

**LEGIONELLA in the  
ENVIRONMENT  
(the cause of Legionnaires'  
disease)**

**A Review of Current Knowledge**

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# LEGIONELLA IN THE ENVIRONMENT (the cause of Legionnaires' disease)

## *A Review of Current Knowledge*

### **Introduction**

Legionnaires' disease was first recognised as a result of an outbreak of acute pneumonia that occurred at the convention of the American Legion in Philadelphia, USA during 1976. The causative organism was later shown to have been a bacterium and it was named, in commemoration of this, the first recognised outbreak, *Legionella pneumophila*. Twenty five years later, legionnaires' disease can still be a significant problem, as is evident from the largest outbreak ever, which occurred in Murcia, Spain in July 2001 with over 370 confirmed cases. Since 1976 over 40 species of *Legionella* have been described and at least eighteen of these have been shown to be associated with respiratory tract infections in humans. The term legionellosis is applied to the disease caused by these organisms. The severity of the disease ranges from typical Legionnaires' disease, an acute form of fulminating pneumonia with low attack rate and relatively high fatality rate, (a low attack rate means that a small proportion, less than 5% of those exposed to the bacteria develop the disease), to Pontiac fever, a mild infection with a high attack rate (a high proportion, usually over 80%, of those exposed to the bacteria develop the disease).

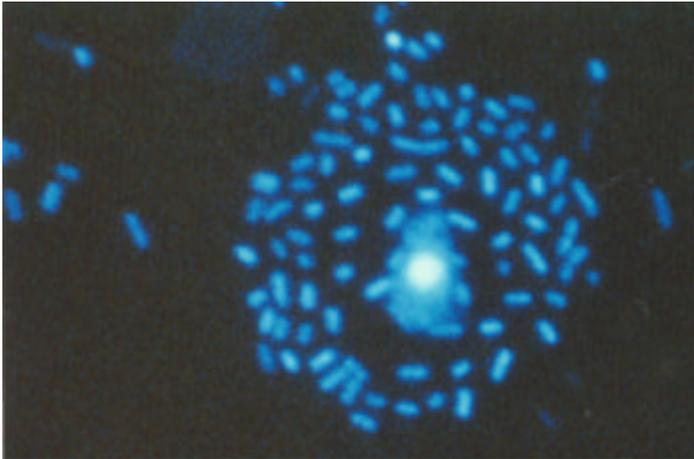
Cases of legionellosis have now been recognised in many countries throughout the world. According to data gathered by the European Working Group on Legionella Infections in Europe, the overall reported attack rate in 1998 was 4.3

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per million population and this rose to 5.4 per million in 1999 (Anon 2000). Between countries the rate ranged from less than 1 to about 21 per million. Denmark has reported the highest attack rate for some years, (21 in 1998 and 17 in 1999), but this probably reflects the efficiency of recognition and reporting, rather than a true difference in incidence. In England and Wales there are about 200 to 250 cases reported each year, with an overall death rate of about 13%. About one half of the cases in the UK are associated with travel, usually abroad. In the U.S. Legionnaires' disease is considered to be a fairly common, but serious form of pneumonia.



*Legionella pneumophila* courtesy of Dr Susanne Surman

The *Legionella* bacterium is one of the top three bacterial agents in the U.S. that cause sporadic community-acquired pneumonia. Because of the difficulty in clinically distinguishing this disease from other forms of pneumonia, many cases go unreported. Although approximately 1,000 cases are reported annually to the

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Center for Disease Control and Prevention, Atlanta, USA, it has been estimated that over 25,000 cases of the illness actually occur. The attack rate for *L. pneumophila* ranges from 0.1 to 5%. The overall case fatality rate ranges from 10 to 20% but can be as high as 30 - 40% in hospital acquired cases, where the population exposed is, of course, particularly susceptible.

Despite extensive epidemiological studies, the source and mode of infection of the outbreak of Legionnaires' disease in Philadelphia was never clearly established, although an association was found between infection and time spent in the lobby of the hotel where the convention was held. This suggested that airborne spread could be a possibility. There is, however, no evidence of transmission from person to person.

When it became possible to detect *L. pneumophila*, and later other *Legionella* species, scientists were then able to study the occurrence and ecology of legionellae. Subsequent investigations have shown that *Legionella* species occur naturally in fresh water, sea water and moist natural environments throughout the world, although they are usually a minor component (less than 1%) of the bacterial population. The prime factor affecting their incidence is water temperature; the organisms having a predilection for warm water that is reflected by their incidence in man-made water systems. The majority of outbreaks have been associated with cooling towers, evaporative condensers, hot and cold water systems, and spa pools (also known as whirlpool spas). Other sources of outbreaks have included cutting oils of machine tools, clinical humidifiers in respiratory equipment, humidifiers in food display cabinets, natural spa baths, fountains and potting composts.

Britain experienced some of the largest outbreaks recorded in the 1980s. As a consequence, the UK was one of the first countries to have legislation governing the control of *Legionella* in water systems. More recently, other countries throughout the world have introduced, or are introducing, standards and guidelines as they have encountered problems in their own countries.

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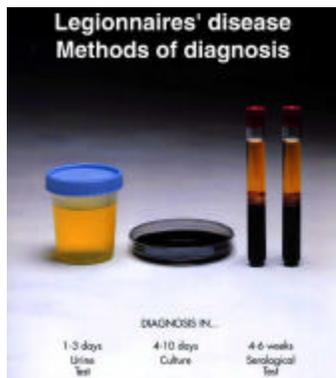
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### Which types of *Legionella* species are most commonly associated with disease

Because at least 18 species of *Legionella* have been observed to be associated with disease, all species are considered to be potentially pathogenic to humans. This is especially true for those whose immune system is severely deficient through disease or, as a result of therapy, e.g. transplant patients. The vast majority of cases, however, particularly those acquired in the community as opposed to hospital, are caused by *L. pneumophila*. This species can be subdivided into at least 16 serogroups, of which serogroup 1 is the most common, both in the environment and in clinical cases.

The serogroup 1 strains of *L. pneumophila* can be further subtyped. Strains of the subtype sometimes called “Pontiac” are more commonly isolated from patients with Legionnaires' disease than the other subtypes of *L. pneumophila* serogroup 1, but are less common in the environment. Thus it appears that they may be better able to cause infections. Why the “Pontiac” strains are the most common cause of community-acquired outbreaks is not certain, but they do appear to survive better in air than the other types (see below).



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### Where do you find *Legionella* species?

#### (a) Naturally

Subsequent to the outbreak of Legionnaires disease, *Legionella* species were discovered and isolated in lakes and rivers in the USA (Fliermans *et al* 1981). They were also found in naturally warm water such as hot springs in North America (Tison *et al* 1983, Campbell *et al* 1984) and in Europe (Bornstein *et al* 1989). It was noted that significantly more were isolated from waters whose temperatures lay between 36 - 70°C, though there were no correlations between the incidence of legionellae and conductivity, pH, dissolved oxygen, chlorophyll 'A' or turbidity. In Europe, they have also been isolated from both surface and groundwaters. Legionellae have also been found in damp or wet soil and in potting composts.

#### (b) In the man-made environment

In a survey carried out in the UK in the early 1980s, legionellae were found in about 60% of cold and hot water systems in large buildings, including hotels, hospitals and business premises. About 55% of hot water systems, 13% of cold water systems and 45% of cooling towers examined contained the organisms. Studies in other countries have shown legionellae to be equally widespread in buildings in other countries in Europe, North America, Singapore and Japan. More recently, legionellae have been shown to be present in private dwellings throughout Europe and North America, their incidence varying according to the type of property, the highest being in multi-occupancy buildings.

