

Monochloramine and chlorine dioxide for controlling *Legionella pneumophila* contamination: biocide levels and disinfection by-product formation in hospital water networks

Isabella Marchesi, Greta Ferranti, Annalisa Bargellini,
Patrizia Marchegiano, Guerrino Predieri, Janet E. Stout and Paola Borella

ABSTRACT

Legionella colonization in hospital hot water distribution networks was evaluated following 36 months of continuous treatment with monochloramine and compared with chlorine dioxide. Nitrite, nitrate, chlorite, chlorate, bromide, trihalomethanes and haloacetic acids as well as the biocide concentration at sampled points were measured. Only 8/84 samples treated with monochloramine were found contaminated and after the first 8 months of treatment no *Legionella* was isolated. Chlorine dioxide was associated with a strong reduction in *Legionella* contamination compared to pre-treatment, but differences according to the device were observed. Monochloramine between 2 and 3 mg l⁻¹ and chlorine dioxide between 0.50 and 0.70 mg l⁻¹ were needed to control *Legionella* colonization. Comparing no- and post-flush samples, a higher frequency of no-flush positive samples was noted using chlorine dioxide, suggesting an increased risk for patients when they open the tap. No increase in chlorite levels and no water nitrification occurred by using monochloramine. Chlorite at levels exceeding the limit requested for drinking water was measured when chlorine dioxide was applied. In conclusion, we highlight that continuous injection of monochloramine should be considered as an effective alternative to chlorine dioxide in controlling legionellae contamination inside hospital water distribution systems.

Key words | chlorine dioxide, disinfection by-products, hospital, hot water distribution system, *Legionella*, monochloramine

Isabella Marchesi (corresponding author)

Greta Ferranti
Annalisa Bargellini
Guerrino Predieri
Paola Borella

Department of Diagnostic, Clinical and Public
Health Medicine,
University of Modena and Reggio Emilia,
Via dei Campi 287, I-41125 Modena,
Italy
E-mail: isabella.marchesi@unimore.it

Patrizia Marchegiano

University Hospital Policlinico of Modena,
Via del Pozzo 71, I-41124 Modena,
Italy

Janet E. Stout

Special Pathogens Laboratory,
1401 Forbes Ave. Suite 209,
Pittsburgh, PA 15219,
USA
and
Swanson School of Engineering,
University of Pittsburgh,
3700 O'Hara Street,
Pittsburgh, PA 15261,
USA

INTRODUCTION

Numerous disinfection methods are suggested for controlling *Legionella* spp. contamination in water networks of big buildings, particularly hospitals, aimed to prevent the occurrence of *Legionella* infections (Lin *et al.* 2011). A comparison of effectiveness and cost-benefit of different treatments has been recently described suggesting that continuous injection of chlorine dioxide is the least expensive procedure, although not the most effective in reducing *Legionella* contamination in hospital (Marchesi *et al.* 2011). In addition, our data suggest that chlorine dioxide can favour *Legionella pneumophila* serogroup 1 (Marchesi

et al. 2012). In Italy, chlorine dioxide is becoming more commonly used as a water system disinfectant due to the frequency of *Legionella* colonization of hospitals, usually located in old buildings and frequently renovated with scarce attention to water networks (Ditommaso *et al.* 2006; Scaturro *et al.* 2007). *Legionella* contamination is thus generally controlled (Casini *et al.* 2008; Tesauro *et al.* 2010), but the biocide levels needed may cause pipe deterioration/corrosion (Chord *et al.* 2011; Yu *et al.* 2011) and data about the formation of disinfection by-products (DBPs) have not received due attention. In drinking water, indeed,

chlorine dioxide forms toxic inorganic DBPs, mainly chlorite, chlorate and chloride ions (Righi et al. 2012). Toxicological studies showed haematological damage mainly due to chlorite exposure, while impairment of sexual and neurological development, soft tissue anomalies and altered thyroid function were observed in association with chlorate exposure (WHO 2011).

Monochloramine is considered more effective than free chlorine in controlling *Legionella*, in part due to its *in vitro* ability to better penetrate biofilms and to inhibit *L. pneumophila* co-cultured with *Acanthamoeba* (Donlan et al. 2005; Dupuy et al. 2011). In the United States, monochloramine used for disinfection of municipal water supplies was associated with a lower *Legionella* colonization of hospital water systems (Moore et al. 2006) and decreased risk of nosocomial outbreaks of Legionnaires' disease (Heffelfinger et al. 2003). Nitrite can occur in the distribution systems at higher concentrations when monochloramine is used to provide a residual disinfectant in drinking water (WHO 2011). The presence of nitrite in a water supply is undesirable because of health concerns such as methaemoglobinemia in infants (Fan 2011). Few studies describe the use of monochloramine on a small scale for controlling *Legionella* colonization in hospital water systems (Finney et al. 2008; Kandiah et al. 2012) and none of them evaluates the DBP formation.

We introduced a new system that continuously injected monochloramine into a hospital water network highly contaminated by *L. pneumophila*, and the first year of application was promising (Marchesi et al. 2012). In this study, we present the results over a 3-year application in comparison with chlorine dioxide applied in three other hot water distribution networks of the same hospital and with untreated networks. The levels of water nitrite, nitrate, chlorite, chlorate, bromide, trihalomethanes (THMs) and haloacetic acids (HAAs) have been measured at pre-established intervals, and the biocide concentration at every sampled point was used to evaluate the minimum concentration associated with a colonization below the detection limit.

METHODS

The study was conducted in a 765-bed hospital consisting of a nine-storey high block (indicated as building 1) and a

separate building (building D) both constructed in the 1970s, plus three other buildings constructed in the 1990s (buildings E, F, G). In building 1, three different water networks (A, B and C) distribute in parallel hot water, while the other four buildings have their own hot water network, as previously described (Marchesi et al. 2011).

In this investigation, we studied the continuous disinfection systems listed below.

- Building 1 (nine-storey, 40 years old):
 - an experimental monochloramine device operating since 2009 on water network C;
 - chlorine dioxide device I (Sanipur S.r.l., Brescia, Italy) operating since January 2005 on water network A;
 - chlorine dioxide device II (Culligan Italiana S.p.A., Bologna, Italy) operating since November 2005 on water network B.
- Building G (four-storey, 13 years old):
 - chlorine dioxide device III (Sanipur S.r.l., Brescia, Italy) installed since 2009 in the hot water line at the appearance of heavy colonization by *L. pneumophila* serogroup 1.
- Building D (four-storey, 40 years old) and E (four-storey, 13 years old):
 - untreated networks as control; the first is under renovation (no patients inside), the second is devoted to operating theatres and patients are not exposed to water. Shock hyperchlorination and/or thermal shock were randomly carried out in these buildings.

Monochloramine is generated *in situ* in the patented system by reaction between a stabilized chlorine-based precursor (Enoxin P10 Plus) and an ammonium salt (Zebion 50), and it is distributed at residual concentrations between 1.5 and 3.0 mg l⁻¹ in the recirculating hot water system. The monochloramine generator is able to keep a constant concentration of monochloramine in the recirculation loop, even at temperatures as high as 60 °C, by a proprietary software. In our study, monochloramine residual levels are in line with those found in drinking water disinfected with this biocide (WHO 2004) and with the guideline value of 3 mg l⁻¹ and the maximum contaminant level of 4 mg l⁻¹, established respectively by the WHO and US Environmental Protection Agency (EPA). Chlorine dioxide is produced *in situ* by using hydrochloric acid and sodium

chlorite in all three devices. A dosing system is set up to add the disinfectant to the recirculating hot water, ensuring concentrations of at least 0.3 mg l^{-1} at distal points. This dose was reported as effective in controlling *Legionella* contamination in European hospitals (Sidari *et al.* 2004).

Routine inspection, cleaning and maintenance of the hot water systems, which includes for example, decalcification and/or replacement of showers/taps, are conducted once a year and when necessary at the hospital.

Sample collection

Since 2000, a programme to assess *Legionella* contamination in the hospitals' water distribution systems was implemented in accordance with National Guidelines (Istituto Superiore di Sanità 2000). These guidelines do not offer advice on the frequency or number of sites to be sampled in non-epidemic situations. Due to the absence of nosocomial-acquired cases in our hospital (Marchesi *et al.* 2011), our sampling strategy took account of hospital and patient characteristics in order to include high and medium risk wards. The protocol provided to sample at least one remote point every 50 beds, reiterating the same sites every 3 or 4 months on the basis of exposure risk and testing other points when high risk patients were hospitalized. The network experimentally treated with monochloramine was monitored more frequently: after 1 week, after the first, third and fourth month, then every 2 months until the second year, and every 4 months the last year.

Over 3 years, we collected hot water samples for bacteriological analysis ($n = 428$) from storage tanks/heater, return loops and distal outlets (showers or taps), without flaming and after 1 min flushing. On a limited number of distal points ($n = 68$), water was collected also without flushing.

At sampling, the biocide levels were measured by using a Palin colorimetric method based on N,N-diethyl-*p*-phenyldiamine Microquant (Merck, Darmstadt, Germany) (Standard Methods; APHA 1992). Monochloramine was expressed as total chlorine. To neutralize residual free chlorine, sodium thiosulphate was added to sterile bottles for bacteriological analysis. Bottles were returned to the

laboratory immediately after sampling, as described elsewhere (Borella *et al.* 2005).

Microbiological analysis

Culture and identification of *Legionella* spp. were carried out following the ISO 11731 method (ISO 1998), as reported in a previous study (Bargellini *et al.* 2011). Briefly, 1 l of water was filtered (0.2- μm -pore-size polyamide filter, Millipore, Billerica, MA, USA), the filtrate was suspended in 10 ml of the original sample water by vortexing for 2 min, and 5 ml of the sample heat-treated. Two aliquots of 200 μl of the original and concentrated samples (heat-treated and untreated) were plated onto GVPC selective medium (Oxoid Ltd, Basingstoke, Hampshire, UK). The plates were incubated at $36 \pm 1^\circ\text{C}$ with 2.5% CO_2 for 10 days and analysed on day 4 with a dissecting microscope. Presumptive *Legionella* colonies were subcultured on BCYE (with cysteine) and CYE (cysteine-free) media (Oxoid) and then incubated at $36 \pm 1^\circ\text{C}$ for 48 h. Colonies grown on BCYE were subsequently identified using an agglutination test (*Legionella* latex test, Oxoid). The results were expressed as CFU l^{-1} , and the detection limit of the procedure was 25 CFU l^{-1} .

Only viable planktonic bacteria detected in the water stream were considered.

Chemical analysis

For the determination of nitrite, nitrate, bromide and DBPs (THMs, HAAs, chlorite and chlorate), municipal cold water, hot water from heater and from the return loop were collected in the hospital water networks treated with monochloramine, chlorine dioxide device I and II, and in an untreated network as control. Sample collection was carried out four times in a year after 5 min of flushing into 40 ml amber glass vials containing sodium thiosulphate (20 mg l^{-1} , for THM analysis) or ethylenediamine (50 mg l^{-1} , for HAAs and other inorganic ions analysis) as preservative.

Trihalomethanes (chloroform, bromodichloromethane, dibromochloromethane and bromoform) were analysed by the Static Headspace Gas Chromatographic technique with a Varian 3380 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a Vocol capillary

column (Supelco), as previously reported (Fantuzzi et al. 2010a). Quantitative and qualitative analyses of THMs were managed using Chromeleon Dionex 6.0 software (Dionex Corporation, Sunnyvale, CA, USA). The detection limit for each THM sample was $0.01 \mu\text{g l}^{-1}$. The Italian legislation fixes a parametric value of $30 \mu\text{g l}^{-1}$ for total THM concentration (reported as the sum of the four single species described above) (Italian Decree 31/2001).

