Legionella pneumophila Control in Water Systems

Executive Summary

Many studies of diverse drinking water systems have documented that Legionella spp. and Legionella pneumophila are common members of the ecosystem of treated drinking water, even in systems meeting and exceeding all regulations and that maintain disinfectant residual throughout their distribution systems. As noted in a National Academies of Science, Engineering and Medicine (NASEM) report, completely preventing occurrence of L. pneumophila in treated drinking water is not feasible and, they may be present in low numbers in well-run and compliant systems that maintain a detectable secondary disinfectant residual. They are environmental organisms that able to persist and grow in environmental niches like biofilms of drinking water distribution system pipes. They can survive in the presence of disinfectants, can grow in low nutrient conditions and have survival strategies such as invasion and growth in free living amoebae, another common member of the treated drinking water microbial community. L. pneumophila appear to be less prevalent in buildings where the secondary disinfectant of the public water system is chloramine rather than free chlorine. The most recent and comprehensive study on L. pneumophila occurrence in distribution systems in the United States determined that L. pneumophila occurrence is more common in systems with free chlorine secondary disinfectant than total chlorine and increases for water with secondary disinfectant concentration < 0.1 mg/L, but increases in residual concentration above 0.1 mg/L did not result in a corresponding decrease in the occurrence of L. pneumophila.

Though L. pneumophila have been detected at distribution system points of entry, published studies indicate that they occur more frequently at sample locations with higher water age, in dead ends with low or no residual concentration and in storage tank sediments. Occurrence surveys of L. pneumophila in distribution systems and connected building water systems remain a critical research need and an opportunity to identify and address conditions that favor L. pneumophila survival and growth. We are unaware of any study on occurrence of L. pneumophila in hydrant leads. Though hydrant leads are dead legs, no data support them being sources of L. pneumophila bacteria and efforts at L. pneumophila management are better directed at known and credible risks such as accumulation in storage tank sediments or intrusion and proliferation associated with improperly managed distribution system disruptions, at least until systematic studies indicate hydrant leads could be a significant source of L. pneumophila.

Studies conducted by the EPA and others demonstrate that L. pneumophila is detected in a greater portion of samples and at higher concentrations in building water systems than in distribution system samples.
That is, \textit{L. pneumophila} is a part of the microbiology of building water systems and conditions specific to building water systems favor their persistence and growth. The most effective risk management strategy focuses on preventing their growth in buildings to problematic concentrations, and development of strategies for minimizing inhalation of aerosolized bacteria. Most studies indicate that chloramine disinfection (in the building water supply) is probably more effective for \textit{L. pneumophila} control in building water systems than free chlorine, though \textit{L. pneumophila} are often detected in buildings with either type of disinfectant. There is no clear indication that higher distribution system residual entering a building affords greater protection, particularly given the rapid disinfectant decay that occurs in most building water systems for both hot and cold water and particularly during periods of stagnation. Other factors associated with increased \textit{L. pneumophila} occurrence and Legionnaires’ disease risk are prolonged stagnation periods and temperature in the optimal growth range \textit{L. pneumophila} \(77^\circ\text{F-108}^\circ\text{F}; 25^\circ\text{C-42}^\circ\text{C}\). Occurrence and growth have been documented for both hot and cold plumbing.

Legionnaires’ disease risks are best managed via partnership between water suppliers and building water system owners and operators, and manufacturers and operators of endpoint devices. The greatest potential for risk reduction lies on the building side of the meter. Though public water systems cannot permanently eradicate \textit{L. pneumophila} from their treated water and distribution systems, they can maintain a detectable residual, identify and remediate potential niches for \textit{L. pneumophila}, and provide information to customers about \textit{L. pneumophila} and steps they can take with the water system to reduce risk. Building water system operators have many options for reducing legionellosis risk and many resources available to help them formulate and execute risk management strategies.

Systematic analysis of \textit{L. pneumophila} occurrence and control confirms the value of our long-held multiple barrier approach to the reduction of risks from drinking water. Especially since so much is not known about the fate and transport of \textit{Legionella} in public water distribution systems. There are bits and pieces of information that leave us as if we were trying to make a jigsaw puzzle and do not know what picture we are making. It is easy to jump to the conclusion of knowing what the picture is, but the fact is, we do not know yet. In these situations, a multiple barrier approach is reasonable. For example, a detectable disinfectant residual, following primary disinfection, could be accomplishing various things: keeping amoeba in their cyst stage preventing uptake and growth of \textit{Legionella}; deterring microbiological regrowth; oxidizing dissolved iron; confirming the freshness of the water. All of these things could be contributing to the control of \textit{Legionella}. After disinfection (primary and secondary), the next steps public water systems already take are to minimize conditions that allow environmental bacteria from entering the water system through activities such as properly repairing and installing water mains (see AWWA C651), minimizing leaks, and covering storage facilities. While it is unknown which practices could contribute to \textit{Legionella} intrusion in a significant way, the multiple barrier approach encourages a robust management approach that is consistent with the control of other risks.

**Document Purpose and Approach**

This white paper describes the role of water systems (both public water systems and building water systems for customers of public water systems) in the occurrence and control of Legionnaires’ disease.
The review is intended to facilitate critical review of regulations proposed in Illinois and elsewhere for improved Legionnaires’ disease risk management and public health protection. Like others in this field, we advocate development of rules most likely to advance public health protection and discourage rules that are unlikely to be associated with improved public health. A systematic review of available information on the occurrence and control of *L. pneumophila* in public water systems and connected buildings can help differentiate the productive rules from unproductive ones. *L. pneumophila* is only one of many hazards, biological and chemical, associated with drinking water and thorough consideration is required to ensure that actions taken for control of one hazard do not increase risks of others.

The document begins by providing a synopsis of basic information related to the bacterium *L. pneumophila*, how it is measured and why it is an important drinking water hazard. Next, the occurrence and control of *L. pneumophila* in treated drinking water and in building water systems are reviewed. The document finishes with a discussion of *L. pneumophila* control, from treated water entry into distribution systems to showerheads in building water systems.

*Legionella pneumophila* and Legionnaires’ Disease

The most comprehensive document on *Legionella* in water systems and associated risks is the recent National Academies of Sciences, Engineering and Medicine (NASEM) report “Management of *Legionella* in Water Systems” (National Academies of Sciences, Engineering, and Medicine, 2019). The executive summary of that document provides a concise statement of why *L. pneumophila* is such a concern for drinking water systems (emphasis added):

“The bacteria in the genus *Legionella* occur naturally in water but have optimal growth at warm temperatures. **Wherever there are water and pipes eventually one can find Legionella including in many human-made building water systems.** However, its exact niche and the factors influencing it to bloom are only now being elucidated. *L. pneumophila* is the species (among many) most often diagnosed as the cause of Legionnaires’ disease. For every case associated with an outbreak there are nine more sporadic cases.”

As indicated in the NASEM report, *L. pneumophila* is a particular concern for treated drinking water because one of the diseases it causes – Legionnaires’ disease – is more severe and has a higher mortality rate (the proportion of ill individuals who die) than the fecal pathogens that have been of greatest concern historically in drinking water production. Legionellae are environmental organisms. They live in natural and engineered environments and do not require human/animal hosts for survival, persistence or growth. They can survive in treated drinking water that meets all regulatory requirements and has a high secondary disinfectant residual concentration. They can, and do amplify to high numbers in building water systems. The incidence of Legionnaires’ disease is increasing in the United States, particularly in the northeast and mid-Atlantic states. As noted in the NASEM report, there are many data gaps in our knowledge of the factors and niches that allow *L. pneumophila* to grow to dangerous levels and the best approaches for controlling them.
**Legionella pneumophila** Occurrence in Water

**Measurement of L. pneumophila in Water Samples**

*Culture techniques remain the gold standard for enumerating L. pneumophila in drinking water samples. Culture techniques (including the relatively new assay Legiolert) report live, viable bacteria and produce data that can be compared against guidelines and standards. At present, data from molecular methods are useful for research studies, but not useful for regulatory monitoring or for water quality assessment in process control (e.g., validation monitoring under a water safety plan).*

A brief discussion of *L. pneumophila* detection and enumeration follows. This discussion is intended to facilitate interpretation of results from the studies reviewed below where different techniques were used to measure *L. pneumophila* concentration. The primary methods used for quantifying *L. pneumophila* and their advantages and disadvantages are presented in Table 1.

**Table 1. Advantages and Disadvantages of the Primary Methods in Use for L. pneumophila Detection and Quantification**

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture (BCYE)</td>
<td>• Measures viable, culturable organisms (organisms of verifiable public health significance).</td>
<td>• Does not detect viable but nonculturable (VBNC) organisms (potential undercount).</td>
</tr>
<tr>
<td></td>
<td>• Gold standard in measurement and produces concentrations that can be compared with standards</td>
<td>• Subject to overgrowth by non-target organisms (potential undercount).</td>
</tr>
<tr>
<td></td>
<td>(all of which are expressed in terms of culture concentrations).</td>
<td>• Requires specialized expertise for sensitive and accurate assays.</td>
</tr>
<tr>
<td>Legiolert</td>
<td>• Much simpler to conduct than other culture assays.</td>
<td>• Relatively new and unfamiliar to the drinking water community.</td>
</tr>
<tr>
<td></td>
<td>• Appears to have better performance than other culture assays (e.g., plating on BYCE agar) for high-concentration samples.</td>
<td>• Might be subject to not counting VBNC organisms (though some reports indicate Legiolert is less prone to undercounting VBNC organisms compared with methods such as plating on BYCE agar.</td>
</tr>
<tr>
<td></td>
<td>• Results are comparable to those from other culture methods and appropriate for comparison against standards.</td>
<td></td>
</tr>
<tr>
<td>Molecular (qPCR)</td>
<td>• Rapid (compared with culture)</td>
<td>• Does not discriminate between genetic material from live and dead cells (likely overcount).</td>
</tr>
<tr>
<td></td>
<td>• More sensitive than culture methods</td>
<td>• Subject to inhibition by substances in water samples (potential for assay to be invalidated).</td>
</tr>
<tr>
<td></td>
<td>• Detects genetic material from viable but non-culturable bacteria.</td>
<td>• Results are not readily interpretable.</td>
</tr>
</tbody>
</table>
Whiley and Taylor (2016) outlined the advantages and disadvantages of culture and quantitative polymerase chain reaction (qPCR) measurement of \textit{L. pneumophila} concentration. Culture determinations can be slow and are subject to overgrowth of plates by organisms other than the target (\textit{L. pneumophila}). Molecular methods (primarily qPCR), but also sequencing target genes such as 16S rRNA, and metagenomic analyses in which all the DNA for all cells in a sample are identified are faster, more sensitive and potentially more specific. However, these methods count genetic material from dead/nonviable cells and their results are difficult to interpret and cannot be compared against any available standard. A relatively new culture method – Legiolert – has been used successfully in studies of \textit{L. pneumophila} in drinking water (Barrette, 2019; LeChevallier, 2019a, 2019b; Mapili et al., 2020; Mapili, 2019; Petrisek and Hall, 2018; Spies et al., 2018) and non-potable water (Rech et al., 2018). Legiolert is similar to other familiar assays such as Colilert and Enterolert and uses defined substrate and specially-designed templates for developing most probable number (MPN) estimates of \textit{L. pneumophila} concentration. The Legiolert assay requires far less expertise than traditional culture methods (e.g., incubation on BYCE agar). All published studies to date indicate that Legiolert results are comparable to results for other culture assays at low concentration and perhaps outperforms the traditional assays when \textit{L. pneumophila} count in a sample is high.

