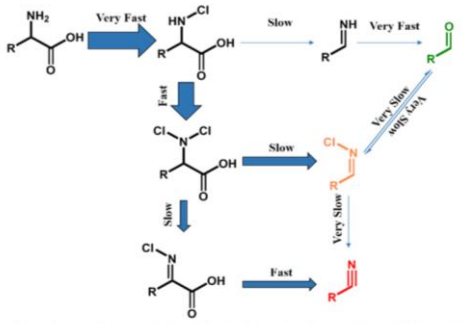

Reaction pathways of the chlorination of amino acids at Cl:AA ≥ 2

Graphical Abstract

CHLORINATION OF AMINO ACIDS: REACTION PATHWAYS AND REACTION RATES

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Abstract

Chlorination of amino acids can result in the formation of organic monochloramines or organic dichloramines, depending on the chlorine to amino acid ratio (Cl:AA). After formation, organic chloramines degrade into aldehydes, nitriles and *N*-chloraldimines. In this paper, the formation of organic chloramines from chlorination of lysine, tyrosine and valine were investigated. Chlorination of tyrosine and lysine demonstrated that the presence of a reactive secondary group can increase the Cl:AA ratio required for the formation of *N,N*-dichloramines, and potentially alter the reaction pathways between chlorine and amino acids, resulting in the formation of unexpected by-products. In a detailed investigation, we report rate constants for all reactions in the chlorination of valine, for the first time, using experimental results and modelling. At Cl:AA = 2.8, the chlorine was found to first react quickly with valine (5.4×10^4 M^{-1} s^{-1}) to form *N*-monochlorovaline, with a slower subsequent reaction with *N*-monochlorovaline to form *N,N*-dichlorovaline (4.9×10^2 M^{-1} s^{-1}), although some *N*-monochlorovaline degraded into isobutyraldehyde (1.0×10^{-4} s^{-1}). The *N,N*-dichlorovaline then competitively degraded into isobutyronitrile (1.3×10^{-4} s^{-1}) and *N*-chloroisobutyraldimine (1.2×10^{-4} s^{-1}). In conventional drinking water disinfection, *N*-chloroisobutyraldimine can potentially be formed in concentrations higher than its odour threshold concentration, resulting in aesthetic challenges and an unknown health risk.

Introduction

Organic chloramines, a subclass of nitrogenous disinfection by-products (N-DBPs), can form in water when the organic nitrogen components of organic matter react with free chlorine[1] or inorganic chloramines.[2, 3] It has been reported that N-DBPs have significantly higher toxicity than the carbonaceous DBPs,[4, 5] and organic chloramines were identified by Bull et al.[6] as a N-DBP subclass of high interest based on their potential carcinogenic hazards. An *in vitro* study by Laingam et al.[7] also found that *N*-chloroglycine, *N*-chlorolysine, *N*-chloroethanolamine and *N*-chlorohistamine were potentially genotoxic and cytotoxic to humans at parts-per-billion concentrations.

Amino acid concentrations in natural waters can range from 20 to 10000 μg L^{-1},[8] accounting for between 2 and 13% of dissolved organic carbon[8] and up to 75% of dissolved organic nitrogen[9]. In general, the concentration of combined amino acids such as proteