Ct values required for degradation of microcystin-LR by free chlorine

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ABSTRACT

Recently, there has been increased interest in microcystin-LR and other fresh water cyanotoxins because of their toxicity and occurrence throughout the world. Although previous studies have shown that free chlorine can degrade microcystin-LR, there are insufficient data to develop the contact times and free chlorine doses that achieve targeted levels of microcystin-LR degradation. Furthermore, there are insufficient microcystin-LR degradation data that would allow for the development of feasible microcystin-LR criteria or standards. To systematically develop this critical information, a total of 34 batch chlorination experiments were performed at different pH values, chlorine doses, and toxin concentrations. For all conditions, *Ct* (C = chlorine concentration, t = contact time) values required for degradation of microcystin-LR were calculated and safety factors were estimated. Twenty seven of the 34 experiments were conducted with reagent-grade water and seven of the 34 experiments were conducted with natural waters. At all pH values tested, the degradation of microcystin-LR increased with increasing *Ct*. For *Ct* values usually observed in drinking water treatment, a 1-log degradation of microcystin-LR in reagent-grade water was observed only at pH 6.0. The results also suggest that the *Ct* values obtained from reagent-grade water experiments are appropriate for application to natural waters that have been subjected to conventional coagulation, flocculation, sedimentation, and filtration prior to chlorine addition.

Key words | blue-green algae, chlorination, *Ct*, cyanobacteria, microcystin-LR, safety factor

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INTRODUCTION

Fresh water toxins produced by cyanobacteria, or blue-green algae, are generally referred to as "cyanotoxins". Blooms of toxic cyanobacteria have occurred worldwide for many years and have led to illness in humans and to the death of animals (Tisdale 1931; Dillenberg & Dehnel 1960; Gorham 1964; Zilberg 1966; Falconer *et al.* 1983*a*; Carmichael *et al.* 1988, 2001; Mahmood *et al.* 1988; Yu 1989; Edmondson 1991; Teixera *et al.* 1993; Kotak *et al.* 1995; Harada *et al.* 1996; Ueno *et al.* 1996). The reported number of cyanobacteria blooms has increased due to increased nutrient loading from farming, urban runoff, treated wastewater, and decreased water flow caused by poor watershed management or drought (Tsuji *et al.* 1997; Westrick 2003). Drinking water treatment plants that treat surface waters are at risk for cyanotoxin contamination if a toxin-producing bloom develops in the source water.

Cyanotoxins may be classified as hepatotoxins (toxins that affect the liver) or neurotoxins (toxins that affect the nervous system), and occurrence data suggest that hepatotoxins are the most prevalent of the cyanotoxins (Westrick 2003). Microcystins are a group of hepatotoxins that were named after the first organism found to produce them, *Microcystis aeruginosa*. Microcystin and microcystin-producing species of cyanobacteria have been found in

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Japan, Portugal, Brazil, Australia, Germany, Canada, Zimbabwe, and the United States (Tsuji *et al.* 2001; Vasconcelos & Pereira 2001; Freitas de Magalhaes *et al.* 2001; Karner *et al.* 2001; Carmichael 2001; Atkins *et al.* 2000; Fromme *et al.* 2000).

Microcystins are hydrophobic, cyclic heptapeptide toxins consisting of seven amino acids with a molecular weight between 800 and 1100. Microcystins can cause death due to liver failure at high doses, or they may promote tumor formation and gastroenteritis at sub-lethal doses (Carmichael 1996; Orr *et al.* 2001). There are approximately 65 known microcystins generated from the *Microcystis* genera (USEPA 2001) and microcystin-LR is the most commonly observed and the most toxic. Microcystin-LR may remain intact in water for one to three months (Kiviranta *et al.* 1991; Tsuji *et al.* 1994).

In 1998, the World Health Organization adopted a provisional guideline value for microcystin-LR of 1.0 μg/L for drinking water systems (WHO 1998). In the United States, microcystin-LR and other cyanotoxins are not yet regulated but they are currently on the USEPA Contaminant Candidate List (USEPA 1997, 1998, 1999*a*,*b*, 2000, 2001, 2004, 2005). A recent survey of water utilities in the United States and Canada confirmed the presence of microcystin-LR in 539 of 677 (80%) source water samples with 4.3% of the samples higher than the WHO guideline of 1.0 μg/L (Carmichael 2001). Similar detection levels were reported in Wisconsin (Karner *et al.* 2001).

A number of water treatment technologies have been evaluated for their effectiveness at removing microcystin-LR from drinking water supplies. Since chlorine is used by more than 95% of the medium and large utilities in the United States (AWWA Water Quality Division Disinfection Committee 2000), oxidation by free chlorine is an easily accessible means of oxidation that is worth investigating further.

Previous work has shown that oxidation effectiveness is reduced in the presence of live cyanobacterial cells (Rositano & Nicholson 1994). If chlorine is applied to raw water containing cyanobacterial cells, the oxidant may cause cell lysis and toxin release. Therefore, it is best to use oxidation to degrade dissolved cyanotoxins after the removal of algal cells by a set of processes like coagulation, flocculation, sedimentation, and filtration (Hart *et al.* 1998).

Initial studies on the chlorine oxidation of dissolved microcystins showed chlorine to be ineffective for microcystin degradation (Hoffmann 1976; Keijola *et al.* 1988; Himberg *et al.* 1989). However, more recent studies have contradicted the earlier findings (Nicholson *et al.* 1994; Hart *et al.* 1998; Bruchet *et al.* 1998). The ineffectiveness of chlorine in the initial studies may have been due to high pH levels or to open systems allowing chlorine release to the air (Lawton & Robertson 1999; Nicholson *et al.* 1994).

Nicholson *et al.* (1994) showed that a chlorine residual of at least 0.5 mg/L was required after a contact time of 30 min to achieve nondetectable microcystin concentrations, as long as pH was maintained below pH 8. The researchers evaluated microcystin concentrations ranging from 130 to 300 μg/L and chlorine doses ranging from 0 to 30 mg/L. Tsuji *et al.* (1997) observed poorer removals, but their initial microcystin concentration was 10,000 μg/L with pH values less than 7.2 and chlorine doses ranging from 0.7 to 2.8 mg/L. Hart *et al.* (1998) tested various contact times using an initial toxin concentration of 6.9 μg/L and an applied chlorine dose of 1.7 mg/L. They observed greater than 93% removal of microcystin-LR at pH 5 and a contact time of 30 min. At pH 9, the reduction in toxin concentration was significantly less, even after a contact time of 22 h. Bruchet *et al.* (1998) observed greater than 80% removal of microcystin-LR when a 1 mg/L chlorine dose was added to water with an initial toxin concentration of 500 μg/L and a contact time of 2 h.

One concern of using chlorine is the possible formation of more toxic chlorination by-products. Tsuji *et al.* (1997) and Senogles-Derham *et al.* (2003) performed mouse bioassays and concluded that the chlorination of microcystins did not result in the formation of harmful products. Senogles-Derham *et al.* (2003) concluded that microcystins were readily removed by chlorine, there was no increase in cancer among the mouse population, and there was no significant increase in trihalomethane or haloacetic acid concentrations.

Although the literature shows that chlorination can degrade microcystin-LR, there are insufficient data to develop the contact times and free chlorine doses that achieve targeted levels of microcystin-LR degradation. Furthermore, there are insufficient microcystin-LR degradation data that would allow the development of feasible

microcystin-LR criteria or standards. Disinfection processes like chlorination are typically designed, operated, and monitored on the basis of the *Ct* concept, where *Ct* refers to the product of the process residence time (*t*) and the chlorine concentration (*C*) remaining at the end of that residence time. This paper describes a systematic approach designed to develop this critical information.

METHODS

Chlorination experiments with reagent-grade water

A total of 27 batch chlorination experiments were performed in 1 L amber glass bottles. These experiments were performed with $NaHCO_3$-buffered reagent-grade (milli-Q) water by adjusting the alkalinity of the reagent-grade water to 100 mg/L as $CaCO_3$. The water was incubated at 11 ± 1°C. Because chemical reaction rates increase with increasing temperature, the results presented above for 11°C are expected to be conservative results. Nine experiments were performed at pH 6.0 ± 0.1, nine at pH 7.5 ± 0.1, and nine at pH 9.0 ± 0.1. The nine experiments at each pH value included a matrix of three toxin concentrations (1.0 ± 0.3 μg/L, 2.2 ± 0.5 μg/L and 8.4 ± 2.5 μg/L) and three chlorine doses (1.0 ± 0.2 mg/L, 2.7 ± 0.5 mg/L, and 9.2 ± 0.8 mg/L). The results in this paper were reported as the average value ± the standard deviation. Chlorination time was varied within each experiment to determine the rate of toxin degradation. For each sample, the chlorine and toxin were allowed to interact for a different amount of time (usually 3, 10, 30, or 100 min). In addition, one sample was spiked with toxin but was not spiked with chlorine. One more sample was used as the control (blank) and was not spiked with toxin or chlorine.

Prior to the experiment, the initial toxin concentration, chlorine dose, and pH value were randomly selected from the values listed above. Six beakers with 1500 mL of reagent-grade water were covered with aluminum foil and placed in an incubator (Fisher Scientific Isotemp Model 626–2, Pittsburgh, PA) to reach 11 ± 1°C. A seventh beaker was prepared for every other experiment in order to have one duplicate to assess experimental repeatability. The 1500 mL samples were then removed from the incubator and 15 mL of stock carbonate buffer (8.4 g $NaHCO_3$ in 1 L of reagent-grade water) was added to each sample. The pH was then adjusted with 1 N NaOH and 1 N HCl solutions to the desired value. The pH was measured with an Orion Model SA520 glass-bulb, liquid-junction electrode, which was calibrated daily with pH 4, pH 7 and pH 10 standards according to *Standard Methods* (APHA, AWWA & WEF 1998).

Samples were put back into the incubator until required for the degradation experiment. To begin the degradation experiment, one sample was taken out of the incubator and the temperature was measured with a Traceable digital thermometer. While stirring, microcystin-LR (Alexis Biochemicals, San Diego, CA; > 96% purity) was added to reach the desired concentration. While continuing to stir, chlorine stock solution (prepared by adding sodium hypochlorite to reagent-grade water) was added to reach the desired concentration. Prior to use, the chlorine stock was adjusted to pH 6.0, pH 7.5 or pH 9.0 with 1 N NaOH and 1 N HCl solutions and the free chlorine concentration was measured. Free chlorine concentration was quantified according to the DPD ferrous titrimetric method (APHA, AWWA & WEF 1998). Timing was started once chlorine was added.

Each sample was poured into one 1.05 L and three 0.130 L amber glass bottles until each bottle was headspace free. Each bottle was placed back into the incubator until the desired time span had ended. At the end of the desired time span, 1250 mg ascorbic acid was added to the 1.05 L bottle to stop the reaction between the chlorine and toxin. At that time, the temperature was also measured. Using the sample in one of the 0.130 L amber bottles, the pH was measured. Using the sample from the second 0.130 L amber bottle, the chlorine residual was measured.