HAAs (monochloroacetic, dichloroacetic, trichloroacetic, monobromoacetic dibromoacetic and tribromoacetic acids), chlorite, chlorate, nitrite, nitrate, bromide ions analyses were performed by ion chromatography (IC) combined with mass spectrometry (MS), as already indicated (Fantuzzi et al. 2010b). The instrument used was an ion chromatograph IC/MS Dionex 600 and data management was performed using Dionex Chromeleon CHM-1 software. The detection limit was $1 \mu\text{g l}^{-1}$ for each HAA, chlorate and bromide, and $20 \mu\text{g l}^{-1}$ for chlorite, nitrite and nitrate. Under Italian and European law, the parametric value is $700 \mu\text{g l}^{-1}$ for chlorite, 0.50 mg l^{-1} for nitrite and 50 mg l^{-1} for nitrate (Decreto Legislativo 2 febbraio 2001 n.31, and next modifications; Council Directive 98/83/EC). In fact, at national and international level chlorate is not

under regulation: only WHO guidelines (WHO 2011) give a provisional value of $700 \mu\text{g l}^{-1}$ for chlorate.

Statistical analysis

Statistical calculations were performed using PASW statistic version 18.0 (SPSS Inc, Chicago, IL, USA). Logarithmic transformations were used to normalize the bacteriological data, and the results are presented as geometric mean values. χ^2 tests, paired t test, one-way analysis of variance and regression analyses were applied when appropriate.

RESULTS

In the examined hospital, only *L. pneumophila* was isolated. Table 1 presents positive points and levels of *Legionella* contamination before and after monochloramine or chlorine dioxide treatment and, for comparison, in untreated networks. Before application of biocide, *Legionella* contamination was similar in every water network showing a high number of both positive and exceeding 10^4 CFU l^{-1} points. During the entire period of the study, the untreated networks remained contaminated at the same levels as

Table 1 | *Legionella* spp. contamination in hospital water networks

Treatments	Positive N (%)		Geom. mean CFU l ⁻¹ (range) ^a		>10 ⁴ CFU l ⁻¹ N (%)	
	Pre	Post	Pre	Post	Pre	Post
Monochloramine: Building 1, network C	22/22 (100.0)	8/84 ^d (9.5)	2.2×10^4 (1.0×10^2 – 9.5×10^5)	3.3×10^{2d} (25 – 4.9×10^3)	16/22 (72.7)	0/8 ^d (0.0)
Chlorine dioxide All chlorine dioxide devices	27/28 (96.4)	96/209 ^d (45.9)	1.9×10^4 (1.2×10^2 – 9.4×10^5)	5.5×10^{2d} (25 – 5.2×10^5)	18/27 (66.7)	9/96 ^d (9.4)
Device I: Building 1, network A	7/7 (100.0)	43/63 ^b (68.3)	9.2×10^3 (1.0×10^3 – 1.5×10^5)	6.9×10^{2c} (25 – 5.2×10^5)	3/7 (42.9)	4/43 ^c (9.3)
Device II: Building 1, network B	12/12 (100.0)	38/68 ^c (55.9)	1.0×10^4 (1.2×10^2 – 2.8×10^5)	7.5×10^{2d} (25 – 2.3×10^4)	7/12 (58.3)	5/38 ^c (13.1)
Device III: Building G	8/9 (88.9)	15/78 ^d (19.2)	1.0×10^5 (1.1×10^4 – 9.4×10^5)	1.3×10^{2d} (25 – 2.1×10^3)	8/8 (100.0)	0/15 ^d (0.0)
Untreated networks: Building D and E	84/85 (98.8)		1.0×10^4 (25 – 1.3×10^6)		45/84 (53.6)	

^aOnly positive samples.

^b $p < 0.05$ vs. pre-treatment.

^c $p < 0.010$ vs. pre-treatment.

^d $p < 0.001$ vs. pre-treatment.

pre-treatment. In the network treated with monochloramine, only 8/84 samples were found contaminated by *L. pneumophila*, none exceeding 10^4 CFU l^{-1} : four during the first month of treatment, another three within 8 months, and the last one in the 15th month, on the occasion of a reduction in monochloramine injection below $1\text{ mg }l^{-1}$. After that, positive samples were never detected until the end of the experiment.