\textbf{Treated Drinking Water Distribution Systems}

\textit{L. pneumophila} is a common member of the microbiome (the microorganisms in a particular environment) of source and treated drinking water and private well water. It has been suggested to be a rare, dynamic species (as opposed to a core species) subject to sporadic growth and detection. Numerous studies have documented presence of \textit{L. pneumophila} in treated water of public water systems using both chloramine and free chlorine secondary disinfection. \textit{L. pneumophila} have been detected at distribution system points of entry, but published data indicate they are more commonly found at sample locations with higher water age. We are unaware of any study on occurrence of \textit{L. pneumophila} in hydrant leads. Though hydrant leads are dead legs, no data support them being important sources of \textit{L. pneumophila} bacteria and efforts at \textit{L. pneumophila} management are better directed at known and credible risks such as accumulation in storage tank sediments or intrusion and proliferation associated with improperly managed distribution system disruptions, at least until systematic studies indicate hydrant leads can be a significant source of \textit{L. pneumophila}.

This section reviews several key papers documenting the common occurrence of \textit{L. pneumophila} as a naturally-occurring environmental organism, common in source and treated water microbiomes and explores factors that favor occurrence. Together, the studies demonstrate that \textit{L. pneumophila} can be a common inhabitant of the distribution system microbial ecology and that organisms occur even in systems that maintain a high disinfectant residual. Risk management efforts should be directed at control, not eradication, of these organisms and at developing partnerships with building water systems operators for managing Legionnaires’ disease risk.

In a relatively small survey of source and treated waters of 25 treatment plants in the US (King et al., 2016), 25\% of source water samples were positive for \textit{L. pneumophila} whereas 4\% (a single sample) of treated water samples were positive. Treated water samples were collected at a sampling point after
final disinfection but prior to the clearwell. Given that *L. pneumophila* is a dynamic member of the treated drinking water microbiome and that the sample size was small, this study indicates the potential for periodic passage of low numbers of *L. pneumophila* into distributions systems, even for well-run plants meeting all regulations.

Lu and co-workers conducted sampling of drinking water distribution system water and sediments in a survey (qPCR methods) of *L. pneumophila* and other opportunistic pathogens in treated drinking water and storage tank sediments (Lu et al., 2016, 2015). *Legionella* spp. were detected in 57% of water samples and 67% of sediment samples. Concentrations of *Legionella* spp. were generally low and the authors advised that “just the detection of these relatively novel OPs [opportunistic pathogens] does not necessarily constitute significant risk.” Similarly, Whiley et al. (2014) observed high occurrence (a high proportion of water samples were positive) of *L. pneumophila* in the bulk water of a chlorinated and a chloraminated distribution system in Australia. Concentrations (as measured via qPCR) were generally low and controlled by disinfectant residual, except for samples collected in a dead end of the distribution system in which disinfectant residual was low. Elevated *L. pneumophila* concentration in dead ends is important since every building connected to a distribution system is a de facto dead end. Using qPCR, Waak et al. (2018) found that *Legionella* occurred less frequently in biofilms (as opposed to bulk water) of a distribution system using chloramine secondary disinfection than a system with free chlorine disinfection.

