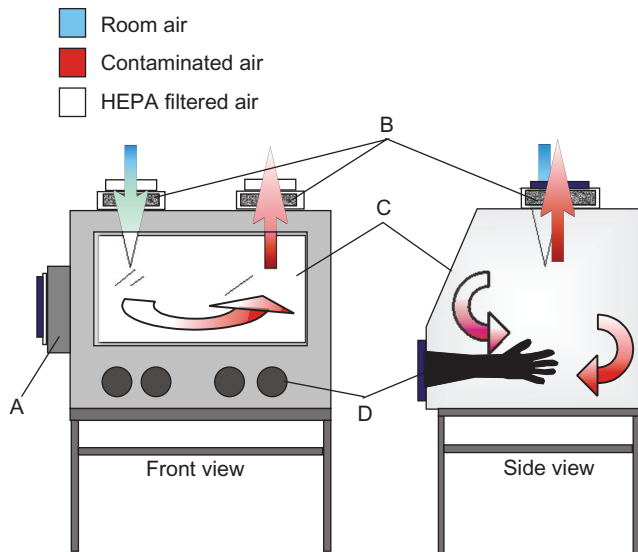


FIGURE 5.18 Schematic representation of a Class III biological safety cabinet. This cabinet is completely sealed from the environment. Any materials entering or leaving the cabinet are passed through a chemical dunk tank or autoclave (A) in order to sterilize them and prevent environmental contamination. Air entering or leaving these cabinets is passed through HEPA filters (B). Access to the workspace is by means of rubber gloves (D) and the workspace is visualized through a sealed window (C). These biosafety cabinets are utilized when working with highly pathogenic microorganisms to protect workers and the environment. Class III cabinets can be used to work with all biohazardous agents except those specifically designated for biosafety level 4 containment.

Laboratory personnel require special training in order to properly use Class II cabinets and to ensure proper containment of bioaerosols. One of the major hazards associated with Class II cabinets is the potential for the disruption of the negative airflow. Many mechanical actions can disrupt the protective airflow, such as repeated insertion and withdrawal of arms, opening or closing of doors in the laboratory, or even someone walking past the cabinet while it is in use. Any of these actions can potentially allow the escape of bioaerosols from the cabinet.

The Class III biosafety cabinet (Figure 5.18) is a completely enclosed environment that offers the highest degree of personnel and environmental protection from bioaerosols. Class III cabinets are used for high-risk pathogens (Table 5.3). All operations in the work area of the cabinet are performed through attached rubber gloves. Class III cabinets use complete isolation to protect workers. All air entering the cabinet is filtered using a HEPA filter, and the air leaving the cabinet is filtered by two HEPA filters in series. The exhaust may also include biocidal treatment such as incineration following the HEPA filtration to further ensure complete biological inactivation. In addition to these safeguards, Class III cabinets are connected with airtight seals to all other laboratory equipment (such as incubators, refrigerators and centrifuges)

TABLE 5.3 Examples of Classification of Biological Agents According to Risk

Class	Type of Agent	Agent	
Class I	Bacterial	All those which have been assessed for risk and do not belong in higher classes	
	Fungal		
	Protozoal		
Class I	Viral	Influenza virus reference strains Newcastle virus	
	Class II	Bacterial	<i>Campylobacter</i> spp.
			<i>Clostridium</i> spp.
<i>E. coli</i> spp.			
<i>Klebsiella</i> spp.			
<i>Mycobacteria</i> spp.			
Fungal		<i>Shigella</i> spp.	
		<i>Vibrio</i> spp.	
		<i>Salmonella</i> spp.	
		<i>Penicillium</i> spp.	
		<i>Cryptococcus</i> spp.	
Class II	Protozoal	<i>Microsporium</i> spp.	
		<i>Cryptosporidium</i> spp.	
		<i>Giardia</i> spp.	
		<i>Encephalitozoon</i> spp.	
		<i>Enterocytozoon</i> spp.	
		<i>Babesia</i> spp.	
		<i>Echinococcus</i> spp.	
		<i>Entamoeba</i> spp.	
		<i>Fasciola</i> spp.	
		<i>Leishmania</i> spp.	
Class II	Viral	<i>Plasmodium</i> spp.	
		<i>Schistosoma</i> spp.	
		<i>Trypanosoma</i> spp.	
		Adenoviruses	
		Corona viruses	
		Cowpox virus	
		Coxsackie A and B viruses	
		Echoviruses	
		Hepatitis viruses A, B, C, D and E	
		Epstein–Barr virus	
Influenza viruses			
Vaccinia virus			
Rhinoviruses			
Class III	Bacterial	<i>Brucella</i> spp.	
		<i>Mycobacterium bovis</i>	
		<i>Mycobacterium tuberculosis</i>	
		<i>Rickettsia</i> spp.	
		<i>Yersinia pestis</i>	
Class III	Fungal	<i>Coccidioides immitis</i>	
		<i>Histoplasma capsulatum</i>	
Class III	Protozoal	None	
		Class III	Viral
Monkey pox virus			
Yellow fever virus			
Class III	Bacterial	None	
Class IV	Fungal	None	
	Protozoal	None	
	Viral	Hemorrhagic fever agents Ebola fever virus Marburg virus	

Adapted from University of Pennsylvania Biological Safety Manual.

that is needed for working with the pathogens while using the cabinet. The Class III cabinet must also be connected to autoclaves and chemical dunk tanks used to sterilize or disinfect all materials entering or exiting the cabinet.

Another type of containment that typically provides the same level of protection as a Class III biosafety hood is the biological safety suit (Figure 5.19). The biological suit, unlike biosafety cabinets, operates under positive pressure created by an external air supply, thus protecting the wearer. Like the biosafety cabinets, the biosafety suit isolates the laboratory worker wearing it from bioaerosols. Biosafety suits are typically used in airtight complete biocontainment areas, and are decontaminated by means of chemical showers upon exiting the biohazard area. Some biosafety suits are portable and can be used in environments outside the laboratory such as “hot zones” (epidemiological areas that are currently under the influence of epidemic cases of diseases caused by high-risk pathogens) so that microbiologists and physicians working in these areas can minimize their risk of exposure to pathogens. As in biosafety cabinets, the air entering and leaving the biosafety suit passes through two HEPA filters.



FIGURE 5.19 Biosafety suit. Source: Centers for Disease Control.

5.9.2 Biosafety Laboratories

Biosafety laboratories are carefully designed environments where infectious or potentially infectious agents are handled and/or contained for research or educational purposes. The purpose of a biosafety laboratory is to prevent the exposure of workers and the surrounding environment to biohazards. There are four levels of biohazard control, which are designated as biosafety levels 1 through 4.

Biosafety level 1, as defined by the Centers for Disease Control (US Department of Health and Human Services: CDC-NIH, 1993), indicates laboratories where well-characterized agents that are not associated with disease in healthy adult humans are handled. In general, no safety equipment is used other than sinks for hand washing, and only general restrictions are placed on public access to these laboratories. Work with the microorganisms can be done on bench tops using standard microbiological techniques. A good example of a biosafety 1 laboratory is a teaching laboratory used for undergraduate microbiology classes.

Biosafety 2 indicates an area where work is performed using agents that are of moderate hazard to humans and the environment. These laboratories differ from biosafety 1 laboratories in that the personnel have specialized training in the handling of pathogens, and access to the work areas is limited. Many procedures that may cause aerosolization of pathogenic microorganisms are conducted in biological safety level II cabinets or other physical containment equipment, to protect the laboratory workers.

Biosafety 3 indicates laboratories where agents that can cause serious or fatal disease as a result of AMB exposure are handled. As with biosafety 2, all personnel are specifically trained to handle pathogenic microorganisms. All procedures involving these infectious agents are conducted in biological safety level II cabinets or other physical containment devices. These facilities also have permanent locks to control access, negative airflow and filtered ventilation in order to protect the public and the surrounding environments. With certain pathogens used in biosafety 3 laboratories, Class III safety hoods may also be used, and clothes must be changed before leaving the premises.

Biosafety 4 is the highest level of control and is indicated for organisms that have high potential for life-threatening disease in association with aerosolization. To work in these facilities, personnel must have specialized training beyond that required for biosafety levels 2 and 3. Biosafety level 4 laboratories are 100% isolated from other areas of a building and may even be separated from other buildings altogether. Work in these areas is confined exclusively to Class III biological safety cabinets unless one-piece positive-pressure ventilation suits are worn, in which case Class II biosafety cabinets may be used. These laboratories are also specially designed to prevent microorganisms from being disseminated into the

environment. The laboratories have complete containment, and require personnel to wear specialized clothing, which is removed and sterilized before leaving the containment areas. Personnel are also required to shower before leaving the facility. In general, all air into and out of these laboratories is sterilized by filtration and germicidal treatment. These facilities represent the ultimate in our ability to control airborne pathogens.

5.9.3 Biological Agent Classification

For any microorganism, defined degrees of risk associated with its use indicate the type of containment needed to ensure the safety of laboratory workers, the public and the environment. There are five classes of organisms. Class I microorganisms are those that pose little or no hazard under ordinary conditions of handling and can be safely handled without special apparatus or equipment. In contrast, Class II are agents of low potential hazard that may cause disease if accidentally inoculated or injected but that can be contained by ordinary laboratory techniques. Class III agents are those that require special containment; they are associated with aerosol disease transmission and special permits are required to import them from outside the country. Class IV agents are those that require extreme containment and are extremely hazardous to laboratory personnel or may cause serious epidemic disease. Finally, Class V agents are restricted foreign pathogens whose importation, possession or use is prohibited by law.

QUESTIONS AND PROBLEMS

- List the major factors important in the survival of microorganisms in aerosols.
- What is the major component of biosafety cabinets that remove microorganisms?
- What is the role of microorganisms in cloud formation?
- Give an example of a continuous linear source and an example of an instantaneous area source of bioaerosols.
- Considering a windspeed of 1.5 m/s, an object that is 12 cm tall and normal air viscosity, determine whether conditions around the object would be considered turbulent.
- Consider an airborne virus and an airborne protozoan with a radius of 30 nm and 1 μm and particle densities of 2.0 and 1.1 g/cm^3 , respectively. Under normal gravitational acceleration calculate the terminal velocity for each.
- Calculate the annual community risk of infection given the following data:
 - aerosolized virus concentration = 7.16/ m^3 of air
 - duration of exposure = 8 hours
 - infectivity constant $r = 0.0253$
 - breathing rate = 0.83 m^3 per hour

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