Comparison of disinfectants for biofilm, protozoa and *Legionella* control

J. F. Loret, S. Robert, V. Thomas, A. J. Cooper, W. F. McCoy and Y. Lévi

ABSTRACT

The aim of this study was to compare the efficiency of different disinfectants applicable to *Legionella* control in domestic water systems. A domestic water supply simulation unit that allowed simulation of real-world conditions was developed for this purpose. The system, consisting of seven identical rigs, was used to compare treatment efficiency under equivalent conditions of system design, materials, hydraulics, water quality, temperature and initial contamination. During the study, each of six loops received continuous application of one of the following disinfectants: chlorine, electro-chlorination, chlorine dioxide, monochloramine, ozone, or copper/silver. The seventh loop was used as a control and remained untreated. Performance evaluation of these disinfectants was based on their ability to reduce not only *Legionella*, but also protozoa and biofilms, which contribute to the establishment and dissemination of these bacteria in water systems, and their resistance to treatments. Regarding these criteria, chlorine dioxide and chlorine (as bleach or obtained by electro-chlorination) were the most effective treatments in this study. However, in comparison with chlorine, chlorine dioxide showed a longer residual activity in the system, which constituted an advantage in the perspective of an application to extensive pipework systems.

Key words | biofilm, disinfection, *Legionella pneumophila*, protozoa

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INTRODUCTION

Domestic water distribution systems are known reservoirs of microbial contamination, including biofilms, protozoa, and opportunistic pathogens such as *Legionella*. Even in the presence of disinfectant residuals, microorganisms may proliferate in these systems, resulting in exposure of the system users to infectious organisms. Many epidemiological studies have identified domestic water systems as the cause of waterborne disease.

Treatments to control *Legionella* in domestic water systems have been evaluated and reviewed (Botzenhart *et al.* 2002; Kim *et al.* 2002). However, these evaluations are based on *in vitro* experiments and the extrapolation of this laboratory data to real-world applications is problematic (Campos *et al.* 2003). This is revealed in the, sometimes, dramatic differences observed for laboratory-derived disinfectant efficacy data compared with actual efficacy observed in water systems.

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Similarly, comparing disinfectant efficacy in real-world applications is difficult given the number of factors that influence microbial growth and system fouling. Factors that influence the potential for microbial proliferation in domestic water systems include water temperature, the age of water tanks and associated distribution pipework, materials of plumbing construction, system hydrodynamics, the chemical constituents of the water itself, and the diversity of microbial flora present.

Increasingly, water system owners, operators and users require scientific data to aid in the selection of domestic water distribution system treatment programmes. The disinfectant efficacy data obtained from *in vitro* tests and real-world applications is very important and should not be underestimated. However, additional data that compares treatment efficacy under controlled, yet realistic conditions can be

helpful. Model plumbing systems have been used with that aim (Muraca *et al.* 1987; Pavey 1996; Pavey & Roper 1998), but none of these studies provided information about comparative performance of the treatments on *Legionella*, their protist hosts, and the biofilm, all in the same study.

The purpose of this study was to provide quantitative comparative performance evaluations of chlorine, electrochlorination, chlorine dioxide, monochloramine, ozone and copper/silver ionization in tightly controlled, reproducible experiments that simulate conditions in typical building (domestic) water service. The choice of these disinfectants was justified by the fact that, although their capacity to reduce *Legionella* is already documented, and although most of them are authorized or recommended for *Legionella* control, their efficiency on real systems is still insufficiently documented, and in some cases, still debated.

MATERIALS AND METHODS

Pilot unit design and construction

The domestic water supply simulation unit developed for this study (Figures 1 and 2) was composed of seven identical rigs. Each rig was designed to reproduce the most common layout for domestic water systems in apartment buildings and hospitals, and included:

- 30 m of re-circulation loop made of galvanized steel (interior/exterior diameter: 26/34 mm, total water volume: 16 l), simulating the re-circulation loop of a five-floor building;
- 13 m of copper dead leg (interior/exterior diameter: 20/22 mm, total water volume: 4 l), simulating the water supply of a distant room.

The materials and jointing polymers used for the construction were standardized and approved for contact with potable water. The entire system was supplied with potable water (Table 1), de-chlorinated through an activated carbon filter. A pressure controller limited the head pressure to 2 bar (200 Kpa). Thermal regulation was achieved through the use of a calorifier for the make-up water to all seven rigs, and of heating cables and insulation sheaths for the pipes. No *Legionella* was detectable during the study in the make-up water delivered by the calorifier. Solenoid valves located on the loops and at the outlet of each dead leg allowed automatic discharges of the water, simulating water consumption. These discharges were performed directly from the loops (flow rate: 15 l min^{-1}) or through the dead legs

