HollandWater

The efficacy of various disinfection methods against *Legionella pneumophila* in water systems

A literature review

Dr. N. Walraven and Dr. C. Chapman (version 22-09-2016)

Samenvatting

Controle en beheer van *Legionella* in water distributiesystemen en koeltorens staat hoog op de agenda van internationale gezondheidsorganisaties. Behandeling van *Legionella* gecontamineerd water is essentieel. Er zijn momenteel diverse disinfectiesystemen op de markt ter bestrijding van *Legionella*, waaronder spoelen met warm water, chloreren, filtratie, behandeling met UV licht, ozonbehandeling en koper-zilver ionisatie. In onderhavig onderzoek zijn de effectiviteit en de voor- en nadelen van deze disinfectie methoden gereviewed.

Van de onderzochte disinfectie methoden zijn koper-zilver ionisatie, warmwaterbehandeling, chloreren en behandeling met chlooramine en chloordioxide geschikt om gehele watersystemen mee te behandelen (bv, complexe waterleidingsystemen). Filtratie, UV licht en ozonbehandeling zijn zogenaamde point-of-use en poortwachtersystemen. Met deze technieken is het niet mogelijk om *Legionella* op afgelegen punten in een watersysteem effectief te bestrijden. Alleen het inkomende water of water op een specifieke locatie (bv., specifieke ruimte in een ziekenhuis) kan worden behandeld. Deze methoden zijn met name geschikt om *Legionella* besmettingen te voorkomen in plaats van te behandelen/bestrijden. Filtratie en UV licht, zijn niet corrosief en vormen bovendien ook geen nevenproducten. Deze methoden zijn echter niet geschikt om *Legionella* besmettingen mee te bestrijden in complexe watersystemen.

De effectiviteit van de gereviewde methoden is aangetoond in diverse *in vitro* en *in vivo* studies. De effectiviteit van koper-zilver ionisatie is uitgebreid getest onder zowel laboratorium als praktijk omstandigheden op diverse locaties gedurende enkele jaren. Dit is het niet het geval voor de andere disinfectie methoden. Langdurige testen op verschillende praktijklocaties ontbreken. Bovendien was de effectiviteit van enkele van deze disinfectie methoden inconsistent (bv, warmwaterbehandeling, chloreren, chloordioxide behandeling en ozonbehandeling). Dit werd, onder andere, veroorzaakt door een laag residueel effect (warmwaterbehandeling; ozon) en de lange duur en hogere dosissen die nodig waren om *Legionella* succesvol te bestrijden (chloreren, chloordioxide).

Elke methode heeft zijn voor- en nadelen. Met chloreren, chloramine en chloordioxide kunnen toxische nevenproducten worden gevormd (bv, trihalomethanen, chloraten en bromaten). Bovendien zijn deze methoden corrosief voor staal. Warmwaterbehandeling geeft kans op brandwonden. Met koper-zilver ionisatie worden geen nevenproducten gevormd. De koper- en zilverconcentraties in het behandelde water zijn bovendien beduidend lager dan de drinkwaternormen.

Abstract

Control of *Legionella* bacteria in water distribution systems and cooling towers is an increasing priority for health authorities world wide. Treatment of *Legionella* contaminated water is essential, and a number of approaches are commonly used such as heat and flush, (hyper)chlorination, point-of-use filtration, treatment with ultraviolet (UV) light and copper-silver ionisation. In this study the efficacy, advantages and disadvantages of these disinfection methods are reviewed.

Of the reviewed disinfection methods, copper-silver ionisation, heat and flush, (hyper)chlorination, treatment with monochloramine and chlorine dioxide are systemic methods that can abate *Legionella* in the entire water system (e.g., complex water distribution systems). Point-of-Use filtration, ozonation and UV light are focal disinfection methods and are most often used as gatekeeper systems (treat incoming water) or for local water disinfection (e.g., specific area in a hospital). These methods are not effective at distal points in water distribution systems and are therefore mostly effective for preventing *Legionella* contamination instead of abating existing contaminations.

Efficacy of the reviewed disinfection methods has been proven in both *in vitro* and *in vivo* studies. The efficacy of copper-silver ionisation has been tested extensively under both laboratory and field conditions at multiple locations for several years. For the other reviewed disinfection methods prolonged studies (years) of the efficacy at multiple locations are currently lacking. In addition, efficacy field tests of some disinfection methods were inconsistent (e.g., heat and flush, (hyper)chlorination, chlorine dioxide and ozonation). Possible causes were, amongst others, low residual effect (heat and flush; ozone) and prolonged time or higher dosing needed to successfully abate *Legionella* ((hyper)chlorination; chlorine dioxide).

The reviewed disinfection methods all have their advantages/disadvantages. (Hyper)chlorination, monochloramine, chlorine dioxide and ozonation can form toxic by-products (e.g., trihalomethanes, bromates and chlorates) and are corrosive to steel. Heat and flush can cause scalding. By-products are not formed when using copper-silver ionisation. The dosed Cu and Ag concentration are well below the international drinking water limits and pose no threat to human health. Corrosion caused by copper-silver ionisation, especially to galvanised steel, is not studied yet. The focal disinfection methods Point-of-Use filtration and UV light are not corrosive and do not form harmful by-products. However, these methods are unsuitable to abate (existing) *Legionella* contaminations in complex water distribution systems.

1) Introduction

Legionella bacteria, usually *L. pneumophila* serogroup 1, can cause Legionnaires' disease. This disease was first described in the 1970s. *Legionella* bacteria were identified as the cause of a pneumonia outbreak at the 1976 American Legion Convention in Philadelphia. 221 of those attending the Convention became ill with pneumonia and 34 of those affected died. The responsible bacterium was named *Legionella pneumophila* to honour the stricken legionnaires and pneumophila from the Greek word meaning 'lung loving'. The pneumonia contracted was named Legionnaires' disease.

Legionella contamination can, amongst others, occur in distribution systems of drinking water, cooling water (e.g., in cooling towers), fountains and swimming pools. In particular, aerosolised water droplets from such contaminated water systems pose significant health risks to people. *Legionella* can cause devastating disease in humans, it is important to prevent water systems from becoming contaminated and to control the risk of exposure. The control of hazardous pathogens, such as *Legionella* in water distribution systems and cooling towers, is therefore a priority for health authorities world wide and leading to increasingly onerous legionella risk assessments and control requirements being placed on owners and operators of water distribution systems. Treatment of contaminated water is essential, and a number of approaches are commonly used such as heat and flush, (hyper)chlorination, point-of-use filtration, treatment with ultraviolet (UV) light and copper-silver ionisation.

To our knowledge, there is not an up-to-date overview of the performance characteristics of the various disinfections methods that are currently on the market. By order of Holland Water, GeoConnect conducted a literature review to summarise and evaluate the available information. The reviewed methods are: copper-silver ionisation, heat and flush, oxidizing substances (e.g., chlorine, monochloramine, chlorine dioxide and ozone), Point-of-Use filtration and UV light.

2) Methods

This literature review is based on an inventory of scientific publications. The literature search was performed on the internet and in databases of university libraries. The libraries and databases which were consulted are listed below. In addition, the keywords used for the search are shown. This literature search doesn't pretend to be comprehensive.

- Libraries: VU Amsterdam, The Netherlands.
- Databases: Google scholar and ScienceDirect
- Keywords: Legionella, pneumophila, disinfection, method, efficacy, water, contamination, copper, silver, ionisation, heat and flush, filtration, UV, light, oxidizing, substances, chlorination, ozone, peroxide, control, abatement, etc.

3) Results

The results of the literature review are summarised in Table 1 - 5.

Table 1. Working principles of the disinfection methods.

Method	Disinfection principle	References
1. Copper-silver ionisation	Positively charged copper and silver ions form electrostatic bonds with negatively charged sites on bacterial cell walls. These electrostatic bonds create stresses leading to distorted cell wall permeability; coupled with protein denaturation this leads to cell lysis and cell death (Pedro-Botet et al., 2007; Bedford, 2012). Importantly, some authors have demonstrated that these ions are able to penetrate the biofilms in which other bacteria, algae, protozoans, and fungi, cohabit with *Legionella* species in water pipes (Liu et al., 1998; Exner et al., 2005).	Liu et al. (1998); Exner (2005); Pedro-Botet et al. (2007); Bedford (2012)
2. Heat and flush	Bacteria, amongst others Legionella, are killed by a heat shock (T > 60°C). By flushing the systems, the heated water reaches all parts of the systems and biofilm is removed.	Groothuis et al. (1985); Lee et al. (1988)
3. Oxidizing substances	Oxidizing substances can oxidize other substances. These substances contain oxygen and/or hydroxyl radicals. These are molecules with one or more unpaired electrons. Radicals are very reactive; they easily pick up electrons from other substances. Radicals react with, amongst others, DNA, lipids and proteins. If there are enough free radicals, the cell is damaged to such an extent that it can no longer function and thus dies.	Westerlaken (2006)
a. Chlorine (hyperchlorination)		Kim et al. (2002)
b. Monochloramine		-
c. Chlorine dioxide		Bernarde et al. (1967); Kim et al. (2002)
d. Ozone		Kim et al. (2002)
4. Point-of-Use filtration	Filters with small enough pore sizes (e.g. 0.2 μm) remove bacteria, amongst other Legionella and Mycobacteria, from the water system.	Lin et al. (2011)
5. UV light	UV-light with a wavelength of 10 to 400 nm kills micro-organisms; 254 nm is the most lethal wavelength. Energy absorption leads to changes in the base-paring in the DNA. This causes creation of thymine dimers and DNA gaps of essential genes of, amongst other, *Legionella*. Theses damages results in the inactivation of *Legionella* bacteria.	Liu et al. (1995); Lin et al. (1998A); Kim et al. (2002); Westerlaken (2006)

Table 2. Application principles of the disinfection methods.

Method	Application principle	References
1. Copper-silver ionisation	The method is based on channelling water (at the point-of-entry) through a device that applies low potential electricity to copper and silver electrodes. This releases positively charged copper and silver ions to the water.	Walraven et al. (2015)
2. Heat and flush	Thermal disinfection is carried out by raising the temperature of the hot water system to at least 60 °C and flushing all outlets, faucets and showerheads for at least 1 hour (?). The hot water circulating loop should be designed to give a return temperature to the calorifier of at least 50 °C, with 55 °C at the supply to the draw-off point farthest away in the circulating system. Others document that the water temperature at the distal outlet must be at least 60 °C (instead of 55 °C).	Anonymous (2006); Moore and Walker (2014); Kelsey (2014) Lin et al. (1998A).
3. Oxidizing substances		
a. Chlorine	Chlorine is added to water as chlorine gas or hypochlorite salts (sodium or calcium hypochlorite). Free chlorine (hypochlorite) can also be generated in situ from 1) chloride naturally present in water or 2) extra added sodium chloride, in an anodic oxidation device. This is, among others, called electrochemical disinfection or anodic oxidation.	Westerlaken (2006) Kraft (2008); CTGB (2016)
b. Monochloramine	Monochloramine is generated on-site by mixing hypochlorous acid with ammonia. Chloramines are derivatives of ammonia by substitution of one, two or three hydrogen atoms. Monochloramine, dichloramine and trichloramine are produced by adding chlorine to a solution containing ammonia.	Lin et al. (2011) Kelsey (2014)
c. Chlorine dioxide	Chlorine dioxide is a gas in solution that is typically generated on site. Methods for producing chlorine dioxide include controlled mixing of chemical precursors (e.g., sodium chlorite and a strong acid) or electrochemical generation.	Kim et al. (2002); Lin et al. (2011).
d. Ozone	Ozone is dissolved into the water system to achieve a dose of about 1 to 2 mg/L, ideally via a generator that produces ozone in proportion to the water flow rather than a generator that produces ozone at a constant rate regardless of demand.	Association of Water Technologies (2000)
4. Point-of-Use filtration	Filters (e.g. 0.2 μm) can be installed at any point in the water system. For example, at the inlet point, but also at the tap-points.	Westerlaken (2006)

Method	Application principle	References
5. UV light	The UV-lamp is placed at the point-of-entry (inlet) of the water system. UV-lamps can also be attached to the tap-points of the system to treat the more distal sites in the water system.	Westerlaken (2006)

Table 3. Efficacy of the disinfection methods.

Method	Efficacy	References
1. Copper-silver ionisation	The recommended concentrations for total *Legionella* eradication are between 0.2 and 0.4 mg/l Cu and between 0.02 and 0.04 mg/l silver.	Liu et al. (1994); Lin et al. (1996); Lin et al. (2011).
	Efficacy proven in *in vitro* studies.	Landeen et al. (1989); Lin et al. (1996); Hwang et al. (2007)
	Efficacy proven in tens of *in vivo* (field) studies.	Colville et al. (1993); Liu et al. (1994, 1998); Biurrun et al. (1999); Stout et al. (2000); Stout and Yu (2003); Kusnetsov et al. (2001); Lee et al. (2002); Cachafeiro et al. (2007); Maki et al. (2007); Modol et al. (2007); Chen et al. (2008); Lin et al. (2000, 2011); Barbosa and Thompson (2016).
	In three field studies copper-silver ionisation was not effective. However, in two of these studies the silver concentration was <10 μg/l (below effective concentration) and in the other a phosphate compound was added to control corrosion (this may have interfered with the biocidal strength of the coper and silver).	Rohr et al. (1999); Mathys et al. (2002); Blanc et al. (2005).
	Copper and silver are also bactericidal (*in vitro*) against Legionella and other waterborne pathogens, including *Pseudomonas auruginosa, Stenotrophomonas maltophilia, Acinetobacter baumannii* and mycobacterial species.	Lin et al. (2011)

Method	Efficacy	References
2. Heat and flush	Temperatures greater than 60 °C are considered inhibitory for *Legionella* spp. and other non-sporulating bacteria.	Kim et al. (2002)
	Efficacy proven in *in vitro* studies.	Dennis et al. (1984); Stout et al. (1986); Sanden et al. (1989)
	Efficacy proven in *in vivo* (field) studies (e.g., several hospitals and domestic buildings).	Plouffe et al. (1983); Groothuis et al. (1985); Vickers et al. (1987); Lee et al. (1988); Alary and Joly (1991)
	However, recolonization *with L pneumophila* has been reported to occur in hospitals after superheat-and-flush procedure, followed by new cases of hospital-acquired legionnaires' disease.	Snyder et al. (1990); Heimberger et al. (1990)
	Superheating (two days > 60 °C) at distal points resulted in a small (but non-significant) reduction in contamination within the first month after which values returned to baseline (*Legionella* contaminated)	Marchesi et al. (2011)
	When maintaining hot water temperatures at 60 °C *Legionella* can be controlled more effectively (longer period)	Muraca et al. (1990); Furuhata et al. (1994); Mermet et al. (1995)
	In a recent study, Kruse et al. (2016) have shown that thermal infection was only effective in fewer than half of the studied buildings (n=718).	Kruse et al. (2016).
3. Oxidizing substances		Westerlaken (2006)
a. Chlorine (Hyperchlorination)	Due to the solubility of chlorine in water, the water temporarily obtains an acidic character. With a pH below 7.6, hypochloric acid appears in its normal form (HOCl) and with a pH of more than 7.6 in ionic form (H+ and OCl-). Kim et al (2002) have shown that HOCl influences (inhibits) micro-organisms more than H+ and OCl-. The recommended concentration for shock	Kim et al. (2002); Westerlaken (2006).

Method	Efficacy	References
	treatment is 10-50 ppm for 12-24 h. For continuous treatment 1-2 ppm is recommended.	Orlando et al. (2005)
	The recommended concentration for in situ chloride generation (by anodic oxidation) is 0.3 mg/l with a minimum and maximum of 0.2 and 0.5 mg/l respectively.	CTGB (2016)
	Continuous hyperchlorination has been used with variable success to control the growth of *L pneumophila* in both *in vitro* and *in vivo* studies.	Lin et al. (1998A) Effective: e.g., Skaliy et al. (1980); Shands et al. (1985); Thomas et al. (1999); McCall et al. (1999) Not effective: e.g., Kilvingston and Price (1990); Hamilton et al. (1996); Marchesi et al. (2011; Legionella returned after 2 months)
	Chlorine may only suppress *Legionella* rather than kill. Especially when Legionella bacteria are associated with protozoa such as amoeba.	Lin et al. (1998A); Kim et al. (2002)
	Limited penetration of chlorine into the biofilm matrix.	De Beer et al. (1994); Chen and Stewart (1996)
	To continuously *control L. pneumophila* in hospital hot water, chlorine concentrations of 2-6 mg/l are reportedly needed. This is much higher than the typical chlorine concentration (~ 1 mg/l) in domestic potable water.	Helms et al. (1988); Snyder et al. (1990); Lin et al. (1998A, 1998B); Kim et al. (2002)
	Tap water spiked with *L. pneumophila* (10^4 cfu) that was passed through an electrolysis cell (in situ generated free chlorine; 0.07 – 0.28 mg/l free oxidants) was completely disinfected. The residual effect reduces *L. pneumophila* but a complete killing was not realised.	Delaedt et al. (2008)
	Efficacy data (peer-reviewed and published) for in situ generated free chlorine (electrochemical disinfection / anodic	

Method	Efficacy	References
	oxidation) under field conditions (at multiple locations for several years) are currently lacking.	
b. Monochloramine	The typical dosage is 1-10 mg/l. Monochloramine is effective against *Legionella in vitro* and against biofilm-associated *Legionella* in model plumbing systems. Monochloramine is effective in reducing Legionella counts in hot water systems in municipal building and in hospitals. It is, however, noted that studies have not yet been conducted over prolonged periods. Increase of Mycobacterium, coliforms and heterotrophic bacteria occurred when monochloramine was introduced in municipal water supplies.	Kim et al. (2002) Gao et al. (2000); Donlan et al. (2002); Lin et al. (2011) Kool et al. (1999, 2000); Shelton et al. (2000); Heffelfinger et al. (2003); Pryor et al. (2004); Flannery et al. (2006); Moore et al. (2006); Marchesi et al. (2009; 2011); Lin et al. (2011) Pryor et al. (2004); Moore et al. (2006)
c. Chlorine dioxide	The target concentration is 0.3 – 0.5 mg/l at the point of use. Efficacy proven in *in vitro* study in continuous flow reactor. Reports on the efficacy based on various field studies are contradictory: in several field studies chlorine dioxide was effective in eradicating Legionella and in others it wasn't. Prolonged time (often >1 year) was necessary to demonstrate significant reductions in the *Legionella* positivity rate.	European Standard BS EN 12671:2000) Botzenhart et al. (1993) Lin et al. (2011) Effective: e.g., Makin (1998); Hood et al. (2000); Sidari et al. (2004); Walker et al. (1995); Zhang et al. (2007); Marchesi et al. (2011; *Legionella* strongly significantly reduced but not eradicated) Not effective: e.g., Hosein et al. (2005); Ricci et al. (2005) Lin et al. (2011); Kelsey (2014)
d. Ozone	The recommended concentration is 1-2 mg/L. Ozone is unstable, and inactivation can	Association of Water Technologies (2000) Farooq et al. (1977)

Method	Efficacy	References
	occur by both gaseous ozone and dissolved ozone. Efficacy proven in *in vitro* studies. Efficacy proven in *in vivo* studies (e.g., model cooling tower). Inconclusive results on the efficacy of ozone in eradication of Legionella in potable water in a hospital building. The mean ozone residual concentration was, however, 0.79 mg/l.	Edelstein et al. (1982); Domingue et al. (1988) McGrane (1995) Edelstein et al. (1982)
4. Point-of-Use filtration	Efficacy proven in a controlled study with contaminated water from a hospital. Point-of-use filters completely eliminated *L. pneumophila* (and *Mycobacterium*) from the hot water samples. No contamination was observed at outlets in an Italian university hospital where filters were used. Efficacy also proven in high-risk areas such as transplant units.	Sheffer et al. (2005) Marchesi et al. (2011) Campins et al. (2000)
5. UV light	UV-light with a wavelength of 10 to 400 nm kills micro-organisms; 254 nm is the most lethal wavelength. Efficacy proven in *in vitro* studies. Efficacy proven in *in vivo* study (model plumbing system). Not effective at distal sites in 2 hospitals. Combination of UV with other treatment modalities was effective for individual hospitals.	Liu et al. (1995); Lin et al. (1998A); Kim et al. (2002); Westerlaken (2006) Gilpin (1984); Yamamoto et al., 1987); Martiny et al. (1989); Lin et al. (1998A, 1998B). Muraca et al. (1987) Eckmanns et al. (2002); Franzin et al. (2002) Matulonis et al. (1993); Franzin et al. (2002); Triassi et al. (2006)

Method	Efficacy	References
	In a new hospital (no biofilm established) UV was effective (over a period of 13 years).	Hall et al. (2003)

Tab le 4a. Disinfection method characteristics: advantages / disadvantages copper-silver ionisation.

Disinfection method	**Advantages**	**References / remarks**	**Disadvantages**	**References / remarks**
Copper-silver ionisation	This method that has been evaluated in tens of peer-reviewed laboratory (*in vitro*) and field studies (e.g., hospitals, long-term care facilities, office buildings, apartment buildings and cooling towers).	Colville et al. (1993); Liu et al. (1994, 1998); Miuetzner et al. (1997); Biurrun et al. (1999); et al. (2000); Stout and Yu (2003); Kusnetsov et al. (2001); Lee et al. (2002); Cachafeiro et al. (2007); Maki et al. (2007); Modol et al. (2007); Chen et al. (2008); Lin et al. (2000, 2011); Walraven et al. (2016; in prep.)	Indications for *Legionella* resistance to Ag. This is, however, based on indirect evidence (non-persistent reduction of *Legionella*). Silver dosing was <10 μg/l. The low Ag concentration (below effective Ag concentration) probably caused the non-persistent reduction instead of resistance of *Legionella* to Ag.	Rohr et al. (1999).
	Residual activity (due to accumulation of Cu and Ag ions inside biofilm)	Liu et al. (1994, 1998)	pH > 7.6 might influence the efficacy (of ionisation).	Lin et al. (2002, 2011)
	May penetrate biofilm	Liu et al. (1994, 1998)	Low ion concentrations may compromise the efficacy of ionisation.	Lin et al. (2011)
	Also effective against other waterborne pathogens (e.g., *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia, Acinetobacter baumannii* and mycobacterial species.	Lin et al. (2011)	Indications for corrosion of galvanised steel. However, copper was highly overdosed in this study (>2 mg/l) and copper pipes were connected to galvanised pipes, which can result in	Loret et al. (2005)

Disinfection method	Advantages	References / remarks	Disadvantages	References / remarks
			galvanic corrosion. Well performed corrosion studies are lacking.	
	Not disadvantaged by heat	Yang (2000); Lin et al. (2011)	Excessive ion levels have led to blackish discoloration of water and lavender discoloration of porcelain sink surfaces.	Lin et al. (1998A); Triantafyllidou et al. (2016)
	Easy installation and maintenance	Lin et al. (2011)	Relatively high concentrations of copper and silver can accumulate in sediment at the bottoms of the hot water tanks.	Miuetzner et al. (1997)

Table 4b. Disinfection method characteristics: advantages / disadvantages heat and flush.

Disinfection method	Advantages	References / remarks	Disadvantages	References / remarks
Heat and flush	Effective against all pathogenic bacteria and protozoa.	Kelsey (2014)	Thermal disinfection is a temporary measure. The water system will be recolonised in weeks when water temperatures are lowered to baseline values (<60 °C).	Lin et al, (1998B)
			Does not work when mixer valves are present that control temperature to a maximum of 44 °C.	Kelsey (2014)
			Added risk of scalding	Kelsey (2014)

Table 4c. Disinfection method characteristics: advantages / disadvantages chlorine (hyperchlorination).