The UV absorbance and dissolved organic carbon (DOC) were measured using the sample in the third 0.130 L amber bottle. The UV absorbance was measured with a Hach DR/4000 Spectrophotometer set to a wavelength of 254 nm (APHA, AWWA & WEF 1998). The DOC concentration was measured with a Tekmar-Dohrmann/Phoenix 8000 TOC Analyzer by using Standard Method #5310.C (APHA, AWWA & WEF 1998).

Analysis of the toxin concentration in the 1.05 L amber bottle was conducted by taking 0.2 mL of sample for ELISA analysis and 600 mL of sample for HPLC analysis. The sample was frozen until ELISA or HPLC analysis

could be performed. The experiments produced a total number of 135 samples for which toxin was added, with 13 of the experimental conditions replicated. The relative standard deviation (defined as the standard deviation over the mean times 100) in microcystin-LR concentration between replicate samples was 10%.

Chlorination experiments with natural waters

Seven natural water samples were collected from various surface waters in the United States to represent geographical diversity and diversity in water quality. Table 1 presents the locations of the surface waters where the natural water samples were taken. A 20 L water sample was collected from the designated location upstream of any treatment process, packed with ice, and shipped by cooler overnight to the laboratory. The pH and turbidity of the raw water were measured. The turbidity was measured with a Hach 2100N Turbidimeter (APHA, AWWA & WEF 1998). A small portion of the raw water was filtered using 0.45 μm membrane filters and the UV absorbance and dissolved organic carbon concentration of the filtered raw water were measured and recorded.

To mimic a conventional water treatment plant, the remaining raw water was prepared using coagulation, flocculation, sedimentation, and filtration before the chlorination experiment was run. Through this treatment process, any intracellular toxin already present in the natural water was expected to be removed (Drikas *et al.* 2001). Alum was used as the coagulant and the optimum dose was determined by running jar tests in which six jars were each filled with 1 L of raw natural water and placed on a gang-mixer (Phipps and Bird Stirrer Model 7790–400, Richmond, VA). A different alum dose was added to each jar and the range of doses used was suggested by the water suppliers. The rapid mix phase was run for 2 min at 200 rpm followed by flocculation for 15 min at 30 rpm and sedimentation for 60 min. The final turbidity was measured for each jar and the alum dose that gave the minimum turbidity was selected.

Enough water, typically 10–12 L, was prepared at the selected alum dose to determine the required chlorine dose and to conduct the toxin degradation experiment. This water was prepared by filling 5–6 jars with 2 L of raw natural water and placing the jars on the gang-mixer. The selected alum dose was added to each jar under the same rapid mix, flocculation and settling conditions noted above. All of the settled water was filtered through 0.45 μm membrane filters. Dissolved organic carbon and UV absorbance were measured for the treated water (see Table 2).

To determine the chlorine dose that would be used in the experiment, five 150 mL amber glass bottles were filled headspace free with the treated natural water. A chlorine dose of 2, 4, 6, 8, and 10 mg/L was added to each respective

Table 1 | Locations of the surface waters where the natural water samples were taken

Source	City	State	Utility
Lake Mendota	Madison	WI	Not applicable
Lake Winnebago	Oshkosh	WI	Oshkosh Water Utility
Lake Michigan	Glendale	WI	North Shore Water Commission
South Bay Aqueduct	San Jose	CA	Santa Clara Valley Water District Penitencia Water Treatment Plant
Lake Del Valle (65%) & South Bay Aqueduct (35%)	Livermore	CA	Zone 7 Water Agency Del Valle Water Treatment Plant
Atkins Reservoir	Amherst	MA	Department of Public Works
Aurora Reservoir	Aurora	CO	Wemlinger Water Treatment Plant

Table 2 | Characteristics of the coagulated, flocculated, settled, and filtered natural waters

Natural water source	Turbidity (NTU)	DOC (mg/L)	UV254 (cm^{-1})
Lake Mendota	0.09	3.6	0.04
Lake Winnebago	0.18	7.1	0.13
Lake Michigan	0.14	1.8	0.02
South Bay Aqueduct	0.25	2.1	0.04
Lake Del Valle (65%) & South Bay Aqueduct (35%)	0.16	2.6	0.04
Atkins Reservoir	0.42	3.1	0.04
Aurora Reservoir	0.05	3.6	0.04

bottle. The bottles were incubated for 100 min at 11°C and the chlorine residual was measured. The residual was plotted versus the dose and a chlorine dose that achieved a 0.5 to 2.0 mg/L chlorine residual was selected for the toxin degradation experiment.

Degradation experiments were run following the same procedure previously outlined for reagent-grade water experiments except that the treated natural water was used instead of the carbonate-buffered reagent-grade water. The treated natural water samples were incubated at 11 ± 1°C, the pH value was adjusted to 7.4 ± 0.2, and 2.9 ± 0.6 μg/L of toxin was added. Table 3 shows the conditions used for each water, along with the average observed chlorine residual during the experiment.

Analytical methods for microcystin-LR

Microcystin-LR concentrations were measured using commercially available enzyme linked immunosorbent assay (ELISA) plate kits (EnviroGard® Microcystins Plate Kit, Strategic Diagnostics Inc., Newark, DE). The kits contained 96-well microtiter plates coated with antibodies specific to microcystins. The ELISA kits were used in accordance with the manufacturer's directions. The plates were read on a

Table 3 | Experimental conditions for the chlorination experiments that used natural waters

Natural water source	Av. temp. (°C)	Initial toxin (μg/L)	Chlorine dose (mg/L)	Average chlorine residual (mg/L)	Average chlorine demand (mg/L)	Av. pH
Lake Mendota	12.0	2.8	3.0	2.2	0.8	7.5
Lake Winnebago	10.0	2.6	3.6	1.3	2.3	7.4
Lake Michigan	10.2	2.3	2.0	1.4	0.6	7.4
South Bay Aqueduct	9.9	2.3	1.6	0.4	1.2	7.5
Lake Del Valle (65%) & South Bay Aqueduct (35%)	10.2	3.2	3.0	0.9	2.1	7.5
Atkins Reservoir	10.4	2.9	3.0	1.5	1.5	7.1
Aurora Reservoir	12.5	4.0	2.5	0.8	1.7	7.7

microtiter plate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 450 nm with a reference wavelength of 650 nm. Microcystin concentrations were calculated from the standard curve using the standards provided with the plate kit.

Microcystin-LR was also quantified using high performance liquid chromatography (HPLC) on 19% of the samples to provide additional confidence in the ELISA results. Analysis of microcystin-LR in water samples by HPLC was based on methods developed by Nicholson *et al.* (1994) and Moollan *et al.* (1996). Microcystin-LR was quantified using an HPLC (Agilent 1090, Wilmington, DE) coupled with photodiode array detection. The results of the two methods were correlated with an $R^2 = 0.904$. There was no tendency observed for one of the methods (HPLC or ELISA) to read consistently higher or lower than the other. The results described in this paper are the ELISA results.

Degradation rate determination

A plausible rate law for the interaction of free chlorine and microcystin-LR is presented in Equation (1). The equation includes the rate constants for the reaction of hypochlorous acid with microcystin-LR (k_{HOCl}) and the reaction of hypochlorite with microcystin-LR (k_{OCl^-}). The equation also includes time (t), microcystin-LR concentration in μg/L ([toxin]), and hypochlorous acid and hypochlorite concentrations ([HOCl] and [OCl^-]):

$$\frac{d[\text{toxin}]}{dt} = -k_{HOCl}[\text{toxin}][\text{HOCl}] - k_{OCl^-}[\text{toxin}][OCl^-]. \quad (1)$$

By combining Equation (1) with the definition of free chlorine (Equation (2)) and the equilibrium constant of the hypochlorous acid/hypochlorite system (Equation (3)), the rate law shown in Equation (4) is derived:

$$[\text{free chlorine}] = [\text{HOCl}] + [OCl^-] \quad (2)$$

$$K_a = \frac{[H^+][OCl^-]}{[\text{HOCl}]} \quad (3)$$

$$\frac{d[\text{toxin}]}{dt} = -\left(\frac{k_{HOCl}[H^+] + k_{OCl^-}K_a}{[H^+] + K_a}\right)[\text{free chlorine}] \times [\text{toxin}]. \quad (4)$$

In Equations (2)–(4), [free chlorine] is the concentration of free chlorine in mg/L as Cl_2, K_a is the equilibrium constant of the hypochlorous acid/hypochlorite system in μmol/L, and [H^+] is the concentration of protons in μmol/L. In experiments performed for this study, the molar free chlorine concentration exceeded the molar microcystin-LR concentration by 3 to 5 orders of magnitude. Because of this, the free chlorine concentration did not vary with time and Equation (4) can be integrated to produce the pseudo-first order rate law shown in Equation (5):

$$\frac{[\text{toxin}]}{[\text{toxin}]_0} = \exp\left\{-\left(\frac{k_{HOCl}[H^+] + k_{OCl^-}K_a}{[H^+] + K_a}\right)[\text{free chlorine}]_0 t\right\}. \quad (5)$$

In Equation (5), $[\text{toxin}]_0$ and $[\text{free chlorine}]_0$ are the initial toxin concentration and free chlorine dose, respectively. Data from several experiments at one pH value and several chlorine doses cannot be used to estimate values for K_a, k_{HOCl}, or k_{OCl^-}. However, they can be fitted with Equation (6), where k is an apparent rate constant that is specific to the pH used in the experiment (see Equation (7)). Equation (6) may be written as Equation (8), where $[\text{free chlorine}]_0 t = Ct$. Therefore

$$\frac{[\text{toxin}]}{[\text{toxin}]_0} = \exp\{-k[\text{free chlorine}]_0 t\} \quad (6)$$

$$k = \frac{k_{HOCl}[H^+] + k_{OCl^-}Ka}{[H^+] + K_a} \quad (7)$$

$$\frac{[\text{toxin}]}{[\text{toxin}]_0} = \exp(-k\,Ct) \quad (8)$$

Ct safety factor determination Clark *et al.* (2002, 2003) described a statistical approach designed to develop safety factors for *Cryptosporidium* oocyst inactivation. The approach was based on the use of statistics to place an upper bound safety factor on a given mathematical equation and is independent of the mechanism by which a substance is degraded. Using their approach, a *Ct* equation was derived to include a factor of safety for the chlorination of microcystin-LR. For data described by Equation (5), the *Ct* at all pH values

is given by the following equation:

$$Ct \leq \exp\left\{\ln\left[-\ln\left(\frac{[\text{toxin}]}{[\text{toxin}]_0}\right)\right] - \left[\ln\left(\frac{k_{HOCl}[H^+] + k_{OCl^-}K_a}{[H^+] + K_a}\right) - Z_a S\right]\right\} \quad (9)$$

where Z_a is the $100(1-a)\%$ cut-off value from the standard normal distribution and S is the estimated standard error of Y, where

$$Y = \ln\left[-\ln\left(\frac{[\text{toxin}]}{[\text{toxin}]_0}\right)/Ct\right]. \quad (10)$$

For a 95% level of confidence, $Z_a = 1.64$. The standard deviation of Y was used as an estimate of S and it was equal to 1.29.

RESULTS AND DISCUSSION

Reagent-grade water chlorination experiments

Microcystin-LR degradation for all twenty-seven experiments is presented in Figure 1, which shows the ratio of final toxin concentration to initial toxin concentration as a function of *Ct*. The *Ct* values were computed by multiplying the residual chlorine concentration with chlorination time. For these reagent-grade water experiments, there was no chlorine demand and the chlorine residual at any given time was equivalent to the chlorine dose. Experiments conducted at the same pH value were grouped together and fitted with Equation (8). The figure shows that the degradation of microcystin-LR increased with increasing *Ct* at all three pH values. In addition, the degradation rates increased as pH decreased. The values of k estimated from Equation (8) were estimated to be $0.065^{\pm}0.007$ L/(mg min), 0.014 ± 0.001 L/(mg min), and 0.005 ± 0.001 L/(mg min) for pH 6.0, pH 7.5, and pH 9.0, respectively. This suggests that HOCl (prevalent chlorine form at pH 6.0) provides faster degradation of microcystin-LR than OCl^- (prevalent chlorine form at pH 9.0). This was further confirmed by fitting all of the data with Equation (5) to obtain estimates for K_a, k_{HOCl}, and k_{OCl^-}. The estimated values were $K_a = 0.176 \pm 0.044$ μμmol/L, $k_{HOCl} = 0.077 \pm 0.010$ L/(mg·min), and $k_{OCl^-} = 0.0036 \pm 0.0005$ L/(mg·min). These results suggest that HOCl was 20 times faster than OCl^- at degrading microcystin-LR.

Figure 2 presents the same data shown in Figure 1, but for pH 6.0 only. The figure includes the best fit line from Equation (5) for the observed data. Based on Equation (9), a *Ct* curve including a safety factor was also estimated and presented in Figure 2. The figure presents results for pH 6.0 only, as an example. Similar trends were observed at pH 7.5 and pH 9.0. Best fit and safety factor *Ct* results for all pH values are summarized in Table 4. Figure 2 shows that 90% degradation of microcystin-LR was observed at a *Ct* value of 35 mg min/L at pH 6.0. When the initial microcystin-LR concentration of the raw water is known, Figure 2 can be used to determine the *Ct* required to achieve a target toxin concentration. For example, if a utility has a microcystin-LR concentration of 10 μg/L in their raw water and needs

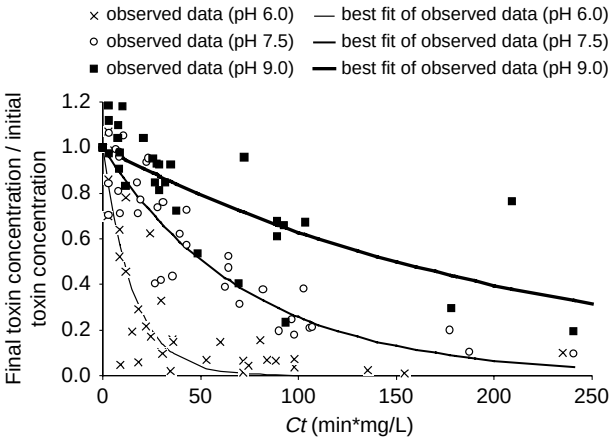

Figure 1 | Degradation of microcystin-LR versus *Ct* in reagent-grade water at 11°C.

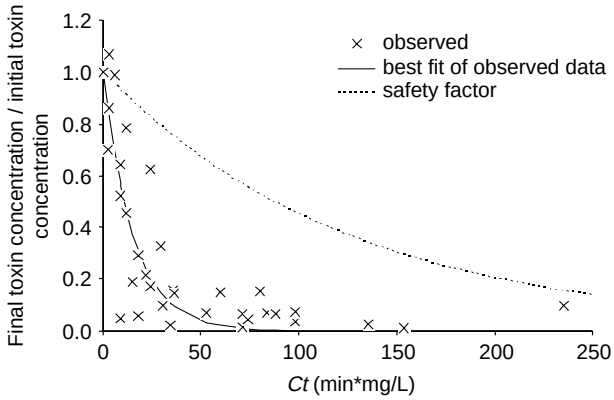

Figure 2 | *Ct* determination for the degradation of microcystin-LR in reagent-grade water at 11°C and pH 6.0.

Table 4 | *Ct* values (mg min/L) for degradation of microcystin-LR by free chlorine in reagent-grade water

	Best-fit estimation			Estimation including safety factor		
Target degradation	**pH 6.0**	**pH 7.5**	**pH 9.0**	**pH 6.0**	**pH 7.5**	**pH 9.0**
50% degradation	11	51	149	89	390	1437
90% degradation	35	169	496	290	1297	4773
95% degradation	46	220	646	378	1687	6210

to achieve a final microcystin-LR concentration of 1 μg/L, they would need to apply *Ct* values higher than 35 mg min/L at pH 6. If a contact time of 100 min is applied at this utility, then chlorine residuals greater than 0.35 mg/L should be maintained. The safety factor analysis indicated that 290 mg min/L would be required to achieve 90% degradation with a 95% level of confidence. This *Ct* value is more than 8 times greater than the value suggested by the best fit of the data.