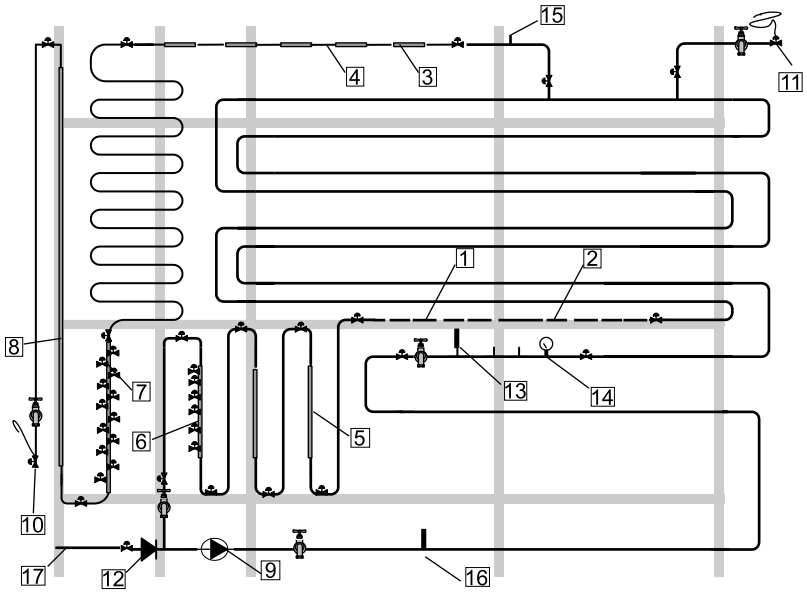

Figure 1 | Domestic water supply simulation unit. 1: galvanized steel coupons, 2: mild steel coupons, 3: copper coupons, 4: brass coupons, 5 and 8: glass beads for biofilm monitoring, 6 and 7: PVC coupons for biofilm monitoring, 9: re-circulation pump, 10 and 11: solenoid valves, 12: non-return valve, 13: temperature probe, 14: pressure gauge, 15: air trap, 16: point of injection of disinfectants, 17: connection to the calorifier.

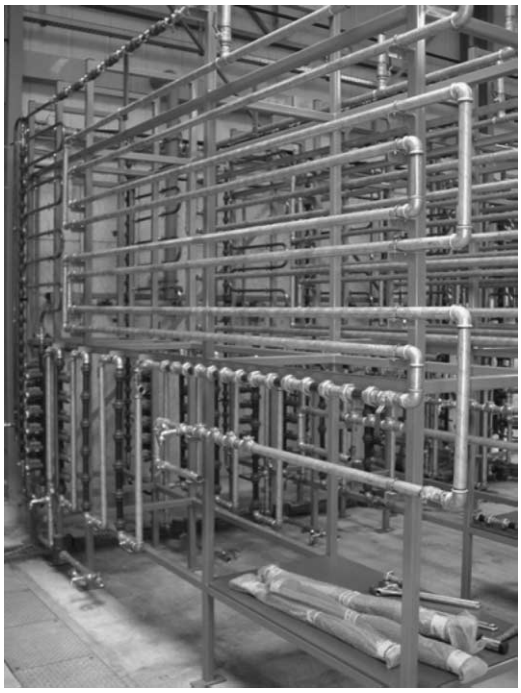

Figure 2 | Domestic water supply simulation unit during construction.

Table 1 | Physicochemical characteristics of the water

	Unit	Value
pH	pH unit	7.6
Turbidity	NTU	0.08
TOC	$mg\,l^{-1}$	0.7
Dissolved oxygen	%	8.6
Oxygen saturation	%	95
Conductivity	$\mu S\,cm^{-1}$	598
Hardness	French grade	21
Ca^{++}	$mg\,l^{-1}$	109.8
Cl^{-}	$mg\,l^{-1}$	32
SO_4^{-}	$mg\,l^{-1}$	69
Fe	$\mu g\,l^{-1}$	<20
Cu	$\mu g\,l^{-1}$	<2
HCO_3^{-}	$mg\,l^{-1}$	256

(flow rate: $0.8\,l\,min^{-1}$). Taps were installed at different places in the system for water sampling. Two types of biofilm monitoring device were installed on the loops and dead legs:

- PVC compartments filled with 5 mm glass beads (700 beads per compartment, representing a contact surface with the water of 550 cm^2), allowing quantitative microbiological analysis.
- PVC Robbins-type devices, with 1-cm^2 coupons, allowing direct microscopic observation of the biofilm.

Corrosion coupons, consisting of 10 cm pipe sections, were connected in series within the re-circulating loops and dead legs. The materials commonly found in domestic water systems were represented: mild and galvanized steel coupons were installed in the loops, admiralty brass and copper coupons in the dead legs.