LeChevallier (2019a, 2019b) sampled 12 drinking water distribution systems for *L. pneumophila* and assayed samples using a culture technique (Legiolert). Source water, distribution system point of entry and distribution system samples were collected for each system. As expected, *L. pneumophila* occurrence in his studies was lower than observed in studies using qPCR for detection. *L. pneumophila* was detected in only one sample (N=576) collected in the winter and was detected in two source water samples (4%), no distribution point of entry samples and 14 (2.4%) distribution system samples collected in the summer. Thirteen out of fourteen positive distribution samples were from systems with free chlorine secondary disinfection. The detection limit for the Legiolert assay was 10 MPN/100 mL. All values greater than 10 MPN/100 mL occurred when free chlorine residuals were less than 0.1 mg/L, whereas the single positive distribution system sample from a chloraminated system had a total chlorine residual concentration of 3 mg/L. The study’s suggestion that 0.1 mg/L might be sufficiently protective for controlling *L. pneumophila* is based on limited data and an incomplete understanding of what a chlorine residual is doing to help minimize *L. pneumophila*; systematic and comprehensive research on the association of distribution systems features and water quality with *L. pneumophila* occurrence and the disinfectant residual concentration required for adequate control remain critical research needs.

A study of water quality in tap and distribution system samples supports a general tendency of low concentration of opportunistic premise plumbing pathogens (OPPPs) in distribution system water yet much higher concentrations in connected building water systems (Wang et al., 2012). After three minutes of flushing, point of use (POU) *L. pneumophila* concentration measured by qPCR fell more than one log compared with first-draw samples. This finding indicates that the building water system
presents better opportunities for managing Legionnaires’ disease risk, either through preventing growth of \textit{L. pneumophila} or through flushing/diverting water that has been stagnant.

Choice of disinfectant (chloramine v. free chlorine) appears more important than concentration of disinfectant provided there is a real, detectable disinfectant residual in occurrence and control of legionellae. Chloramines have been associated with reduced occurrence and abundance of \textit{L. pneumophila}. However, increased occurrence and abundance of mycobacteria (some of which are also environmental pathogens) have been reported (Gomez-Alvarez et al., 2012, 2016; Kool et al., 1999; Pryor et al., 2004; Wang et al., 2014). Flannery et al. (2006) conducted a survey of \textit{Legionella} presence in building plumbing systems before and after a switch from free chlorine to chloramines in a public water system distribution system. Although free and total chlorine concentrations at the building service connections were not provided in the study, the authors documented a drastic reduction in the prevalence of \textit{Legionella}-positive samples when the secondary disinfectant was changed. Pryor et al. (2004), Moore et al. (2006) and Weintraub (2008) found similar reductions in occurrence for both distribution systems and connected plumbing systems after a switch of secondary disinfectant from free chlorine to chloramine. In addition to reducing the occurrence of \textit{Legionella} bacteria, switching public water systems (building supplies) from free chlorine to chloramine has also been reported to reduce the incidence of nosocomial legionellosis in facilities connected to the distribution system (Heffelfinger et al., 2003; J.L. Kool et al., 1999).

A meta-analysis of published data on treated drinking water microbial communities (Bautista-de los Santos et al., 2016) found that \textit{Legionella} spp. occurred in chlorinated and chloraminated treated drinking water samples, but occurred less frequently than in samples from a disinfectant-free system (in a European country) and were relatively less abundant than in samples from a residual-free system. That is, maintaining a disinfectant residual impacts overall microbial ecology and the occurrence of \textit{Legionella} spp., but does not eliminate \textit{Legionella} spp. Other studies (e.g., Bertelli et al., 2018) document that the presence of disinfectant in distribution systems has a marked impact on microbial ecology, with decreased diversity associated with increasing disinfectant concentration and with the potential for selection for antimicrobial resistant organisms. Several studies have proposed that established drinking water distribution system biofilms and bulk water have a core microbial ecology (relatively steady and generally similar spatially within the distribution network) and a superimposed dynamic/transient population of organisms comprising a rare and highly dynamic portion of the overall population (e.g., El-Chakhtoura et al., 2018; Gomez-Alvarez et al., 2016; Zhang et al., 2017). \textit{Legionella} spp. appears to be a member of the rare and dynamic community and \textit{L. pneumophila} is even rarer and more dynamic.

Some bacteria including legionellae are amoeba resisting microorganisms (ARMs). Legionellae can survive and replicate within amoebae that are frequently present in treated and untreated drinking water. Once inside the amoebae, legionellae are protected from disinfectants and can grow rapidly. Several studies have documented the presence of amoebae in treated drinking water as well as the presence of ARMs including \textit{Legionella} spp. in those amoebae (Corsaro et al., 2010; Delafont et al., 2013; Garcia et al., 2013; Loret and Greub, 2010; Thomas et al., 2008).
To date, no published studies have identified likely “hot spots” for L. pneumophila occurrence and growth, but it is presumed that dead ends, storage tank sediments and distribution system portions with no disinfectant or possibly with intermittently absent disinfectant could serve as “hot spots.” Further, no studies have been conducted on risks associated with fire hydrant leads. The best available study that can inform risks associated with hydrant leads was a domestic water system simulation with a rig including recirculating components and dead legs (Loret et al., 2005). At the outset of simulations, no disinfectant residual was maintained in the pilot system and the system was colonized with Legionella. After introduction of disinfectant to the flowing portion of the rig, Legionella concentration fell rapidly in the flowing section of the rig despite high concentrations persisting in dead legs attached to the flowing sections. Potential explanations for low concentration in the flowing section despite high concentration in dead legs are that there is limited exchange between the dead leg and flowing portion and that the disinfectant residual maintained in the flowing part of the rig was sufficient to inactivate and control Legionella contributions originating in the dead leg. The differences between the system described here and a water hydrant lead are too great to assume results in the domestic water study can be extended to hydrant leads without further study. They do indicate the possibility that hydrant leads might not be a significant contributor of Legionella bacteria, but that systematic studies are required to evaluate risks associated with fire hydrant leads and they cannot be assumed a significant source of risk simply because they constitute dead legs.