Disinfection method	**Advantages**	**References / remarks**	**Disadvantages**	**References / remarks**
Chlorine (Hyperchlorination)	Continuous dosing to maintain 1-2 ppm of free (available) chlorine. Provides residual disinfection throughout the entire water distribution system so that colonisation of *L. pneumophila* at the distal sites can be minimised.	Kelsey (2014); Lin et al. (1998A)	Poor penetration of protozoa and biofilm. Chlorine may only suppress *Legionella* rather than kill. Long-term effectiveness is limited.	Lin et al. (1998A, 1998B; Kim et al. (2002); Kelsey (2014)
			Corrosive	Grosserode et al. (1993); Lin et al. (1998A, 1998B); Kelsey (2014)
			Compromised by higher temperatures.	Yang (2000); Lin et al. (2002)
			High residual chlorine can react with organic materials and accelerate the production of trihalomethanes, which is known carcinogen. A positive association has been found for consumption of chlorinated water and cancer in numerous epidemiological studies.	Lin et al. (1998A, 1998B); Nieuwenhuijsen et al. (2000); Kim et al. (2002); Ortolano et al. (2005)

Table 4d. Disinfection method characteristics: advantages / disadvantages monochloramine.

Disinfection method	**Advantages**	**References / remarks**	**Disadvantages**	**References / remarks**
Monochlor-amine	Provides a stable residual (more stable than chlorine) that penetrates biofilm.	Kim et al. (2002); Lin et al. (2011): Kelsey (2014)	Can cause anemia in patients undergoing hemodialysis.	Lin et al. (2011)
	Has a wider working pH range than copper-silver ionisation and chlorine.	Lin et al. (2011): Kelsey (2014)	On-site generation of monochloramine is complicated.	Lin et al. (2011)
			The smell of ammonia in drinking water is unpleasant.	Lin et al. (2011)
			Increased populations of other micro-organisms (e.g., *Mycobacterium* species), presence of nitrogen by-products and increased lead leaching in drinking water.	Lin et al. (2011)
			Less active than chlorine.	Kelsey (2014)
			Low activity against protozoa (and viruses).	Kelsey (2014)
			More difficult to remove from water than chlorine or chlorine dioxide (e.g., water used for dialysis).	Ortolano et al. (2005)

Table 4e. Disinfection method characteristics: advantages / disadvantages chlorine dioxide.

Disinfection method	Advantages	References / remarks	Disadvantages	References / remarks
Chlorine dioxide	Penetrates biofilm more effectively than chlorine.	Lin et al. (2011); Kelsey (2014)	Prolonged treatment before Legionella concentration drops (often >1 year).	Lin et al. (2011); Kelsey (2014)
	Less corrosive than chlorine.	Lin et al. (2011); Kelsey (2014)	Difficult to maintain the effective residual concentration (0.3-0.5 mg/l).	Lin et al. (2011)
	Wider pH range for activity than chlorine and Cu/Ag ionisation	Lin et al. (2011); Kelsey (2014)	Indications that chlorine dioxide kills *Legionella* in the stream but not those inside protozoa.	Marchesi et al. (2011)
	Residual activity	Kelsey (2014)	Conversion to potentially toxic chlorates and chlorites.	Lin et al. (2011); Kelsey (2014)
			Water-soluble gas, explosive when compressed, therefore produced locally	Kelsey (2014)
			Compromised by higher temperatures.	Yang (2000); Lin et al. (2011)

Table 4f. Disinfection method characteristics: advantages / disadvantages ozone.

Disinfection method	**Advantages**	**References / remarks**	**Disadvantages**	**References / remarks**
Ozone	Effective only at point of use; use can be limited to high risk areas or known contaminated taps.	Kelsey (2014)	Effective only at point of use	Kelsey (2014)
	Effectiveness of ozone not markedly affected by pH or temperature. However, others reported that ozone was somewhat more effective at lower temperature and higher pH.	Domingue et al. (1988). Botzenhart et al. (1993)	By-product formation in presence of bromide (bromate) and chlorine (chlorate). The toxicological impact of bromate and chlorate formed, when using ozone, is unclear.	Von Gunten (2003)
			Corrosive to metals	Kelsey (2014)
			Degrades rubber	Kelsey (2014)

Table 4g. Disinfection method characteristics: advantages / disadvantages point-of-use filtration.

Disinfection method	**Advantages**	**References / remarks**	**Disadvantages**	**References / remarks**
Point-of-Use filtration	Effective only at point of use; use can be limited to high risk areas or known contaminated taps.	Kelsey (2014)	Effective only at point of use.	Kelsey (2014)
	Cost- effective and better tolerated by (hospital) patients	Ortolano et al. (2005); Lin et al. (2011)	Potential contamination of the filter sprout.	Kelsey (2014)
	Eliminates also other waterborne pathogens (e.g., Mycobacterium and *Pseudomonas aeruginosa*).	Campins et al. (2000); Trautmann et al. (2008)	Require to be changed frequently (1-3 times monthly or as per manufacturers' instructions).	Kelsey (2014)

Table 4h. Disinfection method characteristics: advantages / disadvantages UV light.

Disinfection method	Advantages	References / remarks	Disadvantages	References / remarks
UV light	Effective only at point of use; use can be limited to high risk areas or known contaminated taps.	Liu et al. (1995); Kelsey (2014)	No residual protection	Liu et al. (1995)
	Easy installation, low expense and no adverse effects on water or plumbing	Liu et al. (1995)	Scale accumulation on UV lamps. Filters needed to prevent scale accumulation.	Liu et al. (1995)
			UV irradiation alone is insufficient to control *Legionella*. Other methods, such as periodic hyperchlorination and heat pasteurization, have to be used along with UV radiation for an effective control of *Legionella*.	Kim et al. (2002)
			Effective only at point of use.	Kelsey (2014)

Table 5. Comparison of the efficacy, disadvantages, installation ease, maintenance and costs of various disinfection methods against *Legionella pneumophila* in water systems

Method	Efficacy Short term (months)	Efficacy Long-term (years)	Disadvantages	Ease of installation	Maintenance	Costs	References
Copper-silver ionisation	Effective	Effective	- The electrodes accumulate scale and must be cleaned regularly. - Level of coper and silver may fluctuate. - Excessive ion levels have led to blackish discoloration of water and lavender discoloration of porcelain sink surfaces. - Long-term treatment with copper and silver ions could theoretically result in the development of resistance to these ions.	Easy	Easy	Low-Moderate	Lin et al. (1998A)
	-	-	- Cu and Ag added to drinking water. - Works well only on water with low dissolved solids content.	Fair	Moderate	Low-Moderate	Ortolano et al. (2005)
	Effective	Effective	- Elevated water pH and low ion concentrations may compromise the efficacy of ionization. - Resistance of *Legionella pneumophila* is theoretically possible. - Various countries require	Easy	Easy	Low-Moderate	Lin et al. (2011)