Sample processing

Water samples were analysed immediately after sampling for planktonic *Legionella* and amoebae (1 l for each analysis). Glass beads for biofilm analysis were soaked in sterile water, sonicated for 2 min, and the resulting suspension was analysed for sessile *Legionella* and amoebae (300 and 400 beads, respectively). *Legionella* were analysed according to ISO 11731 culture plate assay (ISO 1998) (culture on buffered charcoal-yeast extract with 0.1% α-ketoglutarate agar, supplemented with L-cysteine, iron and GVPC, at 37°C). Amoebae were analysed by zone assay on coliform culture, according to the filtration method described in Pernin *et al.* (1998). Plates were incubated at 30°C. This method allows the recovery of amoebae principally in their cystic form. Therefore, all amoebae counts in this study are expressed as cysts.

Coupons for biofilm observation were preserved in water from the corresponding loop for a maximum 2 hours after sampling. Coupons were then submitted for microscopic analysis after using the Live/Dead® *Bac*light™ staining method (Molecular Probes). A Zeiss LSM 510 Meta confocal microscope, equipped with a 40x water immersion objective was used. Samples were scanned at 488 nm. Syto9 (live bacteria) was detected through a 498–530 nm bandpass filter, and propidium iodine (dead bacteria) was detected

through a 594–680 nm bandpass filter. Biofilm was analysed in 1 μm optical slices. At least four microscopic fields were acquired for each coupon. Biofilm thickness was determined by using the ImageJ 1.30v software (The National Institutes of Health, USA). Chlorine concentrations were checked by diethyl-phenylene diamine (DPD) spectrophotometric method (ISO 7393-2) (ISO 2000). Copper was monitored by an electrochemical method (Palintest SA-1000 portable analyser). Corrosion coupons were removed from the system and washed in a solution of passivated acid (10% hydrochloric acid for mild steel, 30% sulfamic acid for galvanized steel, 30% hydrochloric acid for copper and brass, and 10% formaldehyde) to eliminate corrosion residues. Coupons were then rinsed, dried and weight loss was determined. Disinfection by-products were analysed according to ISO 10301 (ISO 1997) for trihalomethanes (THM), USEPA method 300.1 for chlorite (USEPA 1997), and ISO 15061 for bromate (ISO 2001).

System preparation

Sand filtered river water was circulated in the loops and dead legs for two weeks. It was then progressively replaced by tap water, at a renewal rate of 25% per day, until stabilization of the water quality at the outlet of the system (approximately 1 month). This procedure led to the establishment in the system of a natural *Legionella pneumophila* serogroup 1 strain, and an amoebae population consisting of *Hartmannella*, *Acanthamoeba* and *Vahlkampfia*. To increase the *Legionella* population, the natural *Legionella* strain was cultured on BCYE agar with cysteine at 37°C, re-suspended in sterile phosphate buffered saline, and re-injected into the system. The *Legionella* population stabilized at 10^5 (+/− 0.7 log) CFU l^{-1} four weeks after inoculation. The stability of the population was observed for 2 months before starting the disinfection studies. Amoebae concentrations in the system water stabilized at 5×10^3 (+/− 0.6 log) cysts l^{-1}.

Test programme

In order to better distinguish between treatments performance, the main anomalies usually found in domestic water systems were reproduced on the simulation unit:

- Low temperature, favouring *Legionella* growth. The temperature was maintained close to the optimum for *Legionella* growth, at 35°C. Such temperature was also justified by the fact that chemical treatments are of interest principally in situations where a temperature regime above 50°C is not applicable.
- Low water velocity, favouring biofilm growth. Water velocity in the re-circulation loops, at 0.1 m s^{-1} (200 l h^{-1}), was only half of the minimum velocity required by French regulations (0.2 m s^{-1}).
- High retention time of the water in the system. The volume of each loop was renewed by only 100% every day, by steps, in ten sequences per day. During the first two months of the study, the water was only partially discharged through the dead legs. During this period, the water volume within each dead leg was renewed only by 20% per day. A single flushing was performed at the end of the first month on all dead legs using 5 l from each corresponding loop. The total volume of each dead leg was thus totally replaced by disinfected water from the loops. On the third month, the volume of each loop was totally discharged through each corresponding dead leg, in ten sequences per day of 1.5 l each. The total volume of each dead leg was then totally replaced by treated water from the loop approximately four times per day.

During three months, each of the six loops received continuous application of a single treatment. The seventh loop was used as a control and remained untreated. Six disinfectants were studied, with the following targeted doses. Except for ozone, the dose indicates the residual maintained in the re-circulation loop.

- Chlorine (sodium hypochlorite) and electro-chlorination at 2 mg l^{-1} (expressed as free chlorine). This dose was the maximum allowed according to French regulations for *Legionella* control in domestic water systems.
- Monochloramine at 2 mg l^{-1} (expressed as total chlorine). This dose was close to the median concentration observed in a study in which monochloramine was compared with chlorine with respect to its ability to prevent Legionnaires' disease in the USA (Kool *et al.* 2000).
- Chlorine dioxide (expressed as chlorine dioxide) and ozone (at the contact column inlet), at 0.5 mg l^{-1}. This

dose is usually applied in France for both disinfectants, for potable water disinfection.

- Copper/silver at 0.5/0.01 mg l^{-1}. Silver concentration in that case was limited to the maximum allowed in potable water by French regulations.

Water samples for microbiological analyses were taken on each rig every day during the first week, and then once a week throughout the rest of the study. Biofilm samples were taken once a week during the first month, and then once each month. Sodium hypochlorite, electro-chlorination, chlorine dioxide and monochloramine residual concentrations in the loops were monitored by the spectrophotometric method on a daily basis. Copper in the rig treated with copper/silver was checked once a week. Silver concentration was not monitored, since it was designed to be proportional to copper concentration, with a silver/copper ratio of 2%. Corrosion coupons were taken after the second and third months. At the end of the second month, water samples were taken in the loops for disinfection by-products determination.

Disinfectant generation and dosage control

Sodium hypochlorite, electro-chlorination, chlorine dioxide and monochloramine disinfectants were prepared in advance and injected from a stock solution stored in a tank. Five litres were prepared each time, corresponding to a 4–5 day consumption. All stock solutions were prepared in the same concentration range (400–600 mg l^{-1}):

- A 5% sodium hypochlorite solution was diluted to 500 mg l^{-1} solution.
- A 600 mg l^{-1} free chlorine solution was prepared by electro-chlorination, from a 10 g h^{-1} free chlorine capacity generator. The electrolytic cell was operated at 12 V and 10–12 A, and was fed with a 15 g l^{-1} sodium chloride solution. Only the anode stream was collected. Use of a stock solution in that case was necessary, since the generator did not allow on-line injection.
- For chlorine dioxide, an Envirox 1000 electrochemical chlorine dioxide generation unit (5 g h^{-1} capacity, Nalco Company), generating a 500 mg l^{-1} chlorine dioxide solution, was used. The unit was fed with de-ionized water (Millipore Milli RX-20) and a 5% sodium chlorite solution (Nalco Envirox PWT). Only the anode stream was collected. As for electro-chlorination, use of a stock solution was necessary, since the generator did not allow on-line injection.
- Monochloramine was prepared by mixing sodium hypochlorite with ammonia at a chlorine to ammonia molar ratio of 2:1, as described in White (1999).

The stability of the stock solutions was monitored by spectrophotometric methods on a daily basis. Sodium hypochlorite, electro-chlorination and chlorine dioxide stock solutions were stable after preparation and during the 5-day storage time. Monochloramine stock solutions showed a rapid decrease in concentration. Therefore, the stock solution was renewed twice each day. This constraint reduced the study duration of monochloramine to one month, instead of three.

Prominent Gamma 4 diaphragm pumps (capacity: 20–200 ml h^{-1}) were used for the injection of the disinfectants in the loops. Prominent amperometric controllers were used to control the doses of these disinfectants. The following probes were installed in the loops, downstream of the injection points: Dulcotest® CGE 2 mA for chlorine and electro-chlorination, Dulcotest® CDP 1 mA for chlorine dioxide, and Dulcotest® CTE 1 mA for monochloramine.

The amperometric sensor used for chlorine dioxide performed correctly throughout the study. As shown in Table 2, the targeted dosage of 0.5 mg l^{-1} could be achieved accurately in the rig treated with chlorine dioxide, throughout the study. However, the amperometric sensors for chlorine, electro-chlorination and monochloramine failed at the end of the second day. Re-generating the probes and replacing membranes restored their function, but not for more than 48 hours. Therefore, they were replaced by timer devices allowing automatic timed injection of these oxidants. Although continuous disinfection could be achieved by this means, this led to a less precise dosage control in the loops treated with chlorine and electro-chlorination, to over-dosage of these oxidants (2.40 and 2.75 mg l^{-1}, respectively, on average) and to significant variations of the concentrations. However, these variations were deemed acceptable, since variations of the same order are commonly observed in real systems where oxidation-reduction (redox) controllers are used. In the case of monochlora-

Table 2 | Disinfectant dosage achieved in the re-circulating loops

Treatment dose ($mg\,l^{-1}$)	Ozone	Chlorine dioxide	Chlorine	Electro-chlorination	Monochloramine	Copper *	Copper†
Average	0.50	0.50	2.40	2.75	0.50	2.15	0.75
Standard deviation	0.15‡	0.15	2.25	2.10	0.80	2.35	0.35

*Maximum ionisation intensity.
†Half ionisation intensity.
‡Maximum variation.

mine, the average dosage was only $0.5\,mg\,l^{-1}$ instead of the targeted $2\,mg\,l^{-1}$ because of monochloramine instability in the stock solution.

Ozone was produced from a TOGC2 ozone generator ($10\,g\,h^{-1}$ capacity, Ozonia company). The generator was fed with oxygen and produced an ozone concentration of $4\,g\,m^{-3}$ in the oxygen flow. Ozone production was continuously monitored with an on-line BMT 961 ozone analyser (Messtechnik), and injected through an eductor into a 16 l contact column, connected in series to the loop to allow treatment of the entire re-circulating water flow. The ozonated oxygen flow was adjusted to achieve an ozone/-water ratio of $0.5\,mg\,l^{-1}$ at the contact column inlet. No ozone residual was detected at the outlet of the contact column ($<0.1\,mg\,l^{-1}$). Ozone concentration in the oxygen flow showed a maximum variation of 30% around the $4\,g\,m^{-3}$ set point.

Copper and silver were delivered by a LP2-5 system, designed to treat water flows up to $15\,m^3\,h^{-1}$ (Sanichem company). The ionization chamber, containing two electrodes made of copper/silver alloy, was placed in the loop for continuous treatment of the entire flow. The electrodes were cleaned once a month in order to remove the scale deposits, and their polarity was regularly and automatically inversed to ensure a homogeneous ionization of both electrodes. The ionization intensity was set at the maximum level during the first three weeks, and was then reduced by half until completion of the studies. The copper concentration in the loop treated with copper/silver was irregular during the 3-week period of treatment at the high intensity setting on the ionization unit. Reducing the intensity by half on the ionization unit led to a more constant copper residual in the loop, but the average copper level remained higher than the targeted value. Copper-containing sludge, red staining of the water, and copper deposits on the inner surface of the pipes were observed in this loop.

RESULTS

Disinfection in the loops

Unlike the dead legs, which received discontinuous injections of treated water, the loops were treated continuously to maintain disinfectant residual concentrations. The loops exhibited rapid decreases in microbial contamination.

Planktonic *Legionella* (Figure 3)

The planktonic *Legionella* population in the control loop remained stable throughout the 3-month study period, at 2.6×10^5 (+/− 1.1 log) $CFU\,l^{-1}$. Each disinfectant

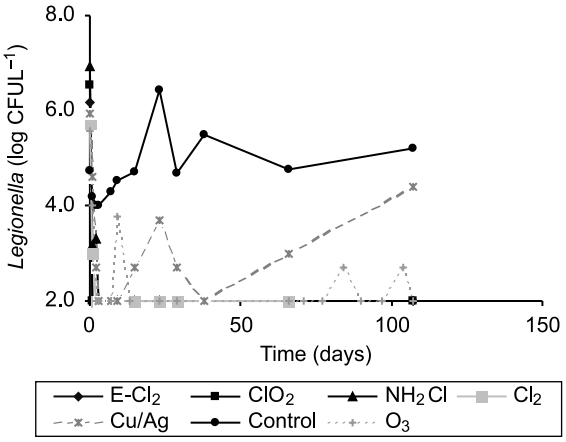

Figure 3 | Changes in planktonic *Legionella* populations within re-circulation loops during treatments (values shown at $100\,CFU\,l^{-1}$ were lower than the detection limit of $500\,CFU\,l^{-1}$).

achieved rapid initial results in the treated loops. *Legionella* populations decreased to undetected levels ($<500\,CFU\,l^{-1}$) within the first three days of treatment, in all cases. However, *Legionella* remained undetected over the whole study period only with sodium hypochlorite, electro-chlorination, chlorine dioxide and monochloramine. Ozone and copper/silver allowed occasional re-emergence of detectable *Legionella* in the bulk water. With ozone treatment, three peaks of contamination were observed during the studies. After two weeks of treatment however, the contamination remained below $10^3\,CFU\,l^{-1}$. A greater number of positive samples were found with copper/silver treatment. Following the initial decrease in *Legionella* concentrations, five of six *Legionella* samples were positive in the copper/silver-treated loop, and *Legionella* concentrations had increased to greater than $10{,}000\,CFU\,l^{-1}$ in this loop by the conclusion of the study.

Sessile *Legionella* (Figure 4)

The sessile *Legionella* population in the control loop remained stable throughout the study period, at 150 (+/− 1.3 log) CFU cm^{-2}. No viable *Legionella* cells were detected in the biofilm after six days of treatment in the loops treated with sodium hypochlorite, electro-chlorination, chlorine dioxide, monochloramine or ozone ($<1\,CFU\,cm^{-2}$). Viable *Legionella* cells were still detected in biofilm from the loop subjected to copper/silver treatment.

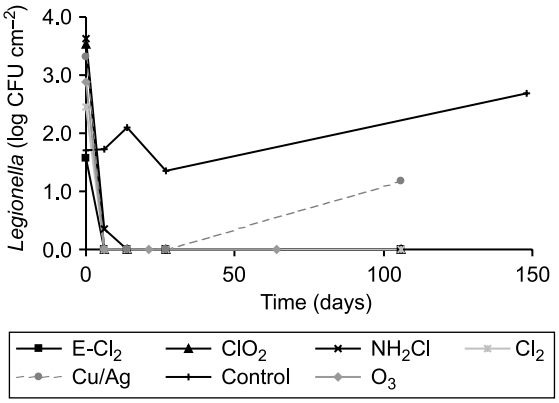

Figure 4 | Changes in sessile *Legionella* populations in re-circulation loops during treatments.

Planktonic amoebae (Table 3)

The planktonic amoebae population in the control loop remained stable during the studies, at 10^4 (+/− 0.8 log) cysts l^{-1}. Copper/silver and monochloramine did not significantly reduce amoebae cyst concentrations. All other treatments reduced cyst concentrations significantly, but no treatment eliminated cysts entirely from the bulk water of each loop.

Sessile amoebae

Biofilms were poorly colonized by amoebae before the treatments were started (10 to 50 cysts cm^{-2}). No clear trends in amoeba population concentrations were observed during the studies and amoebae were still present in the biofilms of all loops at the end of the treatment period (0.1 to 30 cysts cm^{-2}).

Biofilm thickness

Biofilm thickness in the control (untreated) loop increased during the studies from 13 μm to about 35 μm (Figure 5). This same trend was observed in the loop treated with monochloramine, during the 1-month period of application of this disinfectant. Copper/silver had no effect on biofilm thickness while chlorine dioxide reduced it significantly. Ozone, electro-chlorination and chlorine treatments resulted in biofilm thickness below detection limits (<5 μm). In the case of treatments able to reduce biofilm thickness, biofilm reduction was observable only 1 week after treatment initiation.

Disinfection in dead legs

As long as the water renewal rate was maintained at 20% per day, no significant change of the planktonic and biofilm populations was observed in the dead legs. Using this flushing protocol, microbial contamination of the dead legs remained high. All dead legs recovered their initial contamination levels 24 hours after application of a single complete flushing of the dead legs with treated water from the loops. After seven days of applying a flushing programme consisting of totally discharging the loop volumes through the dead legs every day, Legionella became

Table 3 | Changes in planktonic amoebae populations in re-circulation loops during treatments

Amoebae (cysts l^{-1})	Ozone	Chlorine dioxide	Chlorine	Electro-chlorination	Monochloramine	Copper/Silver	Control
log (initial population)	4.2	3.5	4.2	4.2	4.0	2.9	4.2
log (final population)	0.6	1.7	1.4	1.7	3.5	2.6	4.2
log (reduction)	3.6	1.8	2.8	2.5	0.5	0.3	0.0

undetectable in the water (Figure 6) and the biofilm of the dead leg receiving chlorine dioxide. No change in the planktonic or sessile microbial populations was observed in the other dead legs.

Corrosion

Following exposure of the mild and galvanized steel coupons installed in the loops to the various treatments, the coupons had visible corrosion marks on their inner surface. Coupons exposed to monochloramine also showed similar marks, although the contact time was only one month in that case. However, no difference in aspect or loss of weight was noticeable between the coupons from the control loop and the others, except for those in contact with copper/silver. The coupons exposed to copper/silver were covered by copper deposits. Copper deposits are known to initiate pitting corrosion on steel, via a galvanic corrosion mechanism. Although pitting corrosion was not observed during the study period, intense corrosion was observed once the study was complete on the loop treated with copper/silver, in the absence of continued chemical treatment. No corrosion mark or weight loss was observed on the copper and brass coupons installed in the dead legs.

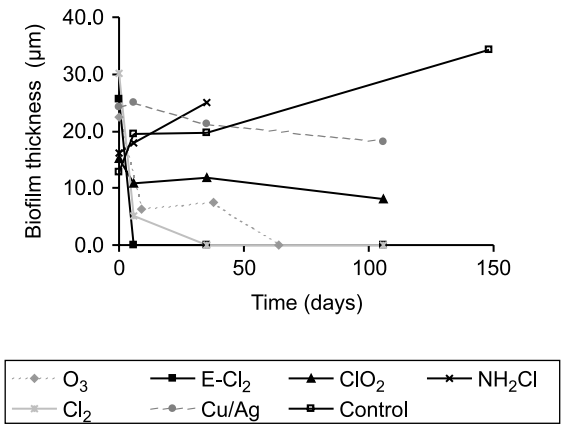

Figure 5 | Changes in biofilm thickness in re-circulation loops during treatments (values shown at 0 µm were lower than the detection limit of 5 µm).