Buildings Water Systems

Studies conducted by the EPA and others demonstrate that L. pneumophila is detected in a greater portion of samples and at higher concentrations in building water systems than in distribution system samples. That is, L. pneumophila is a part of the microbiology of building water systems, and conditions specific to building water systems favor their persistence and growth. The most effective risk management strategy focuses on preventing their growth in buildings to problematic concentrations. Most studies indicate that chloramine disinfection (in the building water supply) is probably more effective for L. pneumophila control than free chlorine, though L. pneumophila are often detected in buildings with either type of disinfectant. There is no clear indication that higher residual affords greater protection, particularly given rapid disinfectant decay that occurs in most building water systems for both hot and cold water and particularly during periods of stagnation.

To protect public health with respect to Legionnaires’ disease, there are a number of effective control strategies along the route from water production through use at the tap. Maintaining a detectable disinfectant residual is one control measure but it is unknown how effective it is compared to other measures such as building water management planning and implementation, training of plumbers, maintaining hot water temperatures outside the optimal L. pneumophila growth range, and routine flushing. Other building water system factors associated with increased L. pneumophila occurrence and Legionnaires’ disease risk are prolonged stagnation periods and temperature in the range of optimal L. pneumophila growth. Occurrence and growth have been documented for both hot and cold plumbing.

Much has been written on the occurrence of L. pneumophila in building water systems. Since L. pneumophila is an environmental pathogen well-suited for persistence and growth under conditions in
building water systems, it is not surprising that studies (particularly those done with qPCR) document frequent occurrence in building water systems. Some key studies, beginning with studies with national scale and conducted by USEPA researchers, are summarized below.

In their 37-state survey of *L. pneumophila* and *L. pneumophila Sg1* in residential and office building water systems, Donohue et al. (2019) observed frequent, but sporadic occurrence of the pathogens, with *L. pneumophila* detected much more frequently than *L. pneumophila Sg1*. Office buildings and residences were equally likely to have persistent *L. pneumophila*. The concentration of *L. pneumophila* in positive samples from office buildings with persistent *L. pneumophila* was greater than concentrations of positive samples for buildings with only sporadic occurrence; the concentration for sporadically- and persistently-positive residence samples were not statistically different. In a prior study of *L. pneumophila* and *L. pneumophila Sg1* in cold water faucet samples by the same research group (Donohue et al., 2014), the incidence of positive samples was similar for buildings with chloramine and free chlorine disinfectant in the building supply and the incidence of repeat positive samples was higher for buildings connected to free chlorine systems than those connected to monochloramine systems. Also, although the incidence of positive samples was high, the counts of *L. pneumophila* were low compared with a standard used for assessing water quality in the European Union (1000 CFU/L) except for samples from a tap for a building not connected to a public water system.

Based on *Legionella*’s ability to grow in oligotrophic treated drinking waters, de Vos et al. (2005) determined that “…in order to control *Legionella* in the environment, focus should be on the eradication of microbial hotspots in which *L. pneumophila* resides [rather than limiting nutrients].” Several studies have identified either hotspots or water quality conditions in premise plumbing systems that are associated with high *L. pneumophila* occurrence and abundance. Those studies are reviewed in this section and inform the selection and design of control strategies.

A study of water samples from 211 houses in Quebec City (Alary and Joly, 1991) identified use of electrical water heaters (rather than oil or gas) as an important determinant of the occurrence of *Legionella* spp. in premise plumbing systems. Factors associated with occurrence of *Legionella* in electric water heaters and connected plumbing systems included age of water heater (old water heaters were associated with higher incidence of *Legionella* spp.) and water heater temperature (low water heater temperature was associated with higher likelihood of detecting *Legionella* spp.), but not water heater volume. Water heater and hot water storage hydraulics are also associated with likelihood that *L. pneumophila* are present. Ciesielski et al. (1984) observed a significant decrease in the occurrence of *L. pneumophila* positive samples in two hot water storage tanks after instituting continuous operation of two storage tanks that had previously been rotated into and out of service. After instituting continuous operation for the two tanks, no *L. pneumophila* positive samples occurred, whereas tanks that were taken offline and not run continuously continued to have *L. pneumophila* positive samples over the 18-month study period. Despite no detections of *L. pneumophila* in hot water storage tank samples, continuous operation of the hot water storage tanks had little to no impact on the prevalence of detection at showerheads and faucets.
Stagnation in branches of plumbing systems also impacts likelihood of microbial growth. Lautenschlager et al. (2010) observed dramatic increases in cell concentrations (planktonic; measured by flow cytometry), HPC and biomass (measured as ATP concentration) after overnight stagnation. Samples were collected from 10 cold water taps of houses with an unchlorinated water supply. HPC varied widely among first flush samples following stagnation periods and frequently exceeded 300 cfu/mL, the guideline value for Switzerland. HPC was much less variable in samples collected after a five-minute flush and no samples exceeded 300 cfu/mL. Similar results were observed in a study of three homes in Tucson, Arizona (Pepper et al., 2004). Although residual disinfectant concentration is not reported by the authors, it is assumed that disinfectant was present in the water supply because some of the water for the Tucson system is treated surface water. The authors determined HPCs at multiple locations and for multiple sample collection events by plating onto Tryptic Soy Agar via membrane filtration and incubating for 3 days at 27°C. Samples were collected from multiple household locations in seven homes over a three-month period. HPC was highly variable and above 500 cfu/mL in 68% of kitchen and bathroom faucet first draw samples. Flushing consistently reduced HPC, sometimes by as much as one log. These studies indicate that building plumbing systems provide environmental conditions conducive to microbial growth for organisms found naturally in water.