Method	Efficacy Short term (months)	Efficacy Long-term (years)	Disadvantages	Ease of installation	Maintenance	Costs	References
			ionization systems to register as a biocide.				
Heat and flush	Good (but temporary)	Poor	- Time consuming procedure. - Numerous personnel involved to monitor distal sites, water tank temperatures, and flushing times. - Scalding can occur. - It is a temporary systemic disinfection method. Recolonization will occur within weeks to months after a superheat-and-flush protocol.			Low (least expensive of all systemic disinfection methods)	Lin et al. (1998A)
	Good	Poor	- Failure to maintain consistent temperature. - Recolonization at low temperature. - Hard to reach all tap points with dead-leg piping and antiscald valves. - Scalding potential. - Recontamination occurs in 30-60 days. - Increase in biofilm sloughing possible. - May not penetrate biofilm.	Easy	Easy	Low	Ortolano et al. (2005)
Chlorine (hyperchlorination)	Effective (shock and	Effective (only	- Chlorine is highly corrosive and cause significant pipe	-	-	Moderate-High (extra	Lin et al. (1998A)

Method	Efficacy Short term (months)	Efficacy Long-term (years)	Disadvantages	Ease of installation	Maintenance	Costs	References
	continuous tretament)	continuous tretament)	damage. - Chlorine may only suppress *Legionella* rather than kill. - High residual chlorine will react with organic materials, which is a known carcinogen.			costs due to corrosion damage)	
	Good	Fair	- Recolinization after system disinfection. - *Legionella* species more resistant to chlorination. - System corrosion causes pipe leaks and can promote biofilm formation. - Carcinogenic byproducts (trihalomethanes). - Chlorine levels checked frequently. - Does not penetrate into center of established biofilm.	Difficult; must hold 10-50 ppm for 12-24 hr in case of shock method; 1-2 ppm for continuous dosing method	Fair-Difficult	High	Ortolano et al. (2005)
	-	-	- Already reviewed by Lin et al. (1998A). Since then virtually all reviewed hospitals (n=17) have since converted to other methods of disinfection. - Hyperchlorination was found to be the most unreliable and also the most expensive disinfection modality. - It has met with increasing	-	-	High	Lin et al. (2011)

Method	Efficacy Short term (months)	Efficacy Long-term (years)	Disadvantages	Ease of installation	Maintenance	Costs	References
			disfavour because of inadequate penetration of the agent into biofilms in piping, persistence of Legionella organisms in hyperchlorinated systems, corrosion of water distribution systems leading to pinhole leaks over time, and introduction of carcinogens into the drinking water.				
Monochloramine	Good	-	- More difficult to remove from water than chlorine or chlorine dioxide (e.g., water used for dialysis). - May not penetrate into biofilm.	Fair	Moderate	Moderate	Ortolano et al. (2005)
	Effective	-	- Monochloramine can cause anemia in patients undergoing hemodialysis. - The on-site generation of monochloramine can be complicated. - The smell of ammonia in drinking water is unpleasant.	-	-	-	Lin et al. (2011)
Chlorine dioxide	Good	Poor	- Unknown corrosive properties. - Unknown maintenance of effective concentration in	Fair	Fair-Difficult	Low-Moderate	Ortolano et al. (2005)

Method	Efficacy Short term (months)	Efficacy Long-term (years)	Disadvantages	Ease of installation	Maintenance	Costs	References
			hot water systems. - Does not penetrate completely into biofilm.				
	Inconsistent results (both effective and not effective)	Inconsistent results (both effective and not effective)	- A prolonged time is necessary to demonstrate significant reductions in the *Legionella* positivity rate (often >1 year). - The residual concentration in hot water is low (<0.1 mg/l) when chloride dioxide is injected into the incoming cold water at a concentration of 0.5 – 0.8 mg/l. Reactions with organic matter and corrosion scale in piping can cause rapid conversion to its byproducts chlorite and chlorate. These byproducts may pose health risks for customers. - Corrosion of galvanized steel pipes can cause loss of chlorine dioxide by reaction with magnetite; this may affect efficacy.	-	-	Low-Moderate	Lin et al. (2011)
Ozone	Good	Poor	- Disinfects only at the point of injection (Decomposes quickly in hot water. Hard to hold effective	Difficult	Moderate	High	Ortolano et al. (2005)

Method	Efficacy Short term (months)	Efficacy Long-term (years)	Disadvantages	Ease of installation	Maintenance	Costs	References
			concentration.)				
Point-of-Use filtration	Good	Good	- Correct installation essential for bacterial removal.	Easy, immediate barrier	Simple	Low	Ortolano et al. (2005)
	Good	Good	- Mainly used for prevention of nosocomial infections due to *Legionella*.	-	-	Low	Lin et al. (2011)
UV light	Effective	Effective	- Point-of-entry-application. Lack of residual protection at distal sites. - Frequent systemic disinfection (e.g., superheat-and-flush) is required to provide additional protection. - The quartz sleeves housing the ultraviolet lamps are susceptible to scale and mineral deposits and must be cleaned regularly. - Prefiltration is strongly recommended to prevent accumulation of scale on the quartz sleeves (that would comprise the intensity of the ultraviolet irradiation).	Easy	-	Low-Moderate (depends on other measures necessary (e.g. prefiltration)	Lin et al. (1998A)
	Good	Fair	- Scale problems. - Poor penetrating power of UV light in established biofilms.	Fair, local effect	Moderate, cleaning for effective energy	Moderate	Ortolano et al. (2005)

Method	Efficacy Short term (months)	Efficacy Long-term (years)	Disadvantages	Ease of installation	Maintenance	Costs	References
					transmission		
	Effective	Effective	- Its point-of-entry application does not allow distal eradication. -	-	-	Low-Moderate (depends on other measures necessary (e.g. prefiltration)	Lin et al. (2011)

4. Discussion

4.1 Efficacy

Three types of disinfection methods for the abatement of *Legionella* can be distinguished, 1) the point-of-use methods, 2) the gatekeeper systems and 3) the methods that can treat the entire water systems, e.g., drinking water distribution system and cooling tower. Point-of-Use filtration is a point-of-use method and ozonation and UV light are gatekeeper systems. Efficacy of the point-of-use methods and gatekeeper systems has been proven in both *in vitro* and *in vivo* studies (Table 3). *Legionella* has only been prevented and/or abated successfully at the point of use or at the gate (water entry). At distal sites in water systems, these methods are not or less effective. Although ozone is dosed to water and transported throughout the entire water system, it decomposes quickly (especially in hot water) and it is therefore hard to hold the effective concentration at distal points.