Disinfection by-products

The disinfection by-products (DBPs) concentrations found in the loops were compared with the maximum levels required by the regulations currently in force in Europe for drinking water. In comparison with the regulatory limits, DBPs were found in excess with the following treatments:

- Chlorine dioxide: chlorite $>0.2\,mg\,l^{-1}$
- Chlorine: THM $>100\,\mu g\,l^{-1}$
- Electro-chlorination: THM $>100\,\mu g\,l^{-1}$, bromate $>10\,\mu g\,l^{-1}$
- Ozone: bromate $>10\,\mu g\,l^{-1}$

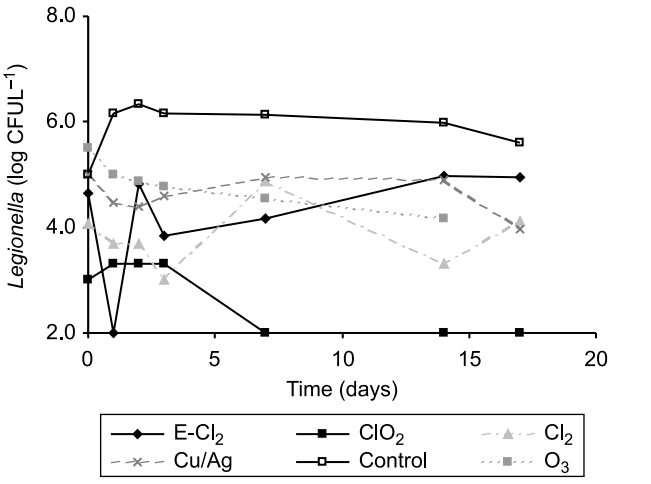

Figure 6 | Changes in planktonic *Legionella* populations in dead legs during treatments (values shown at 100 CFU l^{-1} were lower than the detection limit of 500 CFU l^{-1}).

CONCLUSIONS

The domestic water supply simulation unit developed for this study allowed simulation of the main anomalies that can be found in building domestic (potable) water systems. Anomalies in temperature, water velocity, and retention time of the water in the system were simulated. Reproducing these anomalies favoured the fouling of the system, and allowed us to better distinguish between treatment performances. It is supposed that in domestic water systems where such anomalies are not present, and provided that an efficient dosage control system of disinfectants is in place, the differences between treatment performances should be less significant. The fouling procedure applied to the system led to the establishment of stable biofilm, *Legionella* and amoebae populations in each of the seven rigs composing the pilot unit. Once these populations were established, the system was used for comparative assessment of treatment efficiency and operation, in equivalent conditions of system design, materials of construction, hydraulics, water quality, temperature and initial fouling. Treatment efficiency was assessed not only on *Legionella*, but also on amoebae and biofilms, which contribute to the establishment and dissemination of these bacteria in water systems, and their resistance to treatments. Basing the evaluation on these parameters, differences in treatment efficiencies could be observed among the tested disinfectants.

Chlorine dioxide and chlorine (as bleach or obtained by electro-chlorination) were the most effective treatments in this study. This was revealed in the ability of chlorine and chlorine dioxide to reduce *Legionella* contamination in the water and the biofilm of the re-circulating loops, and to maintain *Legionella* concentrations below analytical detection limits throughout the study. Although electro-chlorination has been described as a more potent disinfectant, in comparison with chlorine, especially for protozoa (Venczel *et al.* 1997), no significant difference was observed in this study between the results obtained with chlorine and electro-chlorination. This could be due to the fact that, in our case, use of a stock solution was necessary to ensure a proper dosage control. As a consequence, the short-lived oxidizing radicals generated by this process were probably lost during the four to five day period of utilization of the stock solution. Further studies would be useful to assess the performance of equipment allowing a direct injection of the generated solution, without the need for a stock solution.

Chlorine dioxide showed less ability than chlorine to remove the biofilm. However, this difference in biofilm removal could have been due to differences in the concentrations applied ($0.5\,mg\,l^{-1}$ for chlorine dioxide versus $2.5\,mg\,l^{-1}$ for chlorine). Compared with chlorine, chlorine dioxide showed a longer residual activity in the system, leading to improved performance in the dead leg. Chlorine dioxide was the only disinfectant able to reduce dead leg *Legionella* concentrations below detection once the flushing protocol began. However, when a single flushing was applied, it was followed by the re-colonization of the dead leg within 24 hours. Consequently on real systems, flushing dead legs with disinfected water once a day appears to be a minimum recommendation. In actual systems, this flushing may be achieved by making outlet flushing part of the normal routine for housekeeping and maintenance functions. Ideally, outlets and associated pipework that are rarely used and not required should be removed or isolated. This observed importance of flushing also shows that controlling the microbial contamination requires a constant maintenance of a disinfectant residual throughout the water system. While 'slug' or 'shock' disinfectant doses may be effective at controlling growth in the short-term or as an emergency decontamination protocol, long-term control requires continuous disinfectant application and flushing to ensure that effective disinfectant concentrations are circulated throughout the system.

In this study, chlorine dioxide dosage control was achieved using an amperometric sensor, which offered accurate and reliable control. Redox potential sensors (not evaluated in these studies) can also be used for chlorine dioxide, but report only a millivolt (mV) value and not a chlorine dioxide concentration ($mg\,l^{-1}$). Since disinfectant dosing and exposure limits are typically expressed as concentrations, the amperometric control device may have greater utility to system operators. In contrast to the chlorine dioxide sensor, the total chlorine amperometric sensors used within this study required heavy maintenance (membrane replacement and calibration every 48 hours), probably because of a rapid fouling of their membrane. Such sensors were not robust enough for on-site

application. Alternative solutions such as redox potential or colorimetric sensors (although not tested in these studies) are preferred for chlorine dosage control.

Ozone at 0.5 mg l^{-1} had an effect throughout the entire re-circulation loop against planktonic and biofilm populations, even in the absence of detectable residual after the contact column. This result was surprising, but could be explained by the presence in the loop system water of an ozone residual below detection limits. Ozone achieved the greatest amoebae reduction in the re-circulation loop and appears therefore to be a promising technology for *Legionella* control. As with the other treatments, other than chlorine dioxide, ozone was ineffective at reducing *Legionella* contamination within dead legs. Further studies would be useful to optimize its dosage and achieve better control of *Legionella* and amoebae. Field studies are also required to assess the feasibility of this technology on real domestic water systems, where pipework systems may be more extensive.

Monochloramine was effective against planktonic and sessile *Legionella*, but showed no effect against amoebae, and no capacity to remove the biofilm. However, the protocol used for monochloramine preparation did not allow maintenance of a stable product. This resulted in insufficient dosing in the re-circulation loop (0.5 mg l^{-1} instead of the targeted 2 mg l^{-1}). The lack of commercially available product or generator for this chemical is a serious obstacle to its potential use for *Legionella* control in domestic water systems. Alternatively, a separate injection of ammonia and chlorine, leading to the formation of monochloramine within the system, could be envisaged, but the feasibility and performance of such a mode of preparation still has to be demonstrated.

Copper/silver over-dosage (0.75 mg l^{-1} of copper, for a target at 0.5 mg l^{-1}) could be due to the fact that the generator was over-sized for the pilot loop (up to 15 m^3 h^{-1} capacity, for a flow of 200 l h^{-1}). However, despite the over-dose applied, and although the French regulatory limit of 10 μg l^{-1} for silver was probably exceeded, the silver concentration in the loop could theoretically not reach the range of 20–40 μg l^{-1} usually recommended for an efficient disinfection (HSE 2001). In these conditions, copper/silver showed poor efficiency on all of the parameters tested. Copper/silver initially reduced planktonic *Legionella* concentrations to below the detection limit, but failed thereafter to control the contamination. Additionally, copper/silver led to a red staining of the water, formation of sludge in the loop, and copper deposits on the pipes. This could be explained by the fact that copper was mainly in the form of suspended particles.

This hypothesis is supported by a recent study showing that copper is able to form insoluble complexes at pH values above 6.0 (Lin *et al.* 2002). According to this study, 90% of the copper should have been present as insoluble complexes in the pilot unit, because of the pH of the water (pH = 7.6). The same study concluded that silver solubility is not influenced by the pH, but is affected by the presence of chloride ions. Hence, 60% of the silver should have been in a precipitate form in the pilot unit, because of the chloride content of the water (32 mg l^{-1}). For these reasons, the real concentration of copper and silver, available in an active form in the loop, remained unknown. Consequently, the application of such a disinfection method should be restricted to waters with low pH and low chloride concentration, and in water systems where such a disinfection method is applied, *Legionella* testing should be continuously performed to detect any drift in disinfection efficiency.

All treatments generated corrosion on mild and galvanized steel. This result was not surprising, as each of the disinfectants evaluated in this study are known to cause corrosion. The relatively short duration of this study, compared with operation of an actual building water system, did not allow for significant corrosion rate data collection. However, the fact that each of the treatments can cause corrosion highlights the need for consistent dosage control at concentrations that reduce microbial fouling, yet minimize the impact on corrosion. In this regard, disinfectants that are effective at low concentrations may be favoured. Copper/silver generated copper deposits which can initiate pitting corrosion on steel. Pitting corrosion, however, was not observed on the pipes, probably because the duration of the study was insufficient to observe such a phenomenon. Nevertheless, the intense corrosion within the copper/silver treated loop that was observed upon study completion suggests that this treatment may lead to corrosion in some circumstances. This corrosion is most probably galvanic. Therefore, mild steel or

galvanized systems treated using copper/silver ionization should be monitored closely for copper deposition and corrosion.

Disinfection by-products (DBPs) were found in excess in the loops treated with chlorine dioxide, chlorine, electro-chlorination and ozone. However, DBP formation is system dependent, and the results observed in this study do not mean that excessive DBP concentrations will be necessarily generated in all systems submitted to such treatments. Indeed, these disinfectants have been successfully applied to domestic water systems without exceeding DBP limits. Nevertheless, the fact that some DBP limits were exceeded in this study signifies that attention must be paid to DBP. This may be particularly important in cases of water systems presenting high oxidant demand (i.e. highly fouled, composed of new materials, with no protective scale deposits or anti-corrosion treatment), and high retention time of the water in the system (i.e. with high storage volumes or low use rates), as simulated in this study.

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