Table 4 summarizes the data obtained with the best-fit estimation of *Ct* and with the *Ct* curve including the safety factor, for all pH values. *Ct* values typically observed in drinking water treatment are less than 200 mg min/L (AWWA Water Quality Division Disinfection Systems Carmichael 1996). With the best-fit estimation, 50% degradation could be achieved with a $Ct < 200$ mg min/L at all three pH values tested. However, 95% degradation could only be achieved at pH 6.0 with a $Ct < 200$ mg min/L. When the safety factor approach was used, a 50% degradation of microcystin-LR was observed only at pH 6.0 with a $Ct < 200$ mg min/L. For all other cases shown in Table 4, the safety factor approach would require *Ct* values greater than 200 mg min/L. These results could have significant practical implications for drinking water utilities and significant policy implications for regulators. A more complete discussion of these implications is provided in the Conclusions section.

Comparison with previous studies

It is worthwhile to determine if either the best-fit approach or the safety factor approach compares favorably with previous research. Microcystin-LR chlorination studies have been published by Nicholson *et al.* (1994); Tsuji *et al.* (1997); Hart *et al.* (1998) and Bruchet *et al.* (1998). For each of these studies, *Ct* values were estimated from reported chlorine doses by assuming that the water had no chlorine demand. Figure 3 shows these estimates and demonstrates that the degradation rates observed in previous studies were similar to those observed in this study. There are some exceptions to this general conclusion, but it is important to note that the literature data and the data obtained in our study were not obtained under identical conditions. For example, the pH values of the literature data were different than ours or not reported at all, and temperatures were higher or not reported. In addition, the molar ratios of chlorine dose to initial toxin concentration were not similar.

For example, Tsuji's group showed that the fraction of toxin remaining leveled off at about 60% in one experiment, 20% in a second experiment and near 0% in a third experiment. These experiments used an initial microcystin-LR concentration of 10 000 μg/L and three chlorine doses ranging from 0.7 to 2.8 mg/L. Chlorine residuals were not reported in their study, but it is plausible that the chlorine residual dropped to zero or near zero in two of the experiments. This is plausible because the initial molar ratio of microcystin-LR to chlorine ranged from 1.0 at the 0.7 mg/L chlorine dose to 0.26 at the 2.8 mg/L chlorine dose. If the chlorine residual dropped to zero, then further microcystin-LR degradation would not be possible. It is also

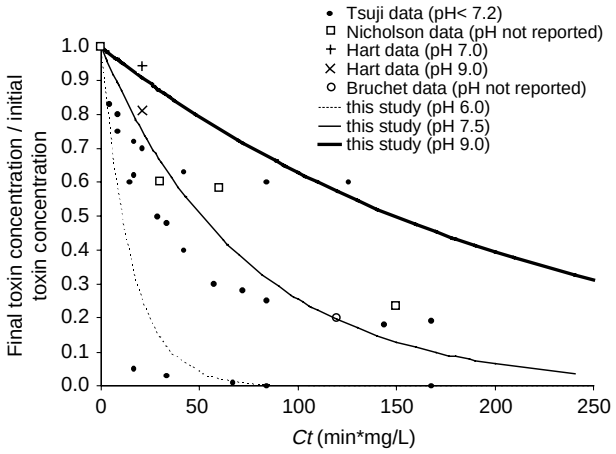

Figure 3 | *Ct* values for degradation of microcystin-LR reported in the literature compared to results from this study.

likely that the conditions used by Tsuji's group would not meet the pseudo-first-order conditions that would normally be expected in drinking water treatment systems.

Figure 4 presents the literature data and a safety factor curve for pH 7.2. This pH value was selected to match the highest pH value used by Tsuji's group. The graph shows that all literature data fall to the left and below the safety factor curve. In other words, all of the observed microcystin degradations were greater than or equal to those predicted by the safety factor curve.

Natural water chlorination experiments

Seven experiments were performed with natural waters. As an example, Figure 5 shows the free chlorine concentration versus time for the experiment with coagulated, flocculated, settled and filtered water from Lake Mendota. As expected, the free chlorine concentration decreased rapidly with time for the first 3 min, presumably due to reactions with substances like natural organic matter. The chlorine degradation rate slowed down after the 3 min contact time. Using the trapezoidal method, the area under the line in Figure 5 was calculated to obtain *Ct* values and the degradation of microcystin-LR versus *Ct* was plotted in Figure 6. The figure shows that the ratio of final toxin concentration to initial toxin concentration rapidly decreased between 0 and 70 mg min/L and then gradually continued to decrease between 70 and 215 mg min/L. Figures 5 and 6 show data for Lake Mendota only but comparable results were observed for the other natural waters. In all cases, free chlorine concentration decreased with time, and the ratio of final toxin concentration to initial toxin concentration decreased as *Ct* values increased.

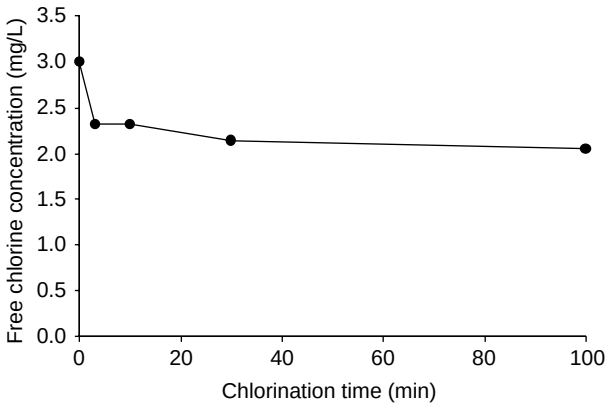

Figure 5 | Free chlorine concentration versus time for the Lake Mendota experiment at 12°C, pH 7.5 and microcystin-LR concentration of 2.8 μg/L.