Disinfection methods that disinfect the entire water system are copper-silver ionisation, heat and flush, chlorination and treatment with monochloramine and chlorine dioxide. Efficacy of these methods has been proven in both *in vitro* and *in vivo* studies (Table 3). However, according to Lin et al. (2011), copper-silver ionisation is the only disinfection technology for the abatement of *Legionella* that has been validated by the 4-step standardised evaluation criteria for disinfection methods:

1. Efficacy against *Legionella* is demonstrated in both *in* vitro and *in* vivo tests (Table 3).
2. Reports of anecdotal experience of efficacy in controlling *Legionella* in individual cases (e.g., individual hospitals) are available.
3. Controlled studies of prolonged duration (years) of efficacy in controlling *Legionella* in individual cases (e.g., individual hospitals) are published in open and peer-reviewed reports.
4. Confirmatory reports of multiple (independent) locations (e.g., multiple hospitals) of prolonged duration of follow-up are available.

For heat and flush, chlorination and treatment with monochloramine and chlorine dioxide prolonged studies (years) on the efficacy at multiple locations are lacking.

Although, efficacy has been proven for copper-silver ionisation, heat and flush, chlorination and treatment with monochloramine and chlorine dioxide, *Legionella* control was not successful at some field locations. For example,

- In 3 field studies copper-silver ionisation was not effective (Rohr et al., 1999; Mathys et al., 2002; Blanc et al., 2005) (Table 3). However, in two of these studies the silver concentration was lower than the recommended concentrations of 0.02 to 0.04 mg/l silver (silver concentration was <10 μg/l). In the other study, a phosphate compound was added to control corrosion. This may have interfered with the efficacy of ionisation.
- In a recent study, Kruse et al. (2016) have shown that thermal infection was only effective in fewer than half of the studied buildings (n=718). In addition, Snyder et al. (1990) and Heimberger et al. (1990) reported that recolonization *with L pneumophila* occurred in hospitals after superheat-and-flush procedure, followed by new cases of hospital-acquired legionnaires' disease.

- To continuously control *L. pneumophila* in hospital hot water, chlorine concentrations of 2-6 mg/l are reportedly needed (Helms et al., 1988; Snyder et al., 1990; Lin et al., 1998A, 1998B; Kim et al., 2002). This is much higher than the typical chlorine concentration (~ 1 mg/l) in domestic potable water. Chlorine may only suppress *Legionella* rather than kill (Lin et al., 1998A; Kim et al., 2002). Especially when *Legionella* bacteria are associated with protozoa such as amoeba. In addition, limited penetration of chlorine into biofilm has been observed by De Beer et al. (1994) and Chen and Stewart (1996). This might explain why in some studies chlorine was not effective in the abatement of *Legionella* (e.g., Kilvingston and Price, 1990; Hamilton et al., 1996).
- The efficacy of monochloramine has not been studied over prolonged periods yet.
- Reports on the efficacy of chlorine dioxide – based on field studies – are contradictory: in several field studies chlorine dioxide was effective in eradicating *Legionella* (e.g., Walker et al., 1995; Makin, 1998; Hood et al., 2000; Sidari et al., 2004; Zang et al., 2007) and in others it wasn't (e.g., Hosein et al., 2005; Ricci et al., 2005). Prolonged time was necessary to demonstrate significant reductions in the *Legionella* positivity rate (Lin et al., 2011; Kelsey, 2014).

The efficacy of disinfection techniques depends on various factors, such as the physical and chemical water composition (including additives such as corrosion inhibitors) and the residual effect of disinfectants (see also Section 4.2). This should be taken into consideration when selecting a disinfection technique for *Legionella* abatement.

4.2 Advantages/disadvantages

All *Legionella* disinfection techniques have some advantages and disadvantages (Table 4-12). The main advantages/disadvantages that can be distinguished are:

1) External conditions that influence efficacy
2) Human health risks
3) Interaction with contact surfaces (e.g., corrosion of piping)

External conditions that influence efficacy

The efficacy of disinfection techniques is influenced by various (external) factors, such as the physical (e.g., temperature) and chemical composition (e.g., pH and ion concentration) of the treated water.

Disinfection techniques that are influenced by water temperature are heat and flush, hyperchlorination and ozonation. The efficacy of heat and flush strongly depends on hot water temperature control (temperatures must be controlled at 60 °C). Failure to reach and/or maintain this temperature can result in recolonization with L*egionella* (Ortolano et al., 2005). Heat and flush does not work when mixer valves are present that control temperature to a maximum of 44 °C (Kelsey, 2014). Hyperchlorination and ozonation are comprised by higher temperatures (Yang, 2000; Lin et al., 2011; Ortolano et al., 2005). Ozone quickly decomposes in hot water which makes it more difficult to hold the effective concentration (at distal sites). The effect of water temperature on the efficacy of chlorination is more complex. Free chlorine in water is present in the form of hypochlorous acid and the hypochlorite ion. The biocidal efficiency of hypochlorous acid is about 100 times greater than that of hypochlorite ion (Government of Canada, 2016). At a given pH, higher temperature leads to greater dissociation of hypochlorous acid (into the hypochlorite ion) which results in a lower efficacy of hyperchlorination.