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### Factors affecting the growth and survival of *Legionella* species

#### Temperature

Temperature is the single most important factor influencing the incidence of legionellae in both natural and man-made water systems. They have been reported to occur in natural waters at temperatures between 5 - 63°C. Significantly more are isolated from warm waters at temperatures of 30°C and above. It has also been shown that temperature has an effect on the pathogenicity of the organism, its virulence being significantly less when grown at 24°C than at 37°C (Mauchline *et al* 1994).

#### Other physico-chemical parameters

In model systems, under natural conditions and along with other organisms *L. pneumophila* grows best at a pH of 5.5 - 6.2 with an oxygen concentration of 6.0 - 6.2 mg per litre. It does not grow, however, at concentrations of oxygen less than 2.2 mg per litre. Legionellae have been reported to survive at temperatures between 4 - 20°C in water with salt concentrations up to 3% NaCl. Between temperatures of 30 - 37°C, a salt concentration over 1.5% NaCl reduced numbers of legionellae significantly. Conversely, small concentrations of salt (0.1 - 0.5%) enhanced the survival of the organisms (Heller *et al* 1998). In recent years several reports have been received of legionellae being detected in cooling systems using seawater.

#### Nutrients and growth in the aquatic environment

Legionellae are quite difficult to grow on artificial media, having relatively complex nutritional requirements. For example, the media must contain iron salts and the amino acid cysteine. Paradoxically, they occur in the aquatic environment where nutrient levels are low. In fact, although they can survive for prolonged periods of time in water, they are unable to grow in it without the support of other organisms (Lee and West 1991). The relationship with other

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organisms is dealt with in more detail below, but clearly they are able to provide legionellae with the organic compounds, such as cysteine, essential for their growth. There is some evidence of legionellae having a higher incidence in water systems with iron pipework. This suggests that the level of iron is important in nature, as well as in artificial media. As legionellae require other organisms for growth, it will be apparent that the conditions that support the growth of micro-organisms in water in general, will also support the growth of legionellae. Thus, growth is affected by the usual factors that govern the productivity of aquatic systems, such as available carbon, nitrogen, phosphorous etc.

### Association with amoebae and other protozoa

Protozoa are ubiquitous in the same natural habitats as legionellae and have a basic role in terrestrial and aquatic environments as predators of bacteria. In 1980 it was observed that legionellae can grow in vegetative amoebal cells and become incorporated in their cysts (*a cyst is a survival form of the organism which is not actually growing and may be relatively resistant to heat, drying and chemical disinfection*). It is now widely accepted that legionellae can infect and grow inside various protozoa including species of *Acanthamoeba* (see photograph Page 2), *Hartmanella*, *Valkampfia*, *Naegleria*, *Echinamoeba*, *Saccamoeba*, *Tetrahymena* and *Cyclidium*. Vesicles within amoebae may contain anything from tens to hundreds or even thousands of cells of *Legionella*. At low temperatures (20°C), limited growth of legionellae occurs within amoebae which may persist but, at 35°C, legionellae multiply rapidly intracellularly.

Protozoa containing legionellae have been detected and isolated directly from river water, dried potting compost and hospital hot water tanks. The growth of legionellae inside protozoa, particularly amoebae, is similar to their method of growth within human cells. The relationship between protozoa and legionellae, particularly *L. pneumophila*, has been the subject of intense study in the past two years that has been reviewed by Harb *et al* (2000).

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The association with protozoa is not only important for the virulence of legionellae, but is of paramount importance to their survival and growth in the environment and, therefore, to our understanding of the control of legionellae. Protozoa provide a sanctuary for legionellae against adverse extra-cellular or environmental conditions such as high temperatures, drying, chlorine and other biocides (Rowbotham 1986, Kilvington and Price 1990, Barker *et al* 1992, Abu Kwaik *et al* 1997).

With 1 mg per litre of chlorine, various bacteria, when contained within *Tetrahymena* and *Acanthamoeba*, required 60 to 200 times more contact time to kill 99% of the bacterial cells than if they were freely suspended in water. *L. pneumophila* has been recovered from *Acanthamoeba* cysts after exposure to 50 mg per litre of free chlorine for eighteen hours (Kilvington and Price 1990). Chlorine at 50 mg/litre for 1 to 4 hours is commonly used to disinfect water systems both for routine purposes and during outbreak investigation. This observation explains how a proportion, albeit small, of a legionellae population may survive disinfection of a water system to re-colonise it subsequently, if conditions permit.

Protozoal cysts also offer a mechanism by which legionellae can be spread via the airborne route over longer distances than the bacteria could survive in aerosols by themselves. Protozoa can enable them to survive drinking water treatment processes, including disinfection and be carried by the mains water distribution into buildings or to be carried into the system in dust through open tanks or during maintenance. Thus, protozoa play an important role in the spread of legionellae in aquatic systems.

### Legionellae and biofilms

It is well established that, rather than grow in the water phase, most aquatic bacteria prefer to colonise surfaces. Bacteria rapidly colonise new surfaces

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where they form a largely polysaccharide matrix around themselves that feels slimy to the touch. This slime layer is called a biofilm. Biofilms may be anything from a few micrometres to several millimetres in depth. A biofilm may be composed of a single species but, more usually, in both man-made and natural water systems is a complex consortium of different species of bacteria growing, together with other micro-organisms. These may include protozoa that graze on the bacteria, fungi or even algae. It is now widely accepted that legionellae, like other aquatic bacteria, grow in biofilms in water systems. All materials ultimately become colonised to some degree, but the nature of the material influences the degree of biofilm formation. In model systems the following materials have been shown to support the growth of biofilms containing *L. pneumophila* (in the decreasing order of the amount of growth supported): natural rubber, synthetic elastomers (washer materials); PVC; polyethylene; polybutylene; glass and copper (West *et al* 1989, 1990, Rogers *et al* 1993).

Biofilms provide a protective environment for the growth of organisms. Nutrients can be trapped in the biofilm and different adjacent organisms may provide nutrients for each other so enabling them to grow, when they might not have been able to do so by themselves. Bacteria growing within biofilms are more resistant to adverse environmental influences, particularly biocides, than they would be if freely suspended in the water. In 1988, Le Chevallier *et al* found that the concentration x time product (CT) required to kill 99% of the heterotrophic bacteria in a biofilm with HOCl was 150 - 3,000 times greater than for the same organisms freely suspended in the water. For monochloramine, however, the difference was only 2 - 100 times.

### **Protozoa versus biofilms as the natural environment for the growth of *Legionella* species**

The recognition that legionellae can grow inside amoebae and other protozoa and the similarity between this and their ability to grow in human cells has led some

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authors to believe that this is the only mechanism of survival in nature. Microcolonies of *Legionella* can, however, be observed in biofilms. Thus, although protozoa clearly have an important role in the ecology of *Legionellae*, the possibility of *Legionella* species growing in the natural environment outside protozoal hosts should not be overlooked.

### Relationship with other bacteria

Several species of bacteria have been shown to support the growth of legionellae on artificial media on which they cannot grow by themselves. Conversely, many species of other bacteria inhibit the growth of legionellae on artificial media, a factor that is important when trying to isolate *Legionella* species.

### Survival in air

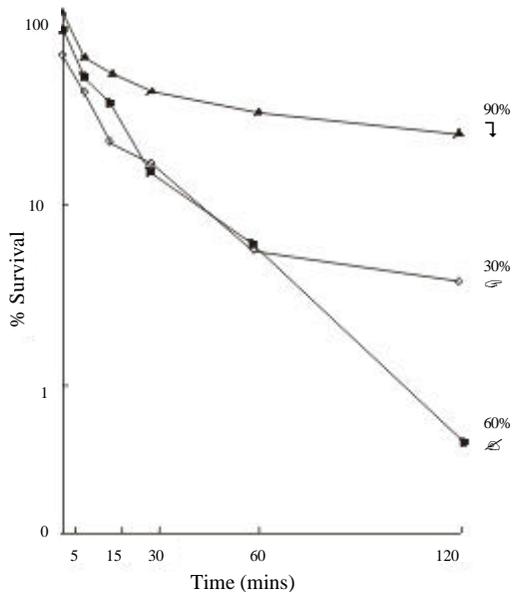
All the available evidence suggests that legionellosis is primarily contracted by inhalation. The survival of *L. pneumophila* in air is thus of great importance. It generally increases with relative humidity, (RH), as may be seen in Fig.1, which shows survival is best at 90% RH. The sub-type, Pontiac, of *L. pneumophila* serogroup 1 that most commonly causes disease, survives better than the other subtypes. About 30% of the population remains viable after 30 minutes at 20°C and 60% RH, which are conditions that are typical of those occurring inside buildings in the UK. Because of the technical difficulties, there has been little work on the aerosol survival of legionellae grown under natural conditions, but it has been shown that some components of the medium in which cyanobacteria have grown, considerably increased aerosol stability (Berendt 1981). In outdoor air, survival would be reduced by UV radiation and the open-air factor (short lived highly toxic compounds generated by the interaction of ozone and the products of internal combustion engines), but the reduction may be ameliorated by organic or inorganic components of the original suspending fluid.

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Containment within amoebal cysts or vesicles may further enhance survival and permit dispersal over a longer distance but, fortunately, many cysts are too large to be inhaled deeply enough to initiate infection.



**Figure 1**

Effect of humidity on aerosol survival of *Legionella pneumophila* serogroup 1 Strain 74/81 at 20°C: ○, 30% RH; □, 60% RH; ▽, 90% RH

**Note:** This figure is taken from Dennis and Lee, 1988, and illustrates the aerosol survival of *L. pneumophila* under different humidities. *Reproduced courtesy of Blackwell Science*

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### Growth in man-made water systems

For *Legionella* species to grow, the conditions must be right for the supporting organisms to grow also. The nutrients for their growth in man-made water systems may be derived from the incoming water, the materials of construction of the system and dirt entering through open tanks or during maintenance. As in natural systems, growth will occur in those parts of the system where the temperature is optimal (30 - 45°C). The presence of sediment, sludge and scale also encourages colonisation and makes control more difficult. Scale too, can increase the surface area available for colonisation and provide crevices and pits that protect organisms and can reduce the penetration of heat or biocides being used to control growth. Scale will reduce the flow in pipes and this may further encourage growth and reduce the effectiveness of control measures as will other causes of stagnation and slow water flow.

### Control in man-made water systems

As has been said, within protozoal cysts, legionellae may survive the processes used in drinking water purification and thereby enter the mains water distribution systems. They can then be transported to water systems in buildings where they may find conditions suitable for their growth. Similarly, containment within protozoal cysts can enable them to survive in dried up soil and sediments and be dispersed in dust blowing around in the wind. Thus legionellae can enter our water systems in the incoming water itself, or as a result of soil or dust entering through open tanks or during repairs etc. The result of this is that there is a high probability that, at some time, legionellae will enter man-made water systems. It is sometimes possible to eliminate the risk completely by using an alternative to water, for example using an air-cooled condenser, rather than a wet cooling tower or evaporative condenser but, for many purposes, it is impossible to eliminate the use of water, for example in hot and cold water systems, in

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buildings. In these cases, (except in very special circumstances such as pharmaceutical water systems), it is impractical to prevent legionellae entering a water system, so that the control of legionellosis must hinge on:

- a) limiting, preventing or killing the growth of those legionellae that enter the system
- b) reducing, so far as is reasonably practicable, exposure to water droplets and aerosols

The control of legionellae in all types of water systems follows the same principles. The system should be designed to prevent stagnation; to be easy to clean and to be constructed of materials that do not encourage the growth of micro-organisms. Systems should be maintained in such a way as to prevent the build-up of dirt and scale. Wherever possible, the growth of legionellae should be minimised by maintaining the temperatures of the system outside the range that permits the growth of legionellae. In cold water systems, this means keeping the temperature below 25°C and ideally below 20°C and, in hot water systems, circulating the water at 60°C, so that it reaches the outlets at 50°C or above. Growth may also be controlled by the addition of biocides and scale and corrosion inhibition should be instituted where appropriate. There should be a programme of regular maintenance, including regular inspection, cleaning and disinfection of tanks and other pieces of equipment such as cooling towers.

### **Cooling water systems**

Cooling towers and evaporative condensers are particularly prone to colonisation by legionellae. They operate as extremely efficient air scrubbers so that it is impossible to prevent contaminants entering from the air. In addition, the continual evaporation of water from the system means that the dissolved solids within it are continually concentrated, unless diluted by fresh water; this

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encourages scale formation. Parts of the system, notably the heat-exchanger, will normally be at temperatures conducive to the growth of legionellae which are continually released into the circulating water. The organisms in this water are then released as an aerosol, which is produced by the action of the water being distributed over the pack or fill by the trough and gutter or spray system. These aerosols become entrained in the air flow and are then ejected in the exhaust from the cooling tower, having first passed through the drift eliminator (see the section on Limiting Dispersal below).

The risk from cooling towers and evaporative condensers can be eliminated by replacing them with direct air-cooled systems or an air-cooled system with a closed secondary loop; this is not, however, always feasible.

### **Legionnaires' disease outbreaks and cooling water systems**

Since 1980, there have been 23 outbreaks caused by cooling water systems in England and Wales, 23 caused by hot water systems, 4 caused by cold water systems, 3 by spa pools and 49 for which the source could not be determined (Public Health Laboratory Service, (PHLS), unpublished results). Cooling towers and evaporative condensers are equally prominent as causes of outbreaks throughout the rest of the world. There are numerous reports of outbreaks in the literature but, unfortunately, few of them give details of the numbers of legionellae present in the water at the time when the tower was infectious, nor detail the disinfection procedures followed. Often the examination of the tower has taken place long after the tower was infectious and, in the interim, the bacterial population may have changed dramatically. For example, in the outbreak of Legionnaires' disease at Stafford District General Hospital in 1985, *L. pneumophila* could only be isolated by the investigation team from a piece of sealant in an air handling unit and not from any water sample. Presumably at the time of the outbreak there had been high numbers of *L. pneumophila* in the cooling water. Between the time of the outbreak and the arrival of the investigation team, however, the cooling water had been shot dosed with biocide

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at least twice and, in addition, the cooling water had been diluted by fresh make-up water. The biocide additions would have reduced the population of legionellae considerably and the addition of fresh make-up water would have further diluted the numbers of legionellae. Very low numbers of *L. pneumophila* were isolated from a sample of the cooling water collected by the water treatment company between the shot doses.

In the early outbreaks in the 1980s, where numbers are given, they have often been determined by direct immunofluorescence microscopy, rather than by culture. These results are unreliable because the reagents used were polyclonal antibodies whose specificity is questionable and their method also detects dead as well as living legionellae. On the other hand, isolation of legionellae by culture tends to underestimate the numbers of legionellae present by at least an order of magnitude.

Samples from the cooling tower incriminated in the BBC outbreak in London in 1988, contained up to  $10^6$  cfu/l (*colony forming units per litre*) by culture. This compares with  $10^9$  cfu/l by immunofluorescence (Westminster Action Committee 1988). The epidemiological investigation indicated people were infected up to 500m away from the cooling tower.

Samples collected from the incriminated tower at British Aerospace in Bolton in 1988, while it was still infectious, contained  $10^5$  cfu/l by culture and  $10^7$  cfu/l by immunofluorescence (Lee, unpublished results).

Addis *et al.* (1989), whilst investigating an outbreak in Wisconsin, sampled cooling towers before they were disinfected and probably while they were still infectious. The incriminated tower contained  $10^6$  cfu/l of *L. pneumophila* serogroup 1 and the epidemiological evidence suggested that patients were infected up to one mile (1.6 km) away from the tower.

Breiman *et al.* (1990) detected  $9 \times 10^6$  cfu/l in the water from the incriminated evaporative condenser in an outbreak at a retirement hotel. They performed air

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sampling around the building while the cooling tower was operating and still infected. Their results suggested that patients had been infected by air containing as little as 0.02 cfu/l. Following the investigation, the evaporative condenser was drained, scrubbed manually and cleaned with a pressure hose to remove sediment and scale, kept dry for 48 hours and then refilled with water containing 50 milligrams per litre of chlorine and a dispersant (dishwasher detergent). After 24 hours the condenser was drained, the process repeated and finally the system was dosed continually with 1 to 2 mg/l of chlorine. *Legionella* species were not isolated again from the condenser over the next six months.

Brown *et al.* (1999) reported on a community outbreak caused by hospital cooling towers in Delaware in 1994. The two incriminated towers were examined while they were still infectious. Water from the main tower contained between  $2.32 \times 10^6$  and  $9.15 \times 10^6$  cfu/l of *L. pneumophila* serogroup 1, whereas water from the small tower contained between 1.05 and  $2.34 \times 10^6$  cfu/l. The epidemiological investigation showed that the risk of illness decreased by 20% for each 0.1 mile increase in distance from the hospital up to one mile away. They concluded that transmission occurred primarily within a 0.25 miles radius of the cooling towers, although the possibility could not be ruled out that some patients were infected further away. They also found that cases were more likely than controls to have had frequent exposure or longer duration of exposure to the source, suggesting that in addition to proximity, cumulative exposure may have been an important risk factor for illness. The cooling towers were disinfected by hyperchlorination and cleaned, but details of the levels of chlorine used were not included.

In the BBC and British Aerospace outbreaks, the cooling towers had severely damaged drift eliminators that would have effectively increased the dispersal of the infectious aerosol from them. None of the other papers described above indicated whether the cooling towers or evaporative condensers were fitted with drift eliminators, (see page 29).

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Bhopal *et al.* (1991) examined the location of the homes of sporadic cases of Legionnaires' disease, (i.e. cases not associated with known outbreaks or travel), in relation to their proximity to cooling towers. They found that there was an inverse association between the distance of the residents from any cooling tower and the risk of infection. The population living within 0.5 km from any tower had a relative risk of infection 3 times greater than people living more than 1 km away.

Bentham and Broadbent (1993) reviewed the common features of some community outbreaks associated with cooling towers. Small towers (less than 300 kW) have predominantly been implicated in outbreaks. Cooling tower-associated outbreaks are most frequent in autumn, and frequently implicated systems have been operated after a period of shut down. They carried out a field study in which they monitored the numbers of legionellae in relation to their mode of operation. In systems that had been shut down, they monitored the numbers of legionellae in samples taken before, ten minutes after and 70 minutes after switching on the circulation. In some cases, switching on the system raised the legionella concentrations from below the detection limit (4,000 cfu/l) to between  $5.0 \times 10^4$  and  $9.5 \times 10^5$  cfu/l within ten minutes. They also examined 30 towers, sampling them twice weekly for two years, and found that the pond (basin) water of operating systems were more frequently positive for *legionellae* than non-operating systems.

In the majority of the outbreaks described above, the cooling systems were associated with air conditioning. In recent years, in the UK, there has been a number of small industrial outbreaks involving systems cooling plastics manufacturing equipment (Joseph *et al.* 1999 and Anon 1999). Such systems tend to have complex piping serving several different machines, each with its own heat exchanger and usually linked to relatively small cooling towers.

In summary the information available suggests that the numbers of legionellae in the water of cooling systems associated with outbreaks is always above  $10^5$  and

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usually greater than  $10^6$  cfu/l when determined by culture. Infection usually occurs within 500m of the tower but exceptionally may occur up to 1.6 km (one mile) away. The incriminated cooling towers are usually relatively small and no evidence has been found incriminating large cooling towers associated with power generation. The risk appears to be associated with systems that operate intermittently. It peaks shortly after startup and is seasonal, with most outbreaks occurring in late summer or early autumn.

### **Maintenance factors affecting colonisation of cooling water systems**

The most extensive field studies on the factors affecting the colonisation of cooling water systems have been done by the Australian Construction Services and are the subject of a report by Bentham *et al.* 1992 and Bentham 1993. A long-term study was carried out on 50 cooling towers in an outer metropolitan district of Adelaide, South Australia and the 34 cooling towers in two suburbs of Melbourne. The ponds of the towers were sampled weekly over three years. Colonisation was greatest during the summer months. Increasing pond (basin) water temperature correlated with the increasing legionellae concentrations above a minimum temperature of 16.5°C. Legionellae concentrations were greater in the operating towers than the non-operating towers even at similar pond water temperatures. They concluded that this indicated that the influence of mode of operation is not wholly attributable to heating the tower water. Towers operating throughout the year showed no seasonal variation in the numbers of legionellae that they could attribute to the heating of the tower water.

Bentham *et al.* (1992) confirmed that sediment and biofilms were the primary sources of legionella multiplication and pointed out that most of these are associated with the pipework and heat exchangers. They also established that water sampling was a more reliable method of determining the colonisation of a system by legionellae, than sampling biofilms or sediment. This was probably because it is difficult to get representative samples of sediment and biofilm, the

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lack of homogeneity in the colonisation of both and the high numbers of competing organisms interfering with the recovery of *Legionella* species from such samples.

Bentham *et al.* (1992) calculated the permanently wet surface area to volume ratio of their systems and found that there was a correlation between increasing concentrations of legionellae with increasing permanently wet surface area-volume ratios. In general, it was found that the smaller the system, the greater the wet surface area-volume ratio. This would suggest that larger systems tend to have lower populations of legionellae.

Bentham *et al.* (1992) also calculated fluid shear stress at pipe walls for various pipe sizes and flow velocities in order to predict the likelihood of biofilm being stripped off from surfaces on start-up. They calculated the shear stresses in cooling systems to be in the range  $0.094\text{N/m}^2$  -  $30\text{N/m}^2$ . This may be compared with the critical range of  $0.1$  to  $0.3\text{N/m}^2$  required to dislodge oral microorganisms from dental surfaces.

These studies also confirmed that dead legs, (spurs of piping without an outlet that are still connected to the system and where there is no flow), in the piping protect the legionellae from effective disinfection and confirmed that chlorination is an essential part of the cleaning process to reduce legionellae concentrations effectively.

### Cooling water treatment

It is generally accepted that the water in cooling systems needs to be treated to prevent corrosion and scale formation. This is essential for the thermal efficiency of the system, but is also a useful adjunct to the control of legionellae. Microbial growth is also commonly controlled by the addition of biocides. It is generally accepted that some type of biocidal treatment is essential for the control of

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growth of micro-organisms including legionellae in cooling systems. Negrón-Alvira *et al.* (1988) in Puerto Rico and Hunt *et al.* 1991 in Gloucester found that the incidence of and populations of legionellae in cooling towers that were treated with biocides was much less than those that were not treated. It is also accepted that biocides cannot be effective unless backed up by a water treatment programme to control scale and corrosion and minimise dirt in the system. However there is less agreement on what is an ideal biocidal regime for the control of legionellae.

### **The use of biocides and other agents to control *Legionella* species in cooling systems**

There have been numerous studies of the sensitivity of *Legionella* species to various biocides under laboratory conditions. Initially biocides were tested against pure suspensions of *Legionella* species and later model systems were used in which the legionellae were grown in conjunction with their supporting microflora. These studies have shown that many biocides can be active against legionellae, particularly *L. pneumophila*, in the laboratory. Studies have also been performed in which populations of artificially grown *Legionella* species have been added to real cooling towers which have then been treated with biocides. However, experience has shown that the results from such laboratory studies and artificially contaminated cooling towers do not reflect experience in the field and that many biocides that were thought to be effective against legionellae in these tests give disappointing results in real, naturally contaminated systems. Therefore only reports on field trials on naturally contaminated systems are described here.

The most extensive trials have again been carried out in Australia by Broadbent *et al.* (1992). This trial utilised sixteen cooling towers contaminated with what they termed "moderate to high levels of *Legionella*" ( $10^5$  to  $10^6$  cfu/l). Twenty-nine challenge experiments were carried out. During the experiments, each tower

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was taken off its respective broad-spectrum biocide (a monomeric quaternary ammonium compound) and dosed for four weeks with one of the three test biocides fentichlor, bromo-chloro-dimethylhydantoin (BCDMH) or bromo-nitropropane diol (BNPD).

### *2-bromo-2-nitropropane-1,3-diol (BNPD)*

Elsmore (1986) reported that 2-bromo-2-nitropropane-1,3-diol (BNPD), a broad spectrum non-oxidising biocide, was effective at controlling *L. pneumophila* in one cooling tower that had previously been ineffectively treated with methylenebis thiocyanate and one other that had previously been treated with an isothiazolone. Broadbent *et al.* (1992) found BNPD significantly reduced *Legionella* concentrations in five of eleven challenges and concluded that the results were equivocal and they could not recommend this as the biocide of choice.

### *Chlorinated phenolic thioether (Fentichlor, Hatacide LP5)*

This non-oxidising biocide was shown to be effective against *L. pneumophila* at 100 to 200ppm of product (Kurtz *et al.* 1982, 1984). It is also fungicidal and bactericidal, though ineffective against pseudomonads. Broadbent *et al.* (1992) found it to be effective in their trials at the recommended dosage giving 200ppm retained in the system for four hours as a weekly slug dose. However, it is costly compared with other biocides and may need an anti-foaming agent in some applications.

### *Quaternary ammonium compounds (quats)*

Quaternary ammonium compounds are recognised to have a "Pseudomonas gap". In Australia they were found to permit the majority of cooling towers to amplify legionellae sometimes to  $>10^6$  cfu/l (Broadbent *et al.* 1992). It has also been experience in the UK, in outbreak investigations, that cooling towers treated with quaternary ammonium compounds often contain significant numbers of legionellae. For these reasons this biocide cannot be recommended for the control of *Legionella* species.

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### *Isothiazolones*

Again in the Australian study this biocide permitted many towers to amplify legionellae. The results from other studies for this biocide are also equivocal.

### *Bromochlorodimethylhydantoin (BCDMH)*

BCDMH is a broad-spectrum oxidising biocide (see below) whose activity depends on bromine and chlorine. It was found to be effective in the Australian studies where the mean concentration of chlorine and bromine determined by the DPD method was 0.33 mg/l (range 0-2 mg/l). Kurtz and Davis (1988) in England also found it effective in the field at concentrations of 1-2 mg/l but in an earlier study in the USA on a single tower it was found to be ineffective at 2 mg/l (Fliermans and Harvey 1984).

The rotation of biocides for use in cooling systems is a recommended practice (DHSS 1988) and Broadbent *et al.* (1992) concluded that the most successful strategy might be the use of BCDMH (oxidising) for one to two months followed by the chlorinated phenolic thioether (Fentichlor) for two to four weeks in rotation. They also thought that rotation of either fentichlor or BCDMH with another broad spectrum biocide such as isothiazolone may be a viable and cheaper alternative. In this case the broad spectrum biocide should be dosed for one to two weeks between doses of the fentichlor or BCDMH.

### *Oxidising Biocides*

Chlorine and bromine are widely accepted as a means of treating cooling waters. They are dosed to give a free chlorine or free bromine reserve. This is a measure of the free halogen, the hypochlorous/hypobromous acid (HOCl/HOBr) and the hypochlorite/hypobromide ion (OCl<sup>-</sup>/OBr<sup>-</sup>). Fliermans *et al.* (1982) found that continuous levels of 1 - 1.5 mg/l of chlorine at pH 6.5 - 7.2 reduced the numbers of legionellae in a cooling tower by two orders of magnitude in 10 hours. Kuchta *et al.* (1985) found that water grown *L. pneumophila* were more resistant to chlorine than those grown on artificial media. At 21°C, pH7.6 - 8.0 and 0.25 mg/l

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chlorine a 99% kill of tap water grown legionellae was achieved in 60 - 90 minutes as opposed to 10 minutes when they were grown on an artificial medium. In the UK, the HSE guidance is that the applied dosage should be sufficient to maintain a free reserve in the range of 0.5 – 1 mg/l chlorine/chlorine dioxide and 1.0 - 2.0 mg/l bromine in the return water (Health and Safety Commission, HSC 2000). Reserves consistently above 2 mg/l free chlorine/bromine should be avoided (excepting in exceptional circumstances) as this may cause system corrosion. The activity (in terms of time taken to have an effect) of chlorine is significantly reduced at alkaline pH and additions of this biocide need to be adjusted to take account of this - this can be overcome by continuous dosing and pH control. It is, in any case, preferable to apply oxidising biocides on a continuous basis but, if they are applied as a shot dose, the effective concentration should be present for at least 4 out of every 24 hours. In large industrial systems, the dosage is based on water recirculation rate. This has to be sustained for a period of time, ranging from a few minutes to several hours, or even continuously, depending on the operating characteristics of the cooling system.

For small systems, such as air conditioning installations, halogen addition would normally be based on system volume. The system and its water chemistry will influence the choice of the best method of addition to obtain effective microbiological control. Once halogenation is stopped, the free halogen reserve is quickly lost, leaving the system open to re-infection and re-population by micro-organisms.

Oxidising biocides are also used for disinfection either in emergency or as part of the routine cleaning programme. For disinfection much higher doses of up to 50 mg/l may be used.

Oxidising biocides have the advantage that they can be readily monitored by simple chemical tests that can be performed on site, are relatively cheap and are easy to neutralise for microbiological monitoring and disposal. Their major

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disadvantage is that they can be corrosive and their activity, particularly for chlorine, is pH dependent.

Other oxidising biocides include chlorine dioxide and monochloramine. Chlorine dioxide can also be used for cooling towers. Monochloramine is more slow acting than chlorine but is more persistent in water systems and more effective at reducing the numbers of biofilm organisms (Camper and McFeters 2000). Kool *et al.* (1999) found that the incidence of legionellae was lower in hospitals supplied with water treated with monochloramine rather than chlorine. Hospitals with a detectable residual chlorine in their water had, however, comparably low levels of legionellae. Monochloramine has not been widely used for cooling towers and its application therein warrants further investigation.

### *Ozone*

*Legionella* species are susceptible to ozone. Several authors have, however, noted problems with its application in cooling towers. These have included corrosion, the inability to maintain a residual, and the presence of *Legionella* (Bird 1987, McDonnell 1989, Keenahan 1990, Ford 1991). In the investigation of the outbreak of Legionnaires' disease at Corby, England the only towers treated with ozone contained significant numbers of *Legionella pneumophila* (PHLS unpublished results). Apart from potential problems with corrosion that will depend on the materials used for the construction of the system, the major problem with ozone is the poor penetration to all parts of the system. It is possible that multiple injection points could overcome this and warrants further investigation.

### *Ultraviolet light*

Ultraviolet (UV) is active against *Legionella* species and has been applied to cooling towers although there is little published evidence of its effectiveness. The efficiency of units is dependent upon the clarity of the water so that it is often installed in conjunction with water filters. UV is only active at the point of

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application and does not control biofilm development downstream. In addition, light stimulated repair of UV damage can occur within bacterial cells. Thus, if UV is used to treat cooling towers, it should be used in conjunction with a biocide such as chlorine.

### *Copper and silver ionisation*

Electrolytically produced copper and silver ions have been extensively applied to the control of *Legionella* species in hot and cold water systems. There is good evidence that they are effective in controlling legionellae in hot water systems. In hard water, however, the electrodes tend to scale up and the effectiveness is reduced at high pH values, factors that reduce its effectiveness in cold water systems and probably cooling towers as well. Although copper and silver ionisation systems have been sold for treating cooling water systems in the US, there are no publications indicating the effectiveness of ionisation in cooling towers.

### *"Activated water"*

A method of electrolytically generating a disinfectant solution from a weak solution of sodium chloride has become available. This technique was originally developed by Russian workers but has been further developed in the UK. In principle, a weak solution of sodium chloride is passed through and electrolysed in a chamber in which the cathode and anode are separated by a ceramic filter containing iridium and other ingredients. The electrolysed product contains chlorine dioxide and various free radical and other molecular species. In the UK, one such system is marketed as "Sterilox". The product of the electrolysis is said to have a half-life of 18 - 24 hours. The chemistry is not clearly understood, but it does have some anti-microbial effects in clean environments. Its effectiveness in relatively dirty environments, such as cooling towers, has not been adequately tested yet, but warrants further investigation.

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### *Lower operating temperature*

Broadbent (1993) found that legionellae did not grow in cooling water when the temperatures were below 16.5°C, but grew well above 23°C. Kusnetsov *et al.* (1997) also produced some limited data that suggests that lowering the water temperature in a cooling system below 20°C by, for example, operating the tower more frequently can also reduce the population of legionellae. Presumably there are some penalties in decreasing the efficiency of the cooling tower, but this approach warrants further investigation.

### **Maintaining cleanliness**

Biocide or other anti-microbial treatments can only work effectively if the water cistern is maintained in as clean a state as possible. The evaporation of water from cooling towers causes the concentration of solutes and suspended matter in the circulating water. Any organic matter and some inorganic materials will react with biocides so reducing their effective concentration. In addition, the build-up of solutes may encourage scale formation as well as microbial growth. It is therefore important that the build-up of dissolved solids and suspended matter is controlled. This is usually done by continuously or intermittently replacing some of the water with fresh make-up water. This may be controlled manually or better, automatically, by a conductivity meter operating an automatic solenoid valve to release water when the build-up of dissolved solids has caused the conductivity to increase to a preset level.

Suspended solids are sometimes also removed by side stream filtration of some kind. In addition, small cooling systems should be cleaned and disinfected by chlorination twice a year. It is important that this cleaning and disinfection is applied to the whole cooling system, including all the pipework and heat exchanger, and not just to the cooling tower itself.

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### Limiting dispersal

The plume emitted from cooling towers consists of water vapour and water droplets. Only the water droplets can carry legionellae and create a risk of infection. The release of droplets in the plume can be reduced by fitting the tower with an efficient drift eliminator. 'Eliminator' is somewhat of a misnomer, as it would more correctly be termed drift reducer. A modern high efficiency plastic drift eliminator traps and reduces the water lost as droplets by at least 90% and even as high as 99% under ideal conditions, provided that they are in good condition and fitted well. They are usually designed for air flows of between 2 and 4 m per second. At lower speeds, for example when the fan is not operating in fan assisted towers, the efficiency will drop off dramatically as they trap the droplets by impaction on the surface. At air speeds below the design speed the small droplets will have insufficient velocity to impact on the surfaces of the eliminator and will be carried through in the air flow. At higher speeds, water on the surface of the drift eliminator will be stripped off and carried into the plume. Without eliminators in place, the maximum release of aerosol from a fan assisted cooling tower occurs when the fan is operating. Consequently, a well-fitted drift eliminator can reduce the risk of infection from cooling towers by appreciably reducing the release of aerosol under these conditions. Drift eliminators, however, cannot entirely prevent the release of aerosols from cooling towers.

Drift eliminators used to be constructed of wood, but these tend not to be as efficient as modern plastic ones that can be designed to create more surfaces for impaction. Some eliminators are made of metal. These can be efficient depending on the design, but again are generally not as effective as well designed plastic eliminators. The advantage of wood and metal eliminators is that they have greater inherent strength than plastic which can be an advantage in larger cooling towers, such as those used traditionally by the power generating industry.

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### Hot and cold water systems

Hot, and less commonly cold, water systems particularly those associated with large buildings such as hospitals and hotels are common sources of Legionnaires' disease. Approximately half of the cases reported annually in the UK are associated with travel and most of these are probably infected in the hotels in which they stay. The importance of hotels and hospitals as sources is a reflection of the complexity of their hot water systems. As we have seen in cooling towers, the greater the surface to volume ratio the greater the chance of the system becoming colonised.



Legionellae may grow in cold water storage tanks, particularly if the temperature exceeds 25°C, there is low water turnover, the tanks are inadequately sealed to prevent the ingress of dirt and not cleaned and disinfected regularly to prevent the build-up of dirt and sediments. The water in storage hot water heaters (calorifiers) invariably displays temperature stratification so that whilst the water temperature at the top may be too high for legionellae to grow, towards the

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bottom there will usually be a band of water at the ideal temperature for their growth. From calorifiers, legionellae can be released into the water leaving the heater, particularly if the system is undersized and the temperature drops during periods of high demand. Within the distribution system, legionellae may grow in the pipework if the temperature permits, but growth occurs particularly at the outlets, on elastomeric seals and washers and in flexible tubing associated with showers etc.

The primary methods of controlling legionellae in hot and cold water systems are: good design and maintenance to prevent the ingress of dirt and build-up of scale and corrosion; the use of materials that are known not to support significant microbial growth; and the limiting of growth by physical (temperature) or chemical means. Legionellae can grow up to about 45°C. However at 50°C in 2 hours 90% of a population of *L. pneumophila* will be killed and at 60°C it only takes 2 minutes. In water systems, temperature is the most common method of control. (*Photograph on Page 30 shows twin forced draught cooling towers courtesy of Dr John Lee*)

Keeping cold water as cold as possible, certainly below 25°C but preferably below 20°C, will minimise the risk of growth. It is important to lag cold water systems to prevent them warming up; to minimise stagnation within pipes; and to minimise storage of cold water wherever possible. It may be useful to add supplementary biocide treatment particularly if the chlorine residual is low in the incoming water in order to ensure that there is a disinfectant residual maintained throughout the system right up to the outlets. Chlorine is probably the most commonly used biocide, but chlorine dioxide can also be used successfully. Silver/copper ionisation can also be applied to cold water, but it is slow to act at low temperatures and hardness salts and high pH can interfere with its effectiveness.

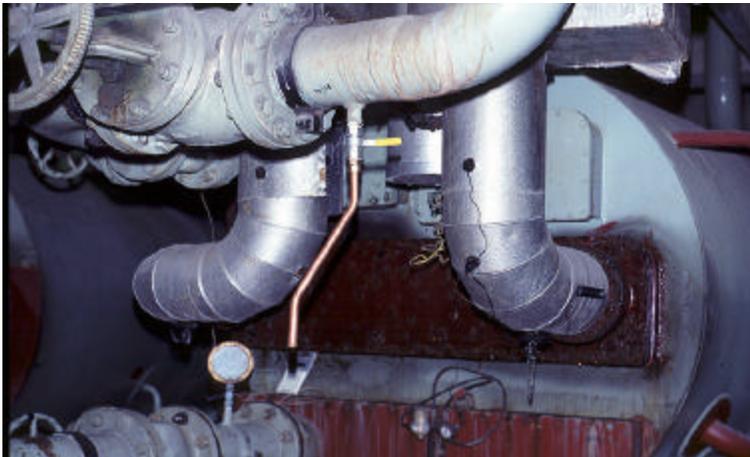
The most common strategy for controlling legionellae in hot water systems is based on keeping the temperature high enough to kill or at least minimise the chance of growth. Thus, in the UK, it is recommended that water should be

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circulated at 60°C so that the temperature at the tap reaches 50°C within a minute of turning it on. Where these temperatures cannot be achieved consistently, copper and silver ionisation or chlorine dioxide have been applied. These technologies are, however, still in their infancy and copper/silver ionisation in particular is still difficult to monitor and control. Chlorine has been used in the past, but this tends to cause problems with corrosion. Periodic disinfection by heating to 70°C, or chlorine at 50 mg/l for 4 hours, has also been used as a strategy but re-colonisation frequently occurs within a few days to weeks. Unfortunately, circulating the water at 60°C increases the risk of scalding, which is of concern in facilities for health care of children, the mentally handicapped or the aged. It is also in conflict with the energy conservation policies of some countries.



*Sampling point on cooling water return pipe courtesy of Dr John Lee*

Hotels are a particular problem because of: variable water demand; long pipe runs; variable occupancy due to the seasonality of the business; the mobility of

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staff which makes maintaining adequate training difficult. Moreover, existing buildings are frequently extended so that hot water systems have to be expanded beyond the design capacity of the water heaters; and, in many of the hot places in the world, there can be a variable water supply due to the necessity for water to be delivered by tanker.

Studies in North America, Germany and the UK have shown that about 10 to 20% of households may contain *Legionella* species. Furthermore, there is clear evidence that some patients are infected at home. The factors associated with colonisation of household water systems are similar to those of larger systems.

More detailed information on the control of *Legionella* in hot and cold water systems can be obtained in the guidance provided by the Health and Safety Commission (HSC 2000), the Chartered Institute of Building Service Engineers (CIBSE 2000) and Freije (1999).

### Spa pools

The water in spa pools (also known as whirlpool spas and more commonly as Jacuzzis which is a trade name of a particular brand) is recirculated, maintained between 35 - 40°C; vigorously aerated and agitated by the air and water jets; and becomes contaminated with skin squames, soaps, sweat and other body secretions which act as nutrients for micro-organisms. They also contain large amounts of plastic tubing that provides the air and water circulation. This tubing is usually difficult or impossible to remove for routine maintenance, provides a large surface area for the development of biofilms and cannot always be penetrated effectively by the biocides in the water. The air jets create an aerosol immediately above the surface of the water in the region of the heads of the bathers. Since the temperature is optimal for the growth of legionellae and there are copious nutrients and surfaces available for biofilm development, it is not surprising that, if they are not rigorously managed, spa pools can support

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significant growths of micro-organisms including legionellae. They have been responsible for many outbreaks of Legionnaires' disease and Pontiac fever in leisure facilities, hotels, private households, cruise liners and even at trade shows where the pools have not been entered by anyone. The market for them is increasing and many are now being installed in private households as well as more public leisure facilities. Despite this, the majority are still constructed in such a way that it is not possible to readily remove the tubing to dislodge physically any biofilm on the internal surfaces. This is unfortunate because it means that biofilm removal relies on chemicals that, as we have seen, can be problematic. Nevertheless, spa pools that have been rigorously maintained according to the guidance provided by the PHLS (PHLS 1994) have never been incriminated in an outbreak of infection caused by *Legionella* or other bacteria. In short, at least half the water in these pools needs replacing each day, public pools should be equipped with swimming pool sand or diatomaceous earth filters and continuously disinfected with an oxidizing biocide, preferably chlorine.

### Monitoring Legionella control

Now that the methods for the culture of legionellae have been improved, sampling is increasingly undertaken as an aid to auditing the effectiveness of control measures. It should be emphasised, however, that culture is a relatively lengthy and inconsistent process and should only be used as an aid to audits of the effectiveness of the treatment employed. Other measures, such as monitoring temperature or the residual concentration of oxidising biocides, are far more effective means of monitoring the operation of control measures and, moreover, provide results instantly or within minutes, in contrast to the 7 to 14 days for the culture of *Legionella* species.

#### *Sampling*

When collecting samples, they should be as representative as possible. Since the purpose is to help estimate the risk from the system, samples should, therefore,

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be collected ideally at the time when, and place where, the risk is likely to be at its greatest.

Samples should be collected when the biocide concentration is at its lowest. In cooling systems the maximum growth, particularly of legionellae, is likely to occur in the warmest part of the system, that is the heat exchanger, so the 'best', (i.e. the most likely to contain infection), samples are obtained from the water leaving that part. It should be noted that this is not the pond that is commonly sampled. Ideally a sample point will be situated near the heat exchanger on the return to the cooling tower.

### Monitoring the general microbial population

Although there is no absolute correlation between the bacterial population and the count of *Legionella*, several studies have shown that the likelihood of a system containing legionellae increases as the total microbial population increases. Thus, monitoring of the bacterial population is commonly performed in cooling systems and spa pools in order to measure the biological control of the system. Using cultural methods to estimate the bacterial population is the most common technique for doing this. The two most common methods are the dip slide and the plate colony count. At best, the dip slide gives only an approximate count and is open to misinterpretation, but at least it can be performed on site with a minimal amount of equipment. The plate colony count is more reproducible, but requires better laboratory facilities and is still subject to considerable sampling errors. Counts are often incubated at 22°C and/or 37°C following the methods commonly used for drinking water analysis. For most cooling systems, however, counts more representative of the majority of the bacterial population are obtained if the temperature is 30°C. Whatever cultural method is used, the count recorded will always be an underestimate of the actual bacterial population, because a large proportion of aquatic bacteria cannot be cultured.

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All living organisms contain adenosine triphosphate (ATP) that rapidly disappears on death. The measurement of ATP is based on the production of bioluminescence using the luciferin/luciferase system. The advantage is that it is rapid, but it does require more specialised equipment and skilful and careful interpretation. Ideally there would be an on-line method of monitoring the bacterial or microbial population and whilst there is research being carried out in this area, no systems are yet commercially available.

The numbers of bacteria in the aqueous phase do not necessarily relate to the biofilm population. Monitoring biofilms, however, is not as easy technically as monitoring the organism in the water. Biofilm development or control can only be monitored by some *in situ* sampling device. These may be directly implanted in the normal pipework, or sidestream devices attached in parallel to the main system. In principle, all these devices rely on having some form of removable studs or coupons. At appropriate intervals, these are removed and replaced with a fresh stud/coupon. The microbial population on the removed stud can then be assayed by a range of methods, including culture, microscopy and bioluminescence. Although such devices are in use in some cooling systems and other water systems, at present they require appreciable technical skills and are not yet really suitable for frequent use as, for example, the dip slide or plate colony count. The use of these devices has recently been reviewed by Percival (2000).

### Monitoring legionellae

Legionellae are fastidious organisms, requiring complex media and it can take from three to ten days for them to grow. In environmental specimens they are usually vastly outnumbered by other bacteria. Isolation, therefore, usually requires concentration of the bacterial flora by filtration, or centrifugation, followed by heat (50°C for 30 minutes) or acid (pH 2.2 for 5 minutes) treatment before inoculation on to media containing antibiotics. Recognition of the colonies of legionella requires skill. Because of the complexity of the procedure

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and the skill required, it is essential that any laboratory used for the isolation of legionella is accredited for the test by the appropriate national accreditation body (e.g. UKAS in the UK). It should, moreover, participate in an external quality assessment scheme for the isolation of legionella, such as that run by the Public Health Laboratory Service of England and Wales.

Legionella bacteria can also be detected by immunological methods in which components of the bacterial cell are detected by antibodies raised against specific components (antigens) of the bacterial cell. Immunofluorescence is one such method in which the cells are stained by fluorescent-tagged antibodies and detected and counted by microscopy. This method can give a result within the working day, but is labour intensive, is difficult to quantify accurately and does not distinguish between living and dead cells. It can, however, be useful in outbreak investigations and for research. Before reliable methods of culture became available, it was widely used, particularly for ecological studies. Its specificity depends upon the quality of the antibody. Modern antibodies, particularly monoclonal ones, are generally more specific than some of the polyclonal antibodies used in the early studies in the late 70s and early 80s.

The urinary test kits, which are used to diagnose Legionnaires' disease by detecting legionella antigens in the patient's urine, can also be adapted to detect legionellae in water. The sensitivity, however, is rather low in comparison with the culture method and the microflora in the water sample will still have to be concentrated in some way.

A more rapid molecular biological technique called Polymerase Chain Reaction (PCR) enables the nucleic acid from legionellae in environmental specimens to be detected, but it is not yet widely available commercially for routine analyses and does not differentiate between live or dead organisms. This method can, however, be of great use in screening samples in outbreak investigations and, in practice, correlates well with the count of legionellae detected by culture.

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### Conclusion

Since the recognition of Legionnaires' disease in 1976 and the subsequent isolation of the causative bacterium, *L. pneumophila*, we have learned much about the ecology of the causative organism. It is one of the best examples of how adapting the environment to suit ourselves can inadvertently create an environment that also favours the growth of an organism that otherwise would not normally be a significant risk to our health. The only natural potential source of infection by legionellae to man is probably from hot springs. The simplest way to eliminate the risk of legionellae growing to a level where they can create a significant risk is by preventing the occurrence of water temperatures that support their growth. Unfortunately this is not practical in many situations.

Most of the precautions that are currently applied for the control of legionellae were originally empirically derived. Even now, many are not based on sound, scientifically designed, controlled studies. One of the problems is that, even in countries such as Denmark, where it is believed that ascertainment is high, the numbers of cases of Legionnaires' disease is relatively small in comparison with other causes of morbidity and mortality. For this reason, funding for research tends to be erratic and is usually provided only in response to a disaster. The recent large outbreaks of Legionnaires' disease in Murcia, Spain, in July 2001, at a flower show in the Netherlands in February 1999, and the outbreak associated with the soccer World Cup in Paris, have been responsible for a resurgence in interest in Legionnaires' disease throughout Europe. As a consequence of this erratic interest in research into the control of Legionnaires' disease, there are still many gaps in our knowledge.

To date the majority of outbreaks of Legionnaires' disease that have been caused by cooling towers or evaporative condensers have been associated with relatively small systems. Large cooling towers, such as those often associated with power generation, have never been shown to be a cause of legionellosis.

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This is probably because the intrinsic risk from such systems is less than from smaller towers because: they are more stable ecosystems; they are often relatively isolated from habitation; and the aerosols are generally released at such a high level that by the time any potentially infectious particles have reached ground level, the organisms within them have died. A less likely, though more pessimistic explanation would be that the lack of an outbreak associated with such systems has occurred by chance, because there are far more small towers than the very large ones. The potential risk from large cooling towers must not, however, be ignored.

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The control of legionellae depends on a good understanding of the ecology of the organisms, but there is still much that we do not know. It is clear that *Legionella* species can multiply inside protozoa and there are many papers on this topic. Following the discovery of the association with protozoa there has been little research on other mechanisms of survival of *Legionella* species in man-made and natural water systems. It is known that *some other bacteria can support Legionella species in vitro*. There is some limited evidence to suggest that legionellae can grow under natural conditions, without having to grow inside amoebae or other protozoa. This work, however, needs extending.

Just as some bacteria can support the growth of legionellae, many others can inhibit them. It could be that some type of biological control is feasible using organisms that are known to inhibit legionellae. It is also possible that some less pathogenic varieties of legionella can outgrow the infectious ones in water systems. Thus, further research into the interactions between *Legionella* species and other organisms could lead to insights into alternative methods of control.

An important component of monitoring the effectiveness of control measures is the monitoring of the levels of *Legionella* species, other bacteria and other micro-organisms in the water. At present these are usually done by culture that can take several days to get a result. Further research is needed on the methods of monitoring microbial activity in cooling water, particularly on-line methods. The development of reliable, rapid and inexpensive methods of determining biofilm development is required. There are some relatively rapid molecular methods available for the detection of *Legionella* species, particularly by PCR, but none of these has been adequately validated at present.

There are relatively few biocides available for the control of legionellae in cooling systems. Of those that are available, the oxidising biocides currently offer the most consistent and controllable means of control in cooling systems. "Activated water" could be more environmentally friendly than other oxidising biocides and warrants further investigation. Similarly, the combination of UV

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with a systemic biocide is another approach worthy of investigation. Although field trials are ultimately essential, laboratory trials under more controlled conditions would be required using *Legionella* species growing under natural conditions in model systems.

In conclusion, there is still much to be learned and much to be validated scientifically. The challenge is to develop more effective, environmentally friendly, cost effective, validated control measures.

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