Organic Chloramines in Drinking Water: An Assessment of Formation, Stability, Reactivity and Risk

Zuo Tong How, Kathryn L. Linge*, Francesco Busetti and Cynthia A. Joll

Curtin Water Quality Research Centre, Department of Chemistry, Curtin University, GPO Box U1987 Perth, Western Australia, Australia 6845

Corresponding author: K. Linge, Email: K.Linge@curtin.edu.au, Tel: +61 (0)8 9266 7534, Fax: +61 (0)8 9266 2300

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Abstract

Although organic chloramines are known to form during the disinfection of drinking water with chlorine, little information is currently available on their occurrence or toxicity. In a recent *in vitro* study, some organic chloramines (e.g. *N*-chloroglycine) were found to be cytotoxic and genotoxic even at micromolar concentrations. In this paper, the formation and stability of 21 different organic chloramines, from chlorination of simple amines and amino acids, were studied, and the competition between 20 amino acids during chlorination was also investigated. For comparison, chlorination of two amides was also conducted. The formation and degradation of selected organic chloramines were measured using either direct UV spectroscopic or colorimetric detection. Although cysteine, methionine and tryptophan were the most reactive amino acids towards chlorination, they did not form organic chloramines at

the chlorine to precursor molar ratios that were tested. Only 6 out of the 21 organic chloramines formed had a half-life of more than 3 hours, although this group included all organic chloramines formed from amines. A health risk assessment relating stability and reactivity data from this study to toxicity and precursor abundance data from the literature indicated that only *N*-chloroglycine is likely to be of concern due to its stability, toxicity and abundance in water. However, given the stability of organic chloramines formed from amines, more information about the toxicity and precursor abundance for these chloramines is desirable.

1. Introduction

Since the discovery in the early 1970s of disinfection by-products (DBPs) in chlorinated drinking water, extensive studies have been undertaken to understand the formation of DBPs and their management (Richardson 2003). Although more than 600 DBPs have now been identified, only minimal information on the occurrence or toxicity of many of these DBPs is available. A recent toxicological study identified several classes of nitrogen-containing DBPs, including haloacetamides, halonitriles and organic halamines, to be of highest interest with respect to potential toxicity (Bull et al. 2011). In addition, Bull et al. (2011) also suggested high priority be given to the characterisation of the toxicological properties of organic halamines that have varying chemical properties (e.g. stability and hydrophobicity). Since then, studies have been conducted on the haloacetamides, halonitriles and many other classes of nitrogen-containing DBPs (Bond et al. 2011, Liew et al. 2012), however there remains very little published information regarding the occurrence or toxicity of organic halamines in drinking water.

Although water-related toxicological studies of organic chloramines are limited, several biomedical studies of the potential for adverse health effects from organic chloramines have

been published. Organic chloramines have been found to cause protein-DNA cross-links (Kulcharyk and Heinecke 2001), inhibit DNA repair (Pero et al. 1996), and affect the rates of cellular apoptosis and the kinetics of the cell cycle (Englert and Shacter 2002, Hosako et al. 2004), which are all common characteristics of carcinogens. A significant *in vitro* cytotoxicity and genotoxicity has also been observed in WIL2-NS cells (human lymphoblastoid) that were treated with *N*-chloroglycine, *N*-chloroethanolamine, *N*-chlorohistamine, and *N*-chlorolysine formed *in situ* at low micromolar concentrations (Laingam et al. 2012).

Organic chloramines may be formed when dissolved organic nitrogen (DON), represented by functional groups such as amino acids, amides and amines within the dissolved organic carbon (DOC), present in water systems reacts with free chlorine (Hunter and Faust 1967) or inorganic chloramines (Isaac and Morris 1983, Snyder 1982). The general equations for the formation of organic (mono/di) chloramines from addition of hypochlorous acid are shown below:

$$R\text{-}NH_2 + HOCl \leftrightarrows R\text{-}NHCl + HOCl \leftrightarrows R\text{-}NCl_2 \quad (1)$$

$$R_2\text{-}NH + HOCl \leftrightarrows R_2\text{-}NCl \quad (2)$$

The reactions between amino acids and free chlorine are believed to be the main contributor to the formation of organic chloramines (Ellis and Soper 1954, Yoon and Jensen 1993). After formation, organic chloramines can degrade to different disinfection by-products, such as aldehydes and nitriles, depending on the chlorine to nitrogen ratio (Nweke and Scully 1989). The stability of different organic chloramines has been reported to be quite variable, ranging from a half-life of 13 minutes to more than 48 hours (Armesto et al. 1996,

Hand et al. 1983, Scully and Bempong 1982). In general, more basic organic chloramines are more stable (Pitman et al. 1969). The presence of an α-hydrogen can reduce organic chloramine stability, as it can promote dehydrohalogenation reactions (Hui and Debiemme-Chouvy 2013).

To accurately assess the risk associated with the presence of organic chloramines in drinking water, it is important to understand both the formation and stability of this class of compounds, in addition to their toxicity. In this study, the formation and degradation of a range of organic chloramines from 21 amino acids and three amines were investigated at pH 7-8. The chlorination of two amides was also studied. A health risk assessment of organic chloramines in drinking water was conducted based on the relationship between the stability and toxicity of the organic chloramines, and also their reactivity and precursor abundance.

2. Methods and Materials

2.1 Chemicals

Amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine) (purity ≥ 97%); amines (dimethylamine, diethylamine and ethanolamine) (purity ≥ 97%); amides (acetamide and acrylamide) (purity ≥ 97%); and aqueous sodium hypochlorite (10 – 15 % chlorine) were purchased from Sigma Aldrich (New South Wales, Australia). The amino acid taurine (purity 99%); was purchased from AK Scientific (California, USA); potassium iodide and formic acid (purity 99%) were purchased from Ajax FineChem (New South Wales, Australia); and glacial acetic acid was purchased from Chem Supply (South Australia, Australia). Monochlor F reagent was purchased from Hach Pacific (Victoria, Australia). The internal standards [^{2}H3] alanine (alanine-d_3), [^{2}H3] leucine (leucine-d_3) and [^{2}H3] glutamic acid (glutamic acid-d_3) were

purchased from CDN Isotopes (Quebec, Canada; distributed by SciVac, Hornsby, Australia); [^{2}H2] glycine (glycine-d_2), [^{2}H5] phenyl [^{2}H3] alanine (phenyl-d_5-alanine-d_3) and *N*-acetylglycine were purchased from Sigma Aldrich. Methanol (ChromHR grade) was purchased from Mallinckrodt Baker (New Jersey, USA). Highly purified water (≥18.2 Ω.cm) was produced using an ion exchange system (IBIS Technology, Western Australia, Australia), followed by an Elga Purelab Ultra system with a 0.2 μm filter (Elga, High Wycombe, UK).

The concentration of sodium hypochlorite in the standard chlorination solution was confirmed by measuring the UV absorbance at 292 nm using a Cary 60 UV-Vis spectrometer from Agilent Technologies (California, USA) with a deuterium arc lamp. A molar absorptivity of sodium hypochlorite of 350 M^{-1} cm^{-1} (Morris 1966) was assumed.

2.2 Formation of organic chloramines

Organic chloramines were prepared by adding sodium hypochlorite to specific precursor aqueous solutions at a molar ratio of sodium hypochlorite to precursors (amino acids and amines) of 0.2. The same ratio was used for the chlorination of the amides. This molar ratio has been shown to minimise side reactions and to promote monochloramine formation (Reckhow 2011). The formation of organic chloramines (and chloramides) was assessed and confirmed by scanning the UV absorbance between λ= 195 and 400 nm, using the Cary 60 UV-Vis spectrophotometer. A peak at λ= 250-280 nm is expected if an organic chloramine (or chloramide) is formed. Some precursors (tyrosine, phenylalanine, tryptophan, dimethylamine, diethylamine, ethanolamine, acetamide and acrylamide) also have UV absorption in the region of λ= 250-280 nm. For these precursors, the formation of organic chloramines (or chloramides) was confirmed by obtaining a UV spectrum (λ= 195 to 400 nm) of the chlorine solution before and after addition of the N-containing precursor. It was

assumed that disappearance of the free chlorine peak at 292 nm indicated that all chlorine had reacted, and that only organic chloramines (or chloramides) were formed. All UV measurements of chlorinated precursors were background subtracted using the UV absorbance from the solvent (highly purified water), cuvette and precursors used in the experiment.

2.3 Measurement of degradation of organic chloramines

The rates of degradation of organic chloramines were measured over 60 minutes at time intervals as indicated in Table 1. The rate was measured four times for each individual organic chloramine. Two different measurement methods described in our previous work (How et al. 2015) were used to determine the rate of formation and degradation of organic chloramines. Briefly, a direct UV method measuring λ= 255 nm was used to measure the degradation of organic chloramines where there was no interference from its precursor or its by-products in the spectral region λ= 250-280 nm. A triidodide colorimetric method was used for the measurement for organic chloramines with interferences from their precursor or their by-products in the spectral region λ= 250-280 nm. For the triiodide colorimetric method, glacial acetic acid (125 µL) and potassium iodide (125 µL of a 15 g L^{-1} solution) were added to 2.5 mL of sample. Organic chloramines will oxidise the iodide into triiodide, producing a pale yellow solution in an acidic environment. The absorbance of the sample was measured at λ= 353 nm and the concentration of organic chloramine was determined using an external calibration. The triiodide colorimetric method will measure the sum of all oxidants including inorganic monochloramine (MCA), inorganic di/tri-chloramines or organic di/tri-chloramines. Monochlor F was used to measure the concentration of inorganic monochloramine and it was assumed that inorganic di/tri-chloramines were not formed under the reaction conditions used. For both measurement methods, the degradation rate constant

was determined from the slope of a least squares linear regression of ln (A_t/A_o) *vs* time where A_o was the absorbance at t=0 and A_t was the absorbance measured at a specific time (t).

2.4 Organic chloramine identification using liquid chromatography-ultraviolet absorbance-high resolution mass spectrometry

In addition to the direct UV method, the formation, and the kinetics of degradation, of *N*-chloroisoleucine and *N*-chlorovaline were investigated using liquid chromatography coupled to both a photodiode array detector and a high resolution mass spectrometer (LC-UV-HRMS). *N*-Chloroisoleucine and *N*-chlorovaline were chosen because their stability (as discussed in Section 3.2) made them suitable for investigation by LC-UV-HRMS. Details of the LC-UV-HRMS system and its operation are presented in the Supporting Information. Instrumental conditions are reported in Table S1.

The organic chloramines for this experiment were formed using a molar ratio of chlorine to amino acids of 0.8 (as discussed in section 3.1) to maximise the formation of monochloramines and to reduce the chance that unreacted amino acids would overload the LC column. The rate of degradation was determined by measuring the response (ratio of peak area of organic chloramines to peak area of internal standard) of the organic chloramines at 5, 50, 95, 140 and 185 min after chlorination. Because the chromatographic separation took 45 min, degradation was monitored for 185 min to ensure that sufficient data points were obtained. The relative abundance of *N*-chloroisoleucine and *N*-chlorovaline was the peak area ratio of the organic chloramine and the assigned internal standard, *N*-acetylglycine. *N*-Acetylglycine was chosen as an internal standard as it has been reported to be unreactive to free chlorine (Deborde and von Gunten 2008), therefore its concentration should not be affected by the presence of free chlorine. The mass spectra of highly purified water, HOCl,

isoleucine and valine were also acquired to ensure that the suspected organic chloramine peak was not originally present in the reagents used. Therefore, any detection of a new peak was assumed to be due to the formation of organic chloramines as the expected degradation products: aldehyde, nitrile and *N*-chloraldimine (Nweke and Scully 1989) do not have an UV absorbance.