Chlorine dioxide was associated with a strong significant reduction in *Legionella* mean levels and percentage of positive samples compared to pre-treatment, but differences according to the device were observed. Compared to chlorine dioxide devices I and II, the third gave better results in terms of the percentage of contaminated points ($\chi^2 = 35.73$, $p < 0.001$) and no sample was $>10^4$ CFU l^{-1} . Monochloramine was significantly more effective than chlorine dioxide (all devices) in reducing *Legionella* positive samples ($\chi^2 = 34.69$, $p < 0.001$).

Figure 1 presents the levels of chlorite and chlorate measured in water. Chlorite formation (panel (a)) was associated with chlorine dioxide in both device I and II, sometimes over the limit of $700\text{ }\mu\text{g }l^{-1}$ requested by Italian law. Chlorate (panel (b)) increased by using both biocides, although high levels associated with monochloramine injection were measured only in November when the tank containing the chlorine-based precursor was nearly finished. A positive correlation between chlorine dioxide concentration in hot water and chlorite levels was found ($r = 0.774$, $p < 0.001$). From regression line parameters, we calculated that chlorite exceeded $700\text{ }\mu\text{g }l^{-1}$ when the disinfectant residual was above $0.30\text{ mg }l^{-1}$. No HAAs and nitrites were detected in the studied water networks, and total THM concentrations were extremely low independently of treatment (hot water mean level was always $<1\text{ }\mu\text{g }l^{-1}$). Nitrates were constant during the entire experimental period, with mean levels of $23\text{ mg }l^{-1}$ in cold water and $22\text{ mg }l^{-1}$ in hot water, regardless of treatment. Also bromide was detected at low levels, with a mean concentration of $0.005\text{ mg }l^{-1}$ in all waters.

Table 2 shows the *Legionella* contamination according to the biocide levels. For monochloramine, $2\text{ mg }l^{-1}$ was necessary at distal outlets to maintain *Legionella* concentration below the detection limit of $25\text{ CFU }l^{-1}$, whereas concentrations between 1 and $2\text{ mg }l^{-1}$ were

sufficient to obtain *Legionella* below $10^3\text{ CFU }l^{-1}$. For chlorine dioxide, levels between 0.21 and $0.50\text{ mg }l^{-1}$ were associated with many positive samples at relatively low *Legionella* concentration (mean = $98.4\text{ CFU }l^{-1}$, only 2 points $>10^3\text{ CFU }l^{-1}$) and concentration $>0.50\text{ mg }l^{-1}$ reduced both number of contaminated sites and their concentration ($\leq 10^2\text{ CFU }l^{-1}$, except one sample contaminated by $6.7 \times 10^2\text{ CFU }l^{-1}$).

Twenty-three out of 68 (33.8%) samples collected immediately after the tap was switched on were contaminated (*Legionella* geom. mean $2.7 \times 10^3\text{ CFU }l^{-1}$). Figure 2 shows *Legionella* concentration in the 23 positive samples collected without flushing compared to the corresponding samples collected after 1 min of flushing. Independently of treatment, samples collected without flushing were significantly more contaminated than those after flushing (paired $t = 5.51$, $p < 0.01$), with a mean 2-log difference. A positive correlation was found between no-flush and post-flush samples ($r = 0.673$, $p < 0.001$). By using monochloramine, only 7.3% of samples negative after flushing were positive without flushing compared to 50.0% of samples treated with chlorine dioxide.

DISCUSSION

Continuous injection of chlorine-derived biocides is in fact a popular method to control legionellae in water networks of healthcare facilities (Lin et al. 2011), but the working conditions and inconveniences associated with long-time application of both chlorine dioxide and monochloramine are not sufficiently described. Following a previous pilot study (Marchesi et al. 2012), we confirm that the monochloramine action in controlling *Legionella* colonization was rapid, and after the first 8 months of treatment, no contamination was observed along a total experimental period of 36 months. In building 1, where both treatments were applied, chlorine dioxide devices I and II showed a lower effectiveness than monochloramine to maintain *Legionella* below the detection limit, as 61% of treated samples remained positive (20.6% at levels $>10^3\text{ CFU }l^{-1}$ and 11.1% $>10^4\text{ CFU }l^{-1}$). Chlorine dioxide device III, installed on building G, a new and small hospital building, gave more satisfactory results compared to devices I and II. In this

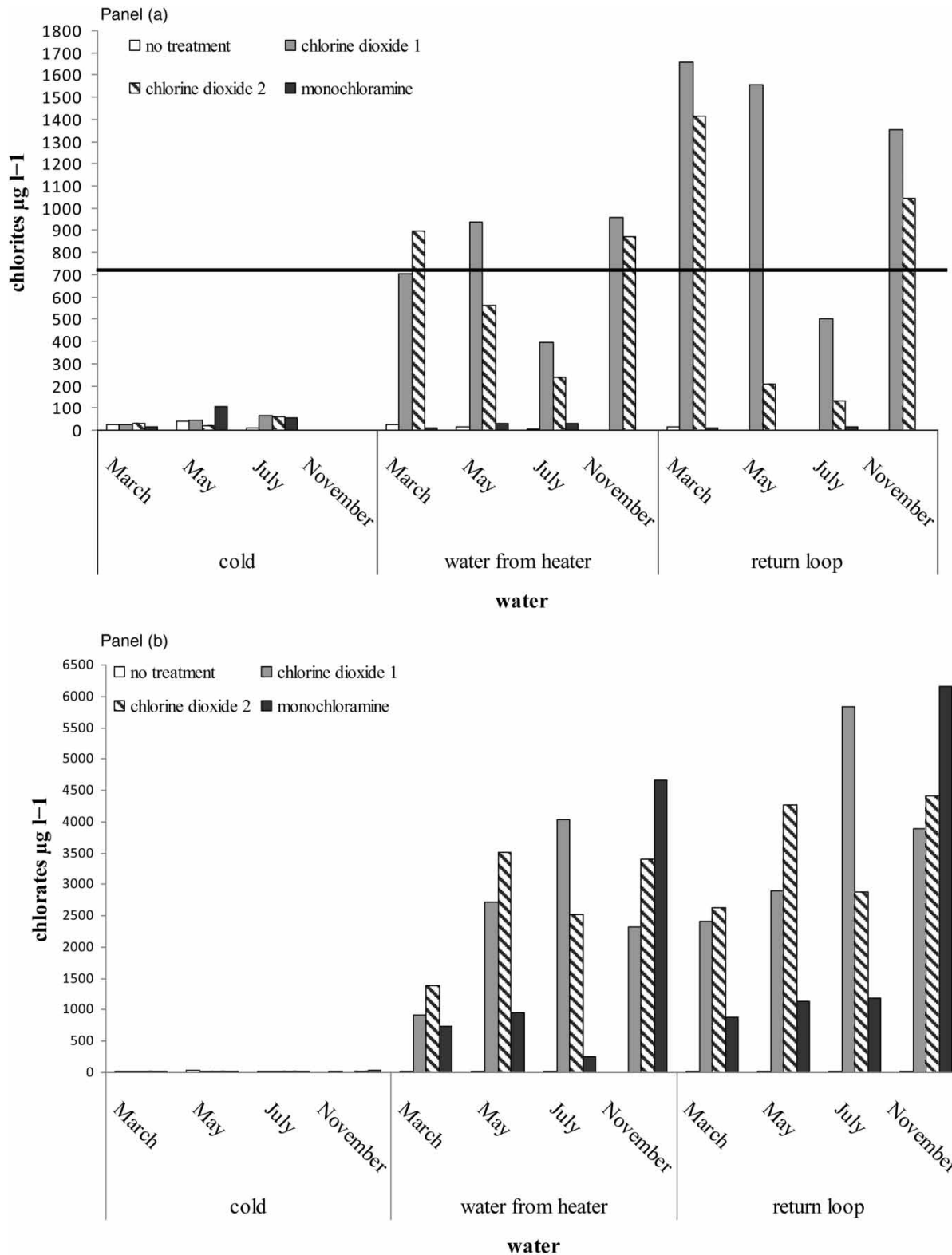
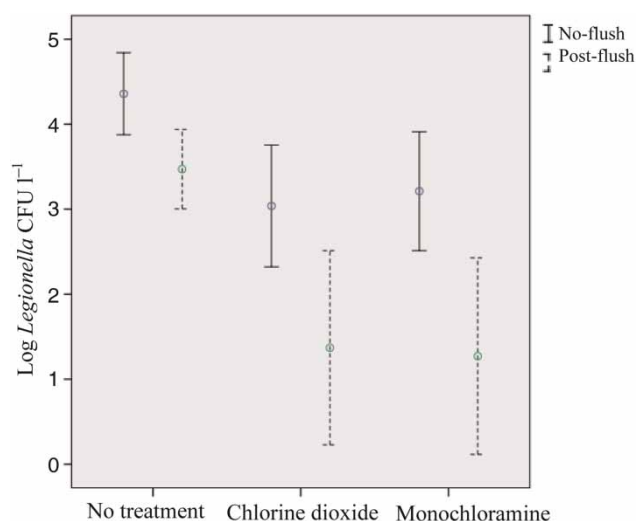