However, hyperchlorination is more pH dependent than temperature dependent. As the pH increases, the ratio of hypochlorous acid to hypochlorite ion decreases. Below a pH of 7.6, hypochlorous acid is the dominant species. Above a pH of 7.6, hypochlorite ion is the dominant species. Since the germicidal efficiency of hypochlorous acid is higher than the hypochlorite ion, the efficacy of hyperchlorination is highest at a pH < 7.6. Copper-silver ionisation is a disinfection technique that is also pH dependent. Elevated water pH (pH > 7.6) may compromise the efficacy of copper-silver ionisation (Lin et al., 2002, 2011). High pH values lead to a shift in the predominant copper species from positively charged to negatively charged. This negatively charged copper species are most likely less effective in eradicating *Legionella* (Lin et al., 2002). pH has no significant impact on the predominant forms of silver ions in solution (Lin et al., 2002). Monochloramine and chlorine dioxide both have a wider working pH range than copper-silver ionisation and chlorine (Lin et al., 2011; Kelsey, 2014).

The physical and chemical water composition have less effect on the efficacy of Point-of-Use filtration and UV light. However, UV light is susceptible to the formation of scale and mineral deposits on the quartz sleeves. This is influenced by the water composition (e.g., by high water hardness and high ionic strength). The quartz sleeves housing the ultraviolet lamps must therefore be cleaned regularly.

Human health risks

Disinfection methods use disinfectants and can form by-products that can be harmful to human health. The effective concentrations of copper and silver are 0.2 to 0.4 mg/l and 0.02 to 0.04 mg/l respectively. These concentrations are well below the international drinking water limits (2000 mg/l for Cu and 100 mg/l for silver) and therefore do not pose health risks to humans. The same accounts for the effective concentrations of chlorine, chloramine, chlorine dioxide and ozone. However, these disinfectants can form hazardous by-products. Chlorine, for example, can react with organic materials and accelerate the production of trihalomethanes, which are carcinogenic (Lin et al., 1998A, 1998B; Nieuwenhuijsen et al., 2000; Kim et al., 2002; Ortolano et al., 2005). A positive association has been found for consumption of chlorinated water and cancer in numerous epidemiological studies. Monochloramine can cause anemia in patients undergoing hemodialysis (Ortolano et al., 2005) and chlorine dioxide can react with organic matter and corrosion scale in piping and form chlorite and chlorate which might pose health risks to consumers (Lin et al., 2011). This also accounts for ozone. In the presence of bromide and chlorine the by-products bromate and chlorate can be formed (Von Gunten, 2003). Bromate is a known carcinogen.

Heat and flush does not produce by-products but can cause scalding (Ortolano et al., 2005). The only reviewed disinfection method without (known) human health risks is Point-of-Use filtration.

Interaction with contact surfaces

The addition of disinfectants to (drinking) water distribution systems, cooling towers, etc. can cause sedimentation, scaling and/or corrosion of the contact surfaces. Excessive levels of copper and silver ions, for example, have led to blackish discoloration of water and lavender discoloration of porcelain sink surfaces (Lin et al., 1998A; Triantafyllidou et al., 2016). Excessive dosing of copper and silver ions can occur when using copper and silver alloys instead of separate copper and silver electrodes in coper-silver ionisation systems (Walraven et al., 2015). This is most likely caused by a difference in the efficiency of the electrolysis process of copper and silver in alloys (silver being more noble). There are also indications for corrosion caused by copper-silver ionisation for galvanised steel (Loret et al., 2005). However, copper was highly overdosed in this study (>2 mg/l) and copper pipes were connected to galvanised pipes, which can result in galvanic corrosion. Well performed corrosion studies for copper-silver ionisation systems are lacking.

Chlorine and ozone are highly corrosive for steel surfaces (Lin et al., 1998A; Ortolano et al., 2005). Ozone also degrades rubber (Ortolano et al., 2005). Chlorine dioxide is also corrosive for steel surfaces, but less than chlorine (Lin et al., 2011). Monochloramine has no known corrosive properties. This also accounts for Point-of-Use filtration and UV light.

5. Conclusions

- Three types of disinfection methods for the abatement of *Legionella* can be distinguished,
    - 1) the point-of-use methods,
    - 2) gatekeeper systems and
    - 3) the methods that can treat the entire water systems, e.g., complex water distribution systems.

  Point-of-Use filtration is point-of-use method and ozonation and UV light are gatekeeper systems. *Legionella* has only been prevented and/or abated successfully with these methods at the point of use and at the gate (water entry). At distal sites in water systems, these methods are not or less effective. Disinfection methods that can disinfect the entire water system are copper-silver ionisation, heat and flush, chlorination and treatment with monochloramine and chlorine dioxide.

- Efficacy of the reviewed disinfection methods has been proven in both *in vitro* and *in vivo* studies. However, copper-silver ionisation is the only disinfection technology for the abatement of *Legionella* that has been validated by the 4-step standardised evaluation criteria for disinfection methods (efficacy extensively tested under both laboratory and field conditions for several years). For the other reviewed disinfection methods prolonged studies (years) on the efficacy at multiple locations are currently lacking. In addition, efficacy field tests of some disinfection methods were inconsistent (e.g., heat and flush, (hyper)chlorination, chlorine dioxide and ozonation). Possible causes were, amongst others, low residual effect (heat and flush; ozone) and prolonged time or higher dosing needed to successfully abate *Legionella* ((hyper)chlorination; chlorine dioxide).

- The reviewed disinfection methods all have their advantages/disadvantages. The main disadvantages that can be distinguished are,
    - 1) external conditions that influence efficacy (e.g., water composition),
    - 2) human health risks (e.g., formation of toxic by-products) and
    - 3) interaction with contact surfaces (e.g., corrosivity to steel).

  Copper-silver ionisation and heat and flush are the only methods that can treat the entire water system without forming toxic by-products. Heat and flush, however can cause scalding and has no residual effect. Hyperchlorination, monochloramine, chlorine dioxide and ozonation can potentially form toxic by-products (such as trihalomethanes and bromates) and are corrosive to steel. Corrosivity of copper-silver ionisation, especially to galvanised steel, is not studied yet. Point-of-Use filtration and UV light are not corrosive and do not form harmful by-products, but are mainly useful for the prevention of *Legionella* contamination instead of abating and eradicating existing ones.

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