2.5 Competition between amino acids during chlorination

Competitive chlorination experiments were undertaken in order to determine the relative reactivity of amino acids with chlorine, to provide insight into the likelihood of specific amino acids forming organic chloramines during chlorination of natural waters. The competitive chlorination experiments were conducted by reacting free chlorine with a mixture of 20 amino acids at equal concentration (30 µM) in 15 mL glass vials, with initial chlorine to total amino acid ratios ranging from 0.5 to 2. The mixture was allowed to react for 3 hours in the dark at room temperature, and then the sample was quenched with ascorbic acid solution to stop further reaction. The residual amino acid concentrations were determined using sub-samples (500 µL) of the reaction mixture using our previously reported LC-MS/MS method (How et al. 2014). Briefly, chromatographic separation was achieved using an Agilent 1100 HPLC system. Amino acids were separated using a Gemini C18 column (250 mm x 3 mm I.D., 3 µm particle size) from Phenomenex (New South Wales, Australia) at a flow rate of 150 µL min^{-1}. The amino acids were detected using a Micromass Quattro Ultima Triple Quadrupole Mass Spectrometer (Manchester, UK) fitted with an electrospray ionisation (ESI) interface operated in positive ion mode. Amino acids were quantified using the ratio of the analyte peak area to the assigned internal standard peak area and an external calibration curve.

3. Results and Discussion

3.1 Formation of organic chloramines

All three amines and 18 out of the 21 amino acids reacted rapidly with hypochlorous acid (HOCl) to form organic chloramines (Tables 2). Neither of the two amides tested formed organic chloramides, most likely due to the low reactivity of most amides with HOCl (Deborde and von Gunten 2008). This suggested that stable *N*-chloramides are unlikely to be formed from chlorination. Three amino acids (cysteine, methionine and tryptophan) did not form organic chloramines at the chlorine to precursor ratio used; for these amino acids, HOCl reacted with other functional groups in the molecules that are more reactive than the amino group (e.g. the thiol group for cysteine and methionine, and the indole group for tryptophan) to form other disinfection by-products like the corresponding sulfoxides from cysteine and methionine (Deborde and von Gunten 2008, Na and Olson 2007) and 7-chlorotryptophan from tryptophan (Flecks et al. 2008). It was found in a previous study by Shang et al. (2000) that organic chloramine can form from cysteine at a chlorine to precursor ratio of 4.4. The UV spectra of organic chloramines formed from precursors that do not have an interference at λ= 250-280 nm (Table 2) confirmed the formation of organic chloramines from the change in the UV spectrum before and after chlorination. All of these organic chloramines, except *N*-chlorolysine which has two primary amine functional groups that can form chloramines, had a UV spectrum similar to that of *N*-chloroglycine (Fig. 1).

In order to investigate the optimal chlorine to amino acids ratio to produce monochloramines, isoleucine was chlorinated at different chlorine to amino acid ratios (0.4 to 2.8) and allowed to react for 30 minutes before direct UV measurement. Fig. 2 shows that, after background subtraction, the intensity of the peak at λ = 256 nm, attributed to *N*-chloroisoleucine increased when the molar ratio of chlorine to isoleucine was increased up to 1.2, but then decreased, completely disappearing at a molar ratio of 2. The increased intensity

of the peak at λ = 292 nm above a molar ratio of 2 was attributed to an increased concentration of residual free chlorine, however no additional peaks were observed that could be identified as organic dichloramines in this study. While organic dichloramines may possess similar UV features as inorganic dichloramine (λ = 295 nm), this finding is in agreement with previous studies which have no identified organic dichloramines by UV-Vis (Conyers and Scully 1993, Freuze at el. 2004, Nweke and Scully 1989).

The increase in absorbance at λ = 256 nm when the molar ratio was 0.4 to 1.2 and the appearance of free chlorine when the molar ratio was above 2 is in agreement with the study of chlorination of isoleucine at different ratios by Nweke and Scully (1989), where they found that the highest concentration of monochloramine was formed at a chlorine to amino acid molar ratio of 1 and a molar ratio above 1 resulted in the formation of the organic dichloramine. Shang et al. (2000) also demonstrated that organic dichloramine was formed above molar ratio of 1.0 chlorine to amino acids, and that free chlorine was observed above molar ratio of 2.8 or higher depending on the type of amino acids. Therefore, a chlorine to amino acid ratio of 0.8 instead of 1.2 was used to maximise the formation of organic monochloramines, while preventing the formation of organic dichloramines (Section 3.3).

3.2 Kinetic study of the degradation of organic chloramines

The degradation of organic chloramines formed at chlorine to precursors' ratio of 0.2 was monitored by either the direct UV method or the triiodide colorimetric method over one hour and empirical rate constants were determined as described in Section 2.3 (Tables 2). All organic chloramines, except *N*-chloroglutamine, *N*-chloroaspartic acid, *N*-chlorotaurine and *N*-chloroethanolamine, followed a first-order degradation reaction, indicating that their degradation rate was independent of the concentration of the precursors. The coefficients of determination for the first-order degradation of organic chloramines are listed in Table S2.

The half-life of each organic chloramine that followed a first-order degradation reaction was determined by calculations using the half-life equation for first-order reactions (Equation 3; Table 3). Whilst relatively rapid, the degradation of *N*-chloroglutamine, *N*-chloroaspartic acid and *N*-chloroethanolamine (Fig. S1) was not linear, indicating that the degradation of these organic chloramines may be dependent upon other factors, such as the concentrations of precursor, by-products or the chloramine concentration, pH or temperature. These factors were not further investigated. The half-life of *N*-chloroglutamine, *N*-chloroaspartic acid and *N*-chloroethanolamine was determined as the time required for the initial concentration in the degradation experiments to be halved (Table 3). As *N*-chlorotaurine did not appear to degrade in the allocated monitoring period of one hour, degradation experiments for *N*-chlorotaurine were repeated over an extended period of 3 days, but the concentration of *N*-chlorotaurine was still greater than 50% after 3 days. While the degradation of *N*-chlorotaurine was not studied over an even longer period, it was apparent that *N*-chlorotaurine is very stable, with a half-life > 3 days.

$$\text{Half-life} = \frac{\ln(2)}{k}, \text{ where } k \text{ is the rate constant} \qquad (3)$$

Most of the degradation rate constants determined in this work were in agreement with previously reported values (Armesto et al. 1996, Hand et al. 1983), with the exception of *N*-chloroproline (Hand et al. 1983), *N*-chlorodimethylamine and *N*-chlorodiethylamine (Scully and Bempong 1982), where the rate constants were about one order of magnitude higher in the current study, compared to the respective previous studies. We note that the relative standard deviation of rate constants that we determined for *N*-chloroproline, *N*-chlorodimethylamine and *N*-chlorodiethylamine, being less than 15% over 4 replicate experiments (Tables 2), indicated good experimental repeatability. One possible reason for

276 the difference between the rate constants in the current study and the previous studies may be
277 differences in the pH. In this study, the pH was about 8 and unbuffered, which is higher than
278 the pH used in previous studies. The degradation rate constant for *N*-chloroproline was
279 previously determined at pH 6.85 using 0.01 M phosphate buffer (Hand et al. 1983), while
280 the degradation rates constants for *N*-chlorodimethylamine and *N*-chlorodiethylamine were
281 previously determined at pH 7 (Scully and Bempong 1982). The results suggested that
282 higher pH destabilised the chlorine to nitrogen bond in organic monochloramines formed
283 from secondary amines resulting in faster degradation of organic monochloramines. In
284 contrast, Armesto et al. (1993) reported that pH had little impact on the stability of organic
285 monochloramines formed from amino acids. However, the in work by Armesto et al. (1993),
286 only amino acids with primary amines were studied. Further study is therefore required to
287 confirm the impact of pH on the degradation of organic monochloramines formed from
288 secondary amines.

289 Eleven of the 18 organic chloramines formed from amino acids had a half-life of less
290 than 90 min (Table 3). In contrast, all three organic chloramines formed from amines had a
291 half-life of greater than 3 hours (Table 3). This suggested that the residence time of drinking
292 water in the distribution system will determine whether these organic chloramines can be
293 detected in drinking water. For example, in many drinking water treatment plants with
294 storage reservoirs, the water may have a residence time of 2-3 days before distribution. This
295 suggests that, if formed, only organic chloramines that are stable for more than 2-3 days, for
296 example *N*-chloroglycine and *N*-chlorotaurine, will enter the distribution system and have the
297 possibility to be detected in distributed drinking water.

298 Overall, the results showed that more basic organic chloramines (based on the pKa
299 values of the amine nitrogen of the non-chlorinated amines; actual pKa values of chlorinated
300 amine nitrogen are unknown but are assumed to follow a similar trend) were more stable, in

agreement with previous research by Pitman et al. (1969), where more basic amines were reported to form more stable organic chloramines. For example, in the current study, basic amino acids like lysine, histidine and arginine formed more stable organic chloramines (half-lives of 275, 60 and 50 min, respectively) compared to acidic amino acids like glutamic acid and aspartic acid (half-lives of 35 and 10 min, respectively). Taurine, the only β-amino acid and also the only sulfonic acid tested, formed a more stable organic chloramine than any of the α-amino acids tested. This result is consistent with the finding of Gottardi et al. (2013) that β-amino acids form more stable organic chloramines compared to α-amino acids. The result also suggested that dehydrohalogenation may be an important mechanism in organic chloramine degradation, since, for example, *N*-chlorotaurine (a β-amino acid) was found to be the most stable organic chloramine, which is consistent with the lower reactivity of the hydrogen involved in the dehydrohalogenation reaction of β-amino acids (in the less reactive β- position to the carboxylic acid group) compared to the α-amino acids (more reactive α-position to the carboxylic acid group). In contrast, dehydrohalogenation becomes increasingly more likely for α-amino acids like *N*-chloroglycine and *N*-chloroleucine, and this is reflected in their decreased half-lives (5775 and 90 minutes, respectively). A similar trend was also reported in the work by Hui and Debiemme-Chouvy (2013) who found that β-amino acids formed more stable organic chloramines compared to α-amino acids. The lower stability of *N*-chloroleucine, compared to *N*-chloroglycine, is attributed to the additional alkane group on the α-carbon, as previous research has shown that α-amino acids with more substituents at the α-carbon are less stable (Hand et al. 1983).

3.3 Identification and confirmation of degradation rates of organic chloramines using liquid chromatography-ultraviolet absorbance-high resolution mass spectrometry

Identification of organic chloramines by LC-UV-HRMS required organic chloramines that would be relatively stable during the chromatographic separation (45 minutes) and detectable by HRMS. While very stable, the organic chloramines formed from the amines selected in this study resulted not detectable by LC-HRMS possibly due to their poor ionisation/degradation in the ion source. Therefore, *N*-chloroisoleucine and *N*-chlorovaline, with half-lives of 90 and 110 min, respectively, were chosen for analysis by the LC-UV-HRMS technique, as they were relatively stable, but had half-lives short enough to allow analysis of degradation rates without more than 24 hours of LC-UV-HRMS instrument time. In addition, LC-HRMS showed good analytical sensitivity for *N*-chloroisoleucine and *N*-chlorovaline by LC-HRMS.

The organic chloramines, *N*-chloroisoleucine and *N*-chlorovaline, were formed using a molar ratio of chlorine to amino acid of 0.8 to maximise the formation of the monochloramine. The rate of degradation was determined by measuring the response (ratio of peak areas of *N*-chloroisoleucine and *N*-chlorovaline to *N*-acetylglycine) of the organic chloramines at 5, 50, 95, 140 and 185 minutes after chlorination. The formation of *N*-chloroisoleucine (Fig. S2) and *N*-chlorovaline (Fig. S3) was confirmed by LC-UV-HRMS. Retention times (t_R) determined by measuring λ= 250 nm on the UV detector (*N*-chloroisoleucine ~30 min and *N*-chlorovaline ~28 min) were similar to t_R determined by monitoring the target mass by HRMS (*N*-chloroisoleucine: *m/z*=166.0628 at ~31 min; *N*-chlorovaline: *m/z*=152.0466 at ~29 min). The mass accuracies of the ions detected were also within the limit of <5 ppm relative error (0.6212 ppm for *N*-chloroisoleucine and 4.754 ppm for *N*-chlorovaline). Identities of the suspected *N*-chloroisoleucine and *N*-chlorovaline were

348 further confirmed by the $HRMS^2$ fragments (structures presented in Table S3) and
349 comparison of measured isotopic patterns to isotopic patterns simulated by the X-calibur
350 software (Figs. S4 and S5).

351 The rate constants for the degradation of *N*-chloroisoleucine and *N*-chlorovaline derived
352 by LC-UV-HRMS were 2.2×10^{-4} and 1.9×10^{-4} s^{-1}, respectively, which was in good
353 agreement with the rate constants obtained from the direct UV measurement (1.3×10^{-4} and
354 1.0×10^{-4} s^{-1}, respectively) and with those reported previously (Table 2), with slight
355 differences potentially attributable to the longer duration of monitoring for LC-UV-HRMS (3
356 hours, compared to 1 hour for the UV measurement).

357

358 **3.4 Competition of amino acids during chlorination**

359 Experiments were conducted to determine the relative reactivity of 20 amino acids with
360 chlorine to provide insight into the likelihood of specific amino acids to form organic
361 chloramines during chlorination of natural waters. The competitive chlorination experiments
362 were conducted by addition of varying free chlorine concentrations to a mixture of 20 amino
363 acids at equal concentration (30 µM each). The reaction mixtures were allowed to react for 3
364 hours in the dark at room temperature. The samples were analysed for their residual amino
365 acids after quenching with ascorbic acid solution to stop further reaction with the free
366 chlorine or combined chlorine. When the chlorine to total amino acid molar ratio was 0.5, the
367 final concentration of most amino acids was greater than 90% of the initial concentration
368 (Table 4). However, the concentrations of cysteine, methionine and tryptophan were below
369 their limits of detection, indicating that these three amino acids were the most reactive and
370 had reacted completely, although not reacting to form organic chloramines (Section 3.1). This
371 high reactivity of cysteine and methionine is in agreement with studies by Shang et al. (2000)
372 and Na and Olson (2007) where sulphur-containing amino acids were found to be the most

373 reactive to chlorination. When equimolar concentrations of chlorine and total amino acids
374 were present (i.e. a molar ratio of 1), six amino acids (isoleucine, leucine, valine, serine,
375 threonine and proline) were still present at concentrations greater than 80% of the initial
376 concentration. This suggested that free chlorine preferentially reacted with the other, more
377 reactive amino acids, or their formed chloramines, rather than form organic chloramines from
378 these six, less reactive amino acids. At a molar ratio of 1.5, most amino acids were found to
379 have reacted with chlorine with the exception of phenylalanine, isoleucine, valine and
380 threonine, where, even at a molar ratio of 2, they were still present at concentrations greater
381 than 20-40% of the initial concentration, indicating that they were the least reactive amino
382 acids with chlorine.

383 Given it is the practice for water utilities in many countries to maintain residual chlorine
384 in the distributed water (chlorine to amino acids ratio much higher than 2), it could be
385 assumed that the chlorine exposure would normally be sufficient to ensure that all amino
386 acids would react with chlorine and is possible that most amino acids would form organic
387 chloramine in distributed water. These results also confirm that the presence of amino acids
388 and amines will increase chlorine demand in drinking water, in agreement with previous
389 research on amino acids which has shown that between 2.6 and 16 mole of chlorine is
390 required to fully react with each mole of amino acid (Hureiki et al. 1994, Shang et al. 2000,
391 White 2010). The commonly reported (Berne et al. 1994, Chinn and Barrett 2000, Dotson and
392 Westerhoff 2009, How et al. 2014, Thurman 1985) free amino acids in natural waters are
393 glycine, tyrosine, valine, threonine, phenylalanine, alanine, serine, leucine, glutamic acid,
394 aspartic acid and proline. Cysteine, methionine and tryptophan, which were found to be more
395 reactive to chlorination, are not commonly detected in natural waters (Berne et al. 1994,
396 Chinn and Barrett 2000, Dotson and Westerhoff 2009, How et al. 2014, Thurman 1985).
397

3.5 Assessing the significance of organic monochloramines in drinking water systems

The significance of organic monochloramine formation from amino acids and amines in drinking water systems was assessed by considering the reactivity of precursors, and formation and stability data collected in this study, to reported data for organic chloramine toxicity (Laingam et al. 2012) and precursor occurrence (Berne et al. 1994, Chinn and Barrett 2000, Dotson and Westerhoff 2009, How et al. 2014, Thurman 1985). The organic monochloramines were ranked by their 1) toxicity, 2) stability (half-life), 3) abundance in natural waters and 4) relative reactivity to chlorination, as described in Table 4. From this assessment, *N*-chloroglycine, *N*-chlorolysine and *N*-chloroethanolamine were of highest significance due to their stability and reported toxicity at micromolar concentrations (Laingam et al. 2012). However, lysine has been reported to be of low abundance in natural waters (Berne et al. 1994, Chinn and Barrett 2000, Dotson and Westerhoff 2009, How et al. 2014, Thurman 1985) and no data is available for the occurrence of ethanolamine in water systems, suggesting that *N*-chloroglycine is most likely to be detected in drinking water systems. More research to understand the occurrence and toxicity of the most stable organic chloramines, *N*-chloroglycine, *N*-chloroethanolamine, *N*-chlorotaurine, *N*-chlorotyrosine and *N*-chlorodimethylamine, in drinking waters is recommended. Given their stability, organic chloramines formed from amines are of particular interest, however, little information is available on their toxicity and abundance of amine precursors in natural waters. Further study of the occurrence of organic chloramines and their precursors, particularly amines, would be beneficial to refine this risk assessment of organic chloramines in drinking water.