Figure 1 | Trend of chlorites (panel (a)) and chlorates (panel (b)) in untreated, treated with monochloramine and treated with chlorine dioxide (devices I and II) water networks. Black line on panel (a) indicates the Italian parametric value of $700 \mu\text{g l}^{-1}$ for chlorites in potable water.

Table 2 | *Legionella* spp. contamination according to the biocide levels (geometric mean on positive samples only)

Monochloramine			Chlorine dioxide		
Biocide (mg l ⁻¹)	Positive N (%) ^a	Geom. mean CFU l ⁻¹ (range) ^c	Biocide (mg l ⁻¹)	Positive N (%) ^b	Geom. mean CFU l ⁻¹ (range) ^d
<1	4/8 (50.0)	1.1 × 10 ³ (3.0 × 10 ² –4.9 × 10 ³)	<0.10	26/33 (78.8)	3.4 × 10 ³ (1.2 × 10 ² –5.2 × 10 ⁵)
1–1.50	2/20 (10.0)	72 (50–1.0 × 10 ²)	0.10–0.20	25/38 (65.8)	4.7 × 10 ² (25–1.0 × 10 ⁴)
1.51–2	1/19 (5.3)	8.5 × 10 ²	0.21–0.30	13/28 (46.4)	97 (25–1.5 × 10 ³)
2.01–2.5	0/15 (0.0)	0	0.31–0.50	11/36 (30.6)	1.0 × 10 ² (25–3.3 × 10 ³)
2.51–3	1/14 (7.1)	25	0.51–0.70	4/25 (16.0)	36 (25–1.0 × 10 ²)
>3	0/8 (0.0)	0	>0.70	1/26 (3.8)	6.7 × 10 ²

^a $\chi^2 = 18.23$, $p < 0.002$.^b $\chi^2 = 51.40$, $p < 0.001$.^c $F = 4.091$, *ns*.^d $F = 12.67$, $p < 0.001$.**Figure 2** | *Legionella* concentration (geometric means and 95% CI) in positive samples collected immediately (unbroken lines) and after 1 min water flushing (dotted lines).

context, we suggest that both size and age of water network can influence the effectiveness of the biocide apparatus.

Among suggested inconveniences of disinfection, the by-products formation can be of interest as they are associated with negative health effects and their regular measurements in potable water is included in EU directives. As documented for drinking water treated either with chlorine dioxide or chloramine (WHO 2011), our study demonstrated that the chlorine dioxide is associated with increased levels of chlorite and chlorate, exceeding the limit requested for the first parameter. In contrast, no increase in chlorite levels was found

in hot water treated with monochloramine. However, high levels of chlorates were found in water disinfected with monochloramine when the chlorine-based precursor was near to finish in the tank. This is in line with the observation that chlorate can also form in hypochlorite solutions on storage (WHO 2011). In our study, the absence of nitrites and the presence of nitrate <50 mg l⁻¹ demonstrate that the use of monochloramine is not associated with the occurrence of water nitrification, a microbiological process described when chloramination is used to provide a residual disinfectant in cold water distribution networks (WHO 2004).