Serrano-Suárez et al. (2013) collected and analyzed 213 samples from hotel and nursing home hot water recirculation systems and conducted regression analysis to determine the factors associated with presence of *L. pneumophila*. While presence/absence is different from growth, presence of *L. pneumophila* indicates that the bacteria are present at a level above the method detection limit and that growth might have occurred. Two sets of samples were collected at each location – first flush and after running taps for 3 min. Higher hot water temperatures were associated with a decrease in *Legionella* detection, whereas higher concentrations of *Pseudomonas aeruginosa* were associated with higher *Legionella* concentration in first-draw samples and higher HPC was associated with higher *Legionella* concentration for samples after 3 minutes of flushing. Other factors such as pH, turbidity, total organic carbon, iron, zinc and copper were not associated with the occurrence of *Legionella*.

The combination of temperature and pipe material determined the ability of *Legionella* to grow in biofilms maintained in dechlorinated filter-sterilized tap water (Rogers et al., 1994). Biofilms were grown in reactors inoculated with sludge from the bottom of a water heater and known to contain *L. pneumophila*. Results of experiments are summarized in Table 2. In general, growth of all organisms and *L. pneumophila* were higher for plastics than copper and the highest *L. pneumophila* growth occurred at 40°C, irrespective of material. For experiments conducted at 20°C, *L. pneumophila* were not detected (detection limit 10 cfu/mL) for model systems containing copper, but were detected for systems containing plastics (polybutylene and chlorinated polyvinylchloride, PVC). At 20°C, several amoeba species, including *Hartmanella vermiformis*, were present. Amoebae and protozoa detected at 20°C were not detected at 40°C or 50°C.
Table 2. Colonization Associated with Different Plumbing Materials and Temperatures (Rogers et al., 1994)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Material</th>
<th>On material surface (cfu/cm²)</th>
<th>In planktonic phase (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total flora</td>
<td>L. pneumophila</td>
<td>Total flora</td>
</tr>
<tr>
<td>20</td>
<td>Copper</td>
<td>2.16×10⁵</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Polybutylene</td>
<td>5.70×10⁵</td>
<td>665</td>
</tr>
<tr>
<td></td>
<td>PVCc</td>
<td>1.81×10⁶</td>
<td>2132</td>
</tr>
<tr>
<td>40</td>
<td>Copper</td>
<td>8.04×10⁴</td>
<td>1967</td>
</tr>
<tr>
<td></td>
<td>Polybutylene</td>
<td>1.18×10⁶</td>
<td>111,880</td>
</tr>
<tr>
<td></td>
<td>PVCc</td>
<td>3.67×10⁵</td>
<td>68,379</td>
</tr>
<tr>
<td>50</td>
<td>Copper</td>
<td>2.26×10⁴</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Polybutylene</td>
<td>4.25×10⁴</td>
<td>868</td>
</tr>
<tr>
<td></td>
<td>PVCc</td>
<td>5.19×10⁴</td>
<td>60</td>
</tr>
</tbody>
</table>

Van Heijnsberg et al. (2015) conducted a literature survey to identify potential reservoirs of *Legionella* with a significant likelihood of causing infection. This study was motivated, in part, by the observation that legionellosis is rare given the ubiquity of infectious *Legionella* in the environment where humans interact. The authors excluded showers and faucets in their study because those sources are the focus of ongoing regulatory efforts in the Netherlands and already established as important reservoirs and routes of infection. Potential reservoirs connected in some way to drinking water supplies and premise plumbing systems include:

- Baths
- Fountains
- Room humidifiers
- Mist machines (at grocery stores)
- Ice/ice machines
- Cooling liquid for machinery
- Foot baths
- Dental units and
- Water used for cleaning.

The level of evidence associating these reservoirs with legionellosis was variable. In general, there is ample opportunity for *L. pneumophila* contamination of appliances and for water uses not directly connected to the building plumbing. The best and most direct way to prevent exposures for these water system features is through maintenance of the features themselves, rather than via changes to the building water system.