4. Conclusions

The results from this study suggest that most amines and amino acids form organic chloramines when treated with chlorine. Amino acids containing more reactive side groups

(e.g. thiols and indole rings) did not form organic chloramines at the chlorine to amino acid molar ratios tested (cysteine, methionine and tryptophan), but potentially could form at the higher chlorine to amino acid molar ratios found in drinking water. A LC-UV-HRMS analytical method was successfully applied to confirm organic chloramine formation and also to determine the degradation rate of *N*-chloroisoleucine and *N*-chlorovaline. While the organic chloramines produced from amines exhibited half-lives of more than three hours, most of the organic chloramines produced from amino acids were found to have a half-life of less than 90 minutes. In general, the formation of organic chloramines will be most significant for water treatment plants with high dissolved organic nitrogen and direct distribution after chlorination, where minimal time exists for degradation of the organic chloramines between chlorination and distribution. However, the presence of amino acids and amines will increase chlorine demand in all drinking water systems.

Based on a screening health risk assessment of the organic chloramines, *N*-chloroglycine is proposed to be the organic chloramine studied of highest risk due to its stability, toxicity and precursor abundance. More information on the toxicity and precursor abundance for other organic chloramines that are likely to be stable in water distribution systems (e.g. *N*-chloramines and *N*-chlorotaurine) is required to provide a more comprehensive health risk assessment. The degradation products of less stable organic chloramines must also be considered, particularly those from amino acids found to be very reactive with chlorine to form organic chloramines.

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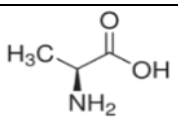
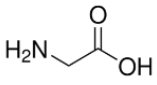
556

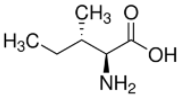
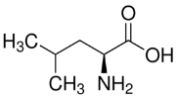
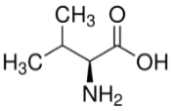
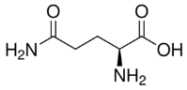
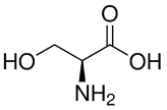
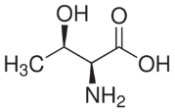
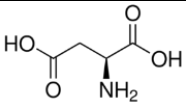
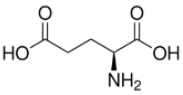
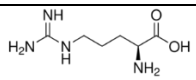
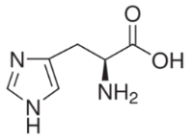
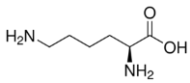
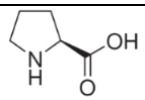
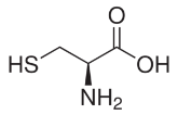
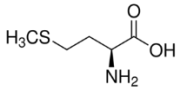
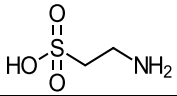
557 **Table 1 - Time interval and duration used for the determination of the stability of selected**
558 **organic chloramines.**

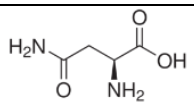
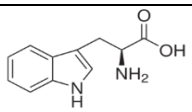
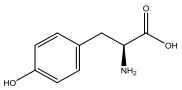
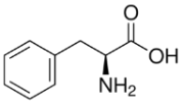
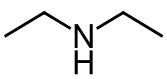
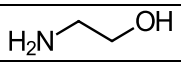
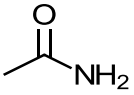
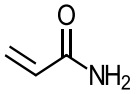
Direct UV method	Time interval	1 min	5 min	10 min
	Duration	1-10 min	10-30 min	30-60 min
Triiodide method	Time interval	5 min		10 min
	Duration	1-30 min		30-60 min

559

560 **Table 2 - Classification, structure, pKa of amine (or amide) nitrogen, and the rate**
561 **constant of organic chloramine degradation from selected amino acids, amines and**
562 **amides. Organic chloramines with interferences from their precursor or by-products in**
563 **the spectral region between λ= 250-280 nm were measured using a triiodide**
564 **colorimetric method, rather than direct UV detection at λ= 255 nm. Organic**
565 **chloramines were formed from all amino acids except cysteine, methionine, tryptophan,**
566 **acetamide and acrylamide.**

Precursor	Classification	Structure	pKa of amine nitrogen	Organic chloramine degradation rate constant k ($x10^{-4}$)(s^{-1})	
				This work k ± SD (n=4)	Literature
Measured using the direct UV method					
Alanine	Non polar		9.71	1.8 ± 0.1	2.7[a], 2.8[b], 3[c]
Glycine			9.58	0.02 ± 0.01	0.04[d]

Isoleucine			9.60	1.3 ± 0.1	1.97 [c], 1.22 [e]
Leucine			9.58	2.5 ± 0.01	3.2 [c], 1.68 [e]
Valine			9.52	1.0 ± 0.2	2 [c], 1.22 [e]
Glutamine			9.00	14 ± 1	Not reported
Serine	Polar		9.05	2.1 ± 0.2	Not reported
Threonine			8.96	1.2 ± 0.04	2 [d]
Aspartic Acid	Acidity		9.66	15 ± 2	Not reported
Glutamic Acid			9.58	3.1 ± 0.3	Not reported
Arginine			9.00, 12.10	2.3 ± 0.2	Not reported
Histidine	Basic		9.09, 6.04	2.0 ± 0.2	Not reported
Lysine			9.16, 10.67	0.42 ± 0.03	Not reported
Proline	Secondary		10.47	56 ± 8	8.8 [d]
Cysteine	Sulfur-containing		10.28	Not Formed	Not reported
Methionine			9.08	Not Formed	Not reported
Taurine	Beta-amino sulfonic acid		9.06	0.02 ± 0.01	Not reported
Measured using the triiodide colorimetric method					

Asparagine	Polar		8.73	6.7 ± 3	Not available
Tryptophan			9.34	Not Formed	Not available
Tyrosine	Aromatic		9.04	0.53 ± 0.2	Not available
Phenylalanine			9.09	1.6 ± 0.03	Not available
Dimethylamine	Secondary amine		10.73	0.39 ± 0.06	0.04[f]
Diethylamine			10.84	0.60 ± 0.09	0.04 [f]
Ethanolamine	Primary amine		9.5	0.05 ± 0.07	Not available
Acetamide	Primary amide		15.1	Not Formed	Not available
Acrylamide			7.9	Not Formed	Not available

SD: standard deviation; [a]Armesto et al. 1996; [b]Stanbro and Smith 1979; [c]Armesto et al. 1993; [d]Hand et al. 1983; [e]Driessen et al. Unpublished; [f]Scully and Bempong 1982.