Among the other possible disadvantages associated with chloramine treatment of drinking water, the formation of N-nitrosodimethylamine (NDMA), an N-nitrosamine classified as probably carcinogenic to humans, is described (Russell *et al.* 2012; Shah & Mitch 2012). N-nitrosation, a reaction between nitrosatable amines and nitrite, is considered the classical nitrosamine formation mechanism (Williams 1988). Furthermore, bromide was reported to produce bromine or bromamine that can react with organic matter and form NDMA (Mitch *et al.* 2003). We have not measured NDMA, but the nitrites below the detection limit and the low bromide levels are in favour of a low NDMA formation potential. Recent studies suggest that NDMA can be formed by the reaction of monochloramine with different precursors also in the absence of nitrite (Chen & Valentine 2006; Kristiana *et al.* 2013), thus our next research will target the measurement of NDMA in water networks treated with this biocide.

One of the aims of our study was to better define the biocide concentrations able to obtain a significant reduction in *Legionella* spp. contamination on hot water. Monochloramine at concentrations between 2 and 3 mg l⁻¹ at distal outlets assured a *Legionella* colonization below 10² CFU l⁻¹. These concentrations are in line with those measured in drinking water treated with monochloramine (WHO 2004). Chlorine dioxide levels between 0.50 and 0.70 mg l⁻¹ were needed to maintain *L. pneumophila* near 10² CFU l⁻¹. However, already at 0.30 mg l⁻¹ of disinfectant residual, chlorite concentration increased above the European parametric value of 700 µg l⁻¹. Studies are in progress to verify whether these two biocides, at our concentrations, are associated with pipe corrosion, as some reports suggest that chlorine dioxide can cause pipe damage and data on monochloramine are lacking (Chord et al. 2011; Yu et al. 2011).

Lastly, by comparing the recovery of *Legionella* according to the sampling method (no-flush vs. 1 min flush), we observed that *Legionella* concentration was 2-log higher in no-flush than in post-flush samples, independently of the biocide, in accordance with similar results obtained by Serrano-Suárez et al. (2013). Compared to monochloramine, however, chlorine dioxide was associated with a higher frequency of no-flush positive samples, suggesting an increased risk for patients when they turn the tap on. In this context, we highlighted that the no-flush sampling, generally mandatory when a case of hospital-acquired legionellosis occurs, is more exhaustive and appropriate than the post-flush one to evaluate both the efficacy of disinfection systems applied in hospital and, even more importantly, the risk to the patients. Thus, we recommend to add no-flush sampling also when routine environmental monitoring of the *Legionella* contamination is performed in hospital, as suggested by some regional guidelines in Italy (Regione Emilia-Romagna 2008; Regione Piemonte 2008).

CONCLUSION

The major achievements of our study are as follows.

- In these 3 years' experience, *Legionella* colonization rapidly decreased after monochloramine treatment and

already after 8 months no *Legionella* contamination was observed.

- Compared to monochloramine, the effectiveness of chlorine dioxide was lower, with different results according to size and age of the water network.
- No increase in chlorite levels and no water nitrification occurred in hot water treated with monochloramine, whereas the use of chlorine dioxide was associated with increased levels of both chlorite and chlorate.
- At distal outlets, monochloramine at concentrations between 2 and 3 mg l⁻¹ and chlorine dioxide between 0.50 and 0.70 mg l⁻¹ can guarantee a satisfactory control of *L. pneumophila* colonization.
- In comparison with monochloramine, chlorine dioxide showed a higher frequency of no-flush positive samples, suggesting an increased risk for patients when the tap is turned on.

In conclusion, we highlight that continuous injection of monochloramine should be considered as an effective alternative to chlorine dioxide in controlling legionellae contamination inside hospital water distribution systems, although further studies are needed to exclude the formation of toxic emerging DBPs such as N-nitrosamines.

ACKNOWLEDGEMENT

The authors thank Sanipur S.r.l. for technical support and equipment provided.

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First received 7 May 2013; accepted in revised form 2 August 2013. Available online 2 September 2013