**Control of *L. pneumophila***

As illustrated in our review of occurrence of *L. pneumophila* in distribution systems and building water systems, Legionnaires’ disease risks are best managed via partnership between water suppliers and building water system owners and operators, and the greatest potential for risk reduction lies on the building side of the meter. Though public water systems cannot guarantee the provision of water free
of *L. pneumophila*, they can maintain multiple barriers such as a detectable residual, identification and remediation of potential niches for *L. pneumophila*, and providing information to customers about *L. pneumophila* and steps they can take with their water system to reduce risk. Water systems should connect with their public health agencies who oversee Legionnaire’s disease cases and follow-up, in order to provide a consistent public health message and to lend their expertise in water microbiology and disinfection to investigations. Building water systems have many options for reducing legionellosis risk and many resources available to help them formulate and execute risk management strategies.

An overview of *L. pneumophila* exposure and risk management, from source to showerhead, is presented in Figure 1.

Public water systems can contribute to risk management by

- Considering *L. pneumophila* control as a factor when selecting their secondary disinfectant,
- Maintaining detectable residual disinfectant in their treated water,
- Maintaining multiple barriers in the water distribution system,
- Minimizing water age, water leakage, and potential for exposure of drinking water and components with the environment, and
- Communicating with their customers about Legionnaires’ disease and what customers can do to reduce their risk in coordination and cooperation with their public health agencies.

Public water systems (PWSs) practice *L. pneumophila* risk management and maintain multiple barriers to *L. pneumophila* occurrence and transmission by complying with regulations and developing their own best practices through participation in programs such as the AWWA Partnership for Safe Drinking Water. *L. pneumophila* is regulated as a primary drinking water contaminant and a treatment technique under the Surface Water Treatment Rule (SWTR). Using their best science and data at the time the rule was developed, EPA believed that if *Giardia* and viruses are removed/inactivated, according to the treatment techniques in the SWTR, *Legionella* will also be controlled (USEPA, 2009). Barriers public water systems maintain for *L. pneumophila* management and management of other contaminants include

- Covering open water storage facilities to prevent environmental cross contamination,
- AWWA Standard C-651 for disinfection of water main installations and repairs, and when its needed,
- Programs for cross connection control to prevent backflow and contamination,
- Total coliform/chlorine residual sampling representative of the distribution system with requirements for follow-up sampling and find-and-fix,
- Corrosion control programs where needed,
- Standards for managing leakage,
- A requirement to provide a detectable chlorine residual throughout the distribution system, and
- Rigorous requirements for disinfection of mains prior to release into service.

Other practices and barriers required in some states or used as best practices by some PWSs include regulation of storage tank turnover/water age (e.g., a Pennsylvania Department of Environmental Protection guideline to maintain turnover time less than or equal to 5 days at all times or establish and
maintain an optimal water turnover rate at each storage facility), routine and systematic distribution system flushing (LeChevallier, 2020), and temperature control (e.g., by blending cooler water; LeChevallier, 2020).

We disagree with requirements enacted or in review in some states for public water systems conducting distribution sampling for *L. pneumophila* (or *Legionella* spp.) as routine follow-up to cases and outbreaks that might have occurred in their service area. Such sampling makes no contribution to risk management and in fact might impede risk management by obscuring the proximal cause of the exposure that caused the illness. As demonstrated in all studies conducted to date, and based on the physiology and lifestyle of *L. pneumophila*, low concentrations of *L. pneumophila* are not uncommon in treated drinking water and biofilms. Finding low concentrations of *L. pneumophila* in a building water supply is of secondary importance, since amplification of *L. pneumophila* in insufficiently managed building water systems produced the dose and exposure that caused the illness. That is, after a disease case, the most productive focus of an investigation is the conditions in the building water system that led to the exposure that caused the illness. At times public health officials will sample building supply water in an investigation; the decision to sample should be made by public health officials and not be a mandatory component of Legionnaires’ disease case follow-up. Another problem with mandatory sampling after cases is that, in the United States, there are no standards against which to interpret results. Absence of standards creates a de facto zero risk tolerance and is an untenable standard. Mandatory distribution system sampling after cases also poses a risk communication complication – explaining the meaning of data to individuals with limited understanding of *L. pneumophila* and how legionellosis occurs. An important element of risk management is effective risk communication (Masters et al., 2018). Regulators, public health agencies and the drinking water community should share information and data with the public in a responsible way – such that it can be placed in context and such that the public can take productive steps in response. Collecting data that will be released publicly without context is irresponsible and not protective of public health.

The scientific, engineering and public health communities can contribute to improved public health and *L. pneumophila* management by sponsoring and conducting research identifying components of distribution systems where conditions favor growth and persistence of *L. pneumophila*, assessing risks for those components and, where merited, developing strategies for improved *L. pneumophila* management. This research is best approached nationally, since it is outside the fiscal means of individual public water systems and addresses a shared national concern.

Building water system operators have many options for Legionnaires’ disease risk management and many resources at their disposal for developing risk management strategies. Some key resources that have been developed to promote control of *L. pneumophila* in building water systems are:

- The Centers for Disease Control and Prevention Legionnaires’ Disease Toolkit (CDC, 2017)
- The World Health Organization Report “Water Safety in Buildings” (Cunliffe et al., 2011)


• Veterans Affairs report: Prevention of Legionnaires’ Disease in VHA Facilities (Department of Veterans Affairs, Office of Healthcare Inspections, 2008)

• Appendix A of VHA Directive 2008-010 (Department of Veterans Affairs, Veterans Health Administration, 2008)

• The US EPA’s literature review of technologies for Legionella control in premise plumbing systems (USEPA, Office of Water, 2016)

These guidances identify many actions building water system operators can take to reduce risk of legionellosis. Those range from simple steps like flushing stagnant water out of the building water system to more complex options such as supplemental disinfection. Irrespective of the specific measures taken for managing legionellosis risks, the measures are most effective if selected and conducted within a comprehensive water management plan and when they are accompanied by ongoing monitoring and assessment.
Low numbers of *Legionella pneumophila* bacteria are often observed in pipes carrying treated drinking water. Why are they there?

- *L. pneumophila* are environmental organisms, meaning they can grow in the environment and do not rely on animal hosts. Because the bacteria can grow in low nutrient conditions and have developed strategies for evading disinfection, treated drinking water is one of their ecological niches.

What can public water systems do about them?

- Eradication is not a practical or even feasible option.
- Maintain a disinfectant residual (i.e., keep chlorine in the water). Disinfectant kills some organisms and prevents organisms from growing to large populations. The most recent suggestion (based on data from 12 drinking water systems) is that at least 0.1 mg/L is needed to reduce the likelihood of finding *L. pneumophila*.
- Look for *L. pneumophila* in their pipes and tanks and develop strategies for improved *L. pneumophila* control when they are found.
- Let their customers know about *L. pneumophila* and measures customers can take to reduce their risk of getting sick.

Sometimes organisms end up in water used in buildings (Intrusion).

The organisms intruding into the building water system from the water supply usually pose a very low risk of making people sick. Current best estimate is that you’d need to breathe in more than 10 viable virulent bacteria in droplets of just the right size over the course of a shower (or some other water use that aerosolizes the water) to have a 50% chance of infection. Only a portion of those infected develop illness. Some people are more susceptible than others (i.e., some people require an even greater dose to have a 50% chance of becoming ill). The greater risk of intrusion is that low numbers of *L. pneumophila* from the water supply can colonize the building water system and grow. Colonizing means attaching to biofilm on pipe walls and fixtures, surviving and becoming part of the plumbing system ecosystem. The plumbing system and ecosystem protect the bacteria from disinfectants and can provide the bacteria with the food and conditions they need to grow.

Once established, *L. pneumophila* are difficult to eradicate. They shelter from chlorine and other adverse conditions inside other organisms (amoebae that are a common and natural part of building water ecology) and in biofilms. Adverse conditions also “select” the organisms that are most resistant to adverse conditions (only the strong survive— and the survivors multiply).

Given the right conditions, *L. pneumophila* in building water systems grow to large and risky populations (amplification).

*L. pneumophila* grow best in warm, stagnant, turbid (cloudy) water without disinfectants.

What can customers do to reduce the chances that *L. pneumophila* amplify?

- Take heart and take charge— there’s a lot customers can do.
- For large buildings, health care facilities, schools and other large plumbing systems, develop a water management plan (WMP). When done right, WMPs lead to more efficient water usage, better chemical water quality, reduced corrosion and longer service life of building. Water system components.
- Turn water over after long stagnation periods. Many drinking water professionals flush their pipes first thing in the morning to clear out water that degraded while sitting stagnant. The fresh water has disinfectant and doesn’t contain high levels of *L. pneumophila* bacteria counts or other contaminants that accumulate in stagnant water.
- For large buildings, recommended water heater set point temperature is 60°C keep the cold water pipes away from the hot water pipes and out of hot spaces.
- Clean fixtures periodically. Especially showerheads.

*L. pneumophila* that grow in building water systems can flow through showerheads, faucets and other fixtures that make droplets containing the bacteria that might be inhaled and retained in lungs and might cause infection.

Illnesses can result from inhaling droplets (aerosols) containing *L. pneumophila* bacteria; oral (swallowing) and dermal (skin) exposures don’t cause human illness.

Only a portion of droplets with bacteria in them pose a credible risk. Large droplets do not penetrate deeply enough in the lungs for the bacteria to be retained or to reach the part of the lungs where they are best able to initiate an infection. Some of the organisms are exhaled and not retained in the lungs.

What are the factors that make infection and illness more likely?

- High numbers of *L. pneumophila* bacteria (each bacterium is another opportunity for infection).
- A large number of very small droplets (aerosols), and
- Some traits and behaviors like smoking, advanced age, suppressed immune systems make people more susceptible to infection.

Figure 1. How *L. pneumophila* Exposures Occur and Actions Water Systems and Building Water System Operators Can Take to Reduce Risk of Legionellosis (ESPRI)
References


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