Table 3 – Significance of organic chloramines for drinking water systems. Ranking is based on organic chloramines stability, toxicity, abundance of precursor and the relative reactivity of the precursor with chlorine expressed as the chlorine to amino acid ratio required to react with more than 50% of the individual amino acid after 3 hours.

Precursor	Half-life (min)	Toxicity at µM concentration [a]	Abundance in natural waters	Relative reactivity
Toxic & stable*				
Glycine	5775	Yes	High [b,c,d,e]	1.5
Ethanolamine	2310	Yes	N.A.	N.A.
Lysine	275	Yes	Low [b,c,d,e,f]	1.0
Stable* & High/unknown abundance of precursors				
Taurine	Not determined[#]	No	N.A.	N.A.
Dimethylamine	295	N.A.	N.A.	N.A.
Tyrosine	217	N.A.	High [f]	1.5
Diethylamine	190	N.A.	N.A.	N.A.
Valine	110	N.A.	High [c]	2.0
Threonine	100	N.A.	High [c]	2.0
Stable but low abundance of precursors; Moderately stable* but high abundance				

of precursors				
Isoleucine	90	No	Low [b,c,d,e,f]	2.0
Phenylalanine	75	No	High [f]	1.5
Alanine	65	N.A.	High [b,c,d,e]	1.5
Serine	55	N.A.	High [b,c,d,e]	1.5
Leucine	45	No	High [f]	1.5
Glutamic Acid	35	No	High [b,c]	1.5
Moderately stable* & low abundance of precursors; very unstable*				
Histidine	60	No	Low [b,c,d,e,f]	1.0
Arginine	50	No	Low [b,c,d,e,f]	1.0
Asparagine	15	N.A.	Low [b,c,d,e,f]	1.5
Glutamine	10	N.A.	Low [b,c,d,e,f]	1.5
Aspartic Acid	10	N.A.	High [b,c]	1.5
Proline	2	N.A.	High [d]	1.5

[a]Laingam et al. 2012, [b]Thurman 1985, [c]Berne et al. 1994, [d]Chinn and Barrett Sylvia 2000, [e]Dotson and Westerhoff 2009, [f]How et al. 2014

*Stable = half-life ≥ 90 min, moderately stable = half-life of 16-89 min and unstable = half-life ≤ 15 min

[#]Half-life of *N*-chlorotaurine was not determined as there was no significant decrease in concentration after 3 days

N.A. = No available data

High abundance = Reported as top 5 contributors to total amino acids in water; low abundance = Amino acids that were not reported as main contributors to total amino acids.

Table 4 - Percentage of amino acids remaining 3 hours after chlorination at different ratios of chlorine to total amino acids.

Amino acids	Percentage (%) amino acid remaining at different ratios of chlorine to amino acid				Number of reactive sites	Second reactive functional group	Chlorine demand (Hureiki et al. 1994)
	0.5	1.0	1.5	2.0			
Non polar							
Alanine	90±20	60±7	30±4	9±1	1	-	2.8
Glycine	100±9	70±7	40±10	10±6	1	-	5.6
Isoleucine	100±6	80±10	50±6	30±0.5	1	-	2.6
Leucine	100±8	80±8	30±9	9±2	1	-	2.6
Valine	100±10	80±10	60±10	40±0.6	1	-	2.7
Polar							
Asparagine	100±10	50±10	10±3	8±0.7	1	-	5.6
Glutamine	100±10	60±10	20±4	9±0.4	1	-	3.8
Serine	100±20	90±10	30±9	1±1	1	-	5.3
Threonine	100±8	80±9	50±10	40±2	1	-	5.6
Basic							
Arginine	70±10	30±4	10±2	3±0.3	2	guanidinium	8.2
Histidine	50±5	9±3	0.8±0.2	0.4±0.2	2	imidazole	12
Lysine	70±10	30±5	8±2	2±0.8	2	amine	3.8
Acidic							
Aspartic acid	100±7	50±9	10±4	2±0	1	-	5.5
Glutamic acid	100±20	60±8	20±3	4±1	1	-	2.4
Secondary							
Proline	100±10	80±9	40±10	2±2	1	-	5.4

Aromatic							
Phenylalanine	80±8	50±10	30±7	20±0.6	2	benzyl	2.7
Tryptophan	4±6	0.1±0.1	0.2±0.1	0±0	2	phenol	16
Tyrosine	100±9	70±9	30±7	10±2	2	indole	13.4
Sulfur-containing							
Cysteine	0.7±0.1	0.7±0.4	0.3±0.02	0±0	2	thiol	6.2
Methionine	0.3±0.1	0.4±0.1	0.5±0.3	0.1±0.5	2	thiol	6.0