Ct values were calculated for all of the natural water experiments in a manner similar to that used for the Lake Mendota experiment. Figure 7 shows the degradation of microcystin-LR versus *Ct* for all natural water experiments. This figure demonstrates that the rate of reaction between chlorine and microcystin-LR was site-specific, with the fastest degradation occurring in water from the South Bay Aqueduct at San Jose, California. There was no statistically significant correlation between the degradation rate of microcystin-LR and dissolved organic carbon concentrations. Similarly, the degradation rate of microcystin-LR was not significantly correlated with turbidity, absorbance

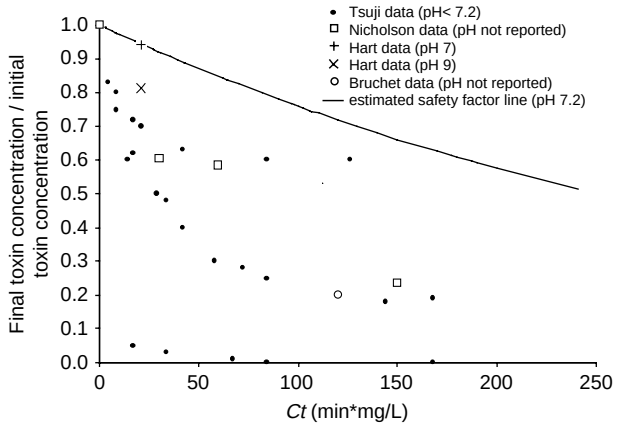

Figure 4 | *Ct* values for degradation of microcystin-LR reported in the literature, compared with safety factor equation.

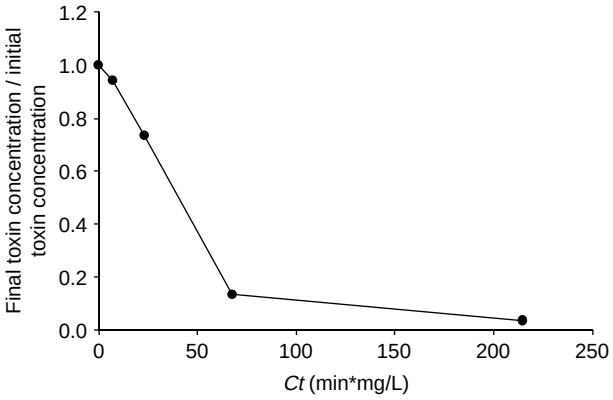

Figure 6 | Degradation of microcystin-LR versus *Ct* for the Lake Mendota experiment at 12°C, pH 7.5 and microcystin-LR concentration of 2.8 μg/L.

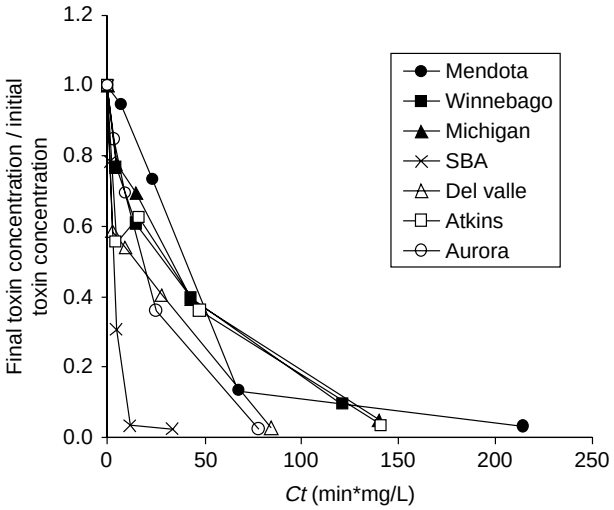

Figure 7 | Degradation of microcystin-LR versus *Ct* for 7 natural water experiments.

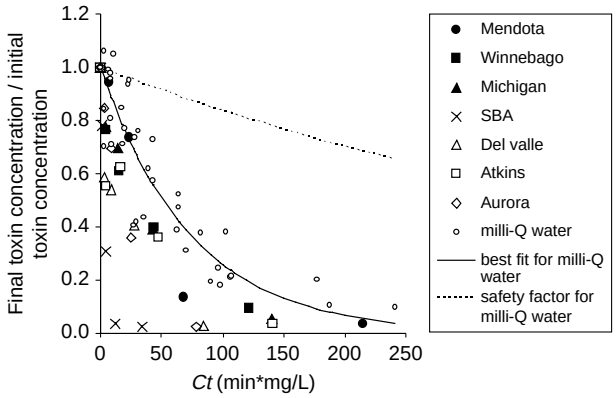

Figure 8 | Comparison of degradation results for natural waters with degradation results for reagent-grade water at pH 7.5.

of 254 nm light (UV254), chlorine dose, or with the chlorine demand of the water. Therefore, there was no evident explanation for the site-specific nature of microcystin-LR degradation. It is plausible that the interaction between chlorine and microcystin-LR could be influenced by a number of parameters that were not measured in this study. For example, free chlorine behaves as an electrophile, attacking electron-rich structures in the microcystin-LR molecule (Tsuji *et al.* 1997). Substances present in natural water could conceivably bind with microcystin-LR and change the availability of sites for electrophilic attack by chlorine. Further study is required to elucidate the mechanism responsible for the site specificity of microcystin-LR degradation by free chlorine.

Figure 8 compares the degradation of microcystin-LR for the natural water experiments with the degradation of microcystin-LR for the reagent-grade water experiments at 11.2°C and pH 7.5. This comparison shows that degradation of microcystin-LR occurred faster in the natural waters than it did in the reagent-grade water. At first glance, this is a surprising result given the differences in chlorine demand between reagent-grade water and the natural waters. However, the discussion in the previous paragraph pointed out that the degradation rate was not correlated with chlorine dose or chlorine demand. Again, there was not a sufficient amount of data to explain this observation but it is plausible that there may be something present in the natural waters (e.g., a catalyst or a promoter of radical formation) that is capable of accelerating the reaction rate. Clearly, the mechanism behind the observed results can only be elucidated with additional studies. Despite this, the results presented in Figure 8 suggest that best-fit *Ct* values obtained from the reagent-grade water experiments may be conservative for natural waters that have been subjected to conventional coagulation, flocculation, sedimentation, and filtration prior to chlorine addition.

SUMMARY AND CONCLUSIONS

Kinetic modeling was performed to develop *Ct* values needed to achieve target microcystin-LR degradation levels by free chlorine. The modeling was based on experiments performed with reagent-grade water having no chlorine demand. *Ct* values were developed with and without safety factors. The safety factor approach clearly produced conservative *Ct* values, as illustrated by Figures 2, 4, and 8. Because microcystin-LR degradation was significantly faster in the natural waters than it was in the reagent-grade water, the *Ct* values obtained without a safety factor were also conservative for the natural waters examined in this study (see Figure 8).

As noted earlier, the *Ct* values developed in this research could have significant implications for both practitioners and regulators. To provide a realistic assessment of these implications, it is important to determine how much removal might be needed in a given water treatment

plant. Occurrence studies performed to date have shown that only 4% of raw waters in the United States have microcystin-LR concentrations that are greater than the current World Health Organization guideline of 1 μg/L (Carmichael 2001). Assuming that this accurately reflects the true national occurrence, 96% of the water utilities in the United States would not need chlorination or some other treatment technology to meet the 1 μg/L target.

For those few utilities having raw waters that exceeded the 1 μg/L guideline, microcystin-LR concentrations in the plant influents ranged from 2 to 17 μg/L (Carmichael 2001). If a utility had a raw water microcystin-LR concentration of 2 μg/L, then that utility would need to achieve a 50% removal of microcystin-LR to reach the 1 μg/L target. A 94% removal would be required for a utility that needed to reduce microcystin-LR concentrations from 17 μg/L in the treatment plant influent to 1 μg/L in the treatment plant effluent. Therefore, when needed, chlorination would typically be used to achieve 50–95% degradation of microcystin-LR.

Assuming that the best-fit estimates are conservative enough for the natural waters, *Ct* values of 11 to 46 mg·min/L would be needed at pH 6 and 11°C for 50–95% degradation of microcystin-LR, respectively. As noted earlier, these *Ct* values are within the range of values typically achieved by most surface water treatment systems in the United States. Interestingly, these systems are already required to meet *Ct* values for inactivation of *Giardia lamblia*. For comparison, 12 to 16 mg·min/L of free chlorine is needed at pH 6 and 10°C to achieve 0.5-log inactivation of *Giardia* (USEPA 1991). This level of disinfection for *Giardia* is typically required of treatment systems that employ conventional filtration. Therefore, at pH 6, it appears that 50% degradation of microcystin-LR could be achieved with a *Ct* value similar to the *Ct* value needed for 0.5-log *Giardia* inactivation. However, if 95% microcystin-LR degradation is needed, the required *Ct* would increase by a factor of 3 over the *Ct* values needed for *Giardia* inactivation. If the utility was not able to meet this with their current chlorination facilities, then the facility would need to upgrade their chlorination facilities or find an alternative method (e.g., activated carbon adsorption) of removing the microcystin-LR. The alternative method would also be needed if an upgrade of chlorination facilities would cause violations of trihalomethane and haloacetic acid standards.

Similar calculations can be performed at pH 7.5 and pH 9.0. Table 4 shows that *Ct* values of 51 and 220 mg min/L are needed to achieve 50% and 95% removal of microcystin-LR, respectively, at pH 7.5 and 11°C. The *Ct* needed for 0.5-log inactivation of *Giardia* at pH 7.5 and 10°C ranges from 21 to 28 mg min/L. Therefore, the *Ct* values needed for microcystin-LR degradation are 2 to 9 times larger than the *Ct* values needed for 0.5-log *Giardia* inactivation. At pH 9 and 11°C, the 149 mg min/L needed for 50% microcystin-LR degradation is 3 to 4 times larger than the *Ct* values of 35 to 49 mg·min/L needed for 0.5-log *Giardia* inactivation at pH 9 and 10°C. For 95% degradation of microcystin-LR at pH 9.0 and 11°C, the required *Ct* value of 646 mg min/L is 13 to 18 times greater than the *Ct* value needed for 0.5-log *Giardia* inactivation. The increased *Ct* values needed for microcystin-LR degradation at higher pH values are more difficult to meet with the typical range of *Ct* values observed in surface water treatment plants. Again, if a utility was not able to meet these higher *Ct* values with their current chlorination facilities, then the facility would need to upgrade their chlorination facilities or find an alternative method of removing the microcystin-LR.

To summarize, a small fraction of surface water facilities in the United States would need to provide treatment to achieve a microcystin-LR target of 1 μg/L in water entering the distribution system. For these utilities, free chlorination should be an acceptable microcystin-LR treatment method if chlorination is performed at pH values less than 7.5. For some waters, the pH of the water being treated may need to be reduced for adequate toxin degradation at reasonable *Ct* values. Utility managers should run lab experiments with their specific source water to determine the optimal *Ct* value for sufficient microcystin-LR degradation.

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