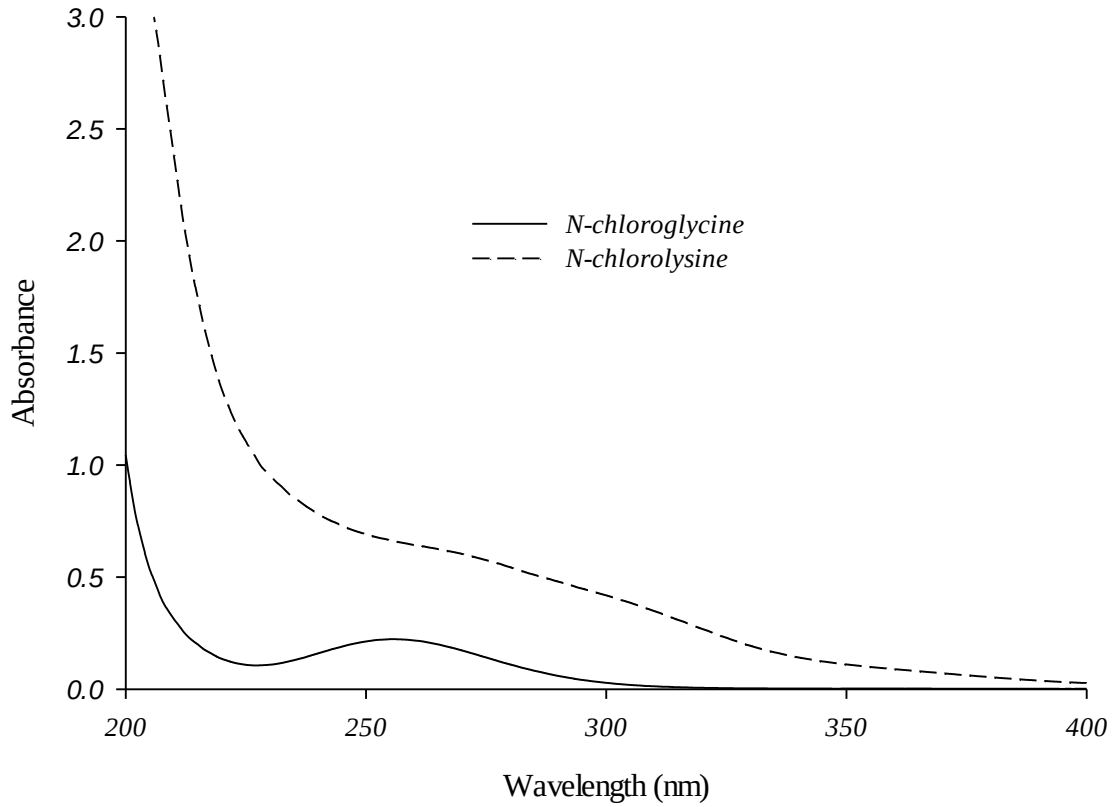
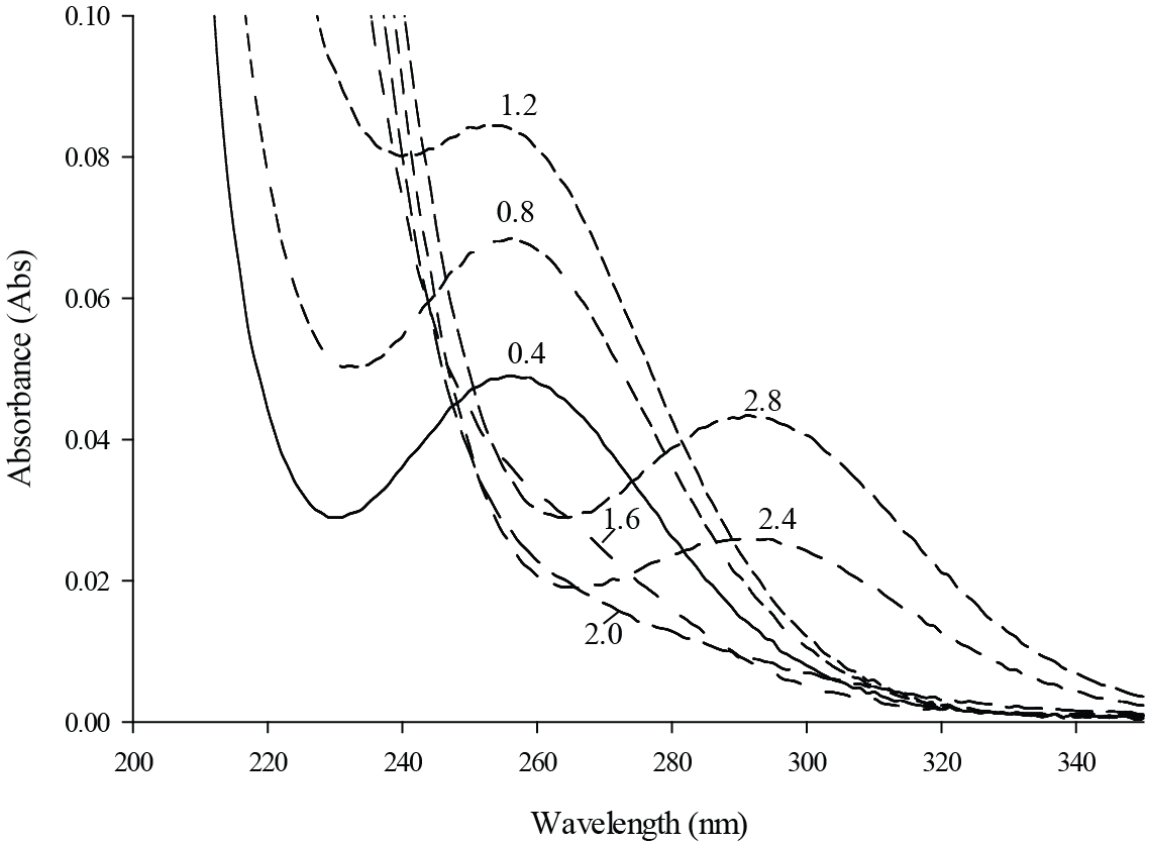


Fig. 1- UV spectra of *N*-chloroglycine and *N*-chorolysine (0.6 mM). Organic chloramines formed from non-aromatic amino acids have similar UV spectra to *N*-chloroglycine.



589
590 **Fig. 2- UV spectra from chlorination of isoleucine (0.6 mM) at different chlorine to isoleucine**
591 **molar ratios (0.4-2.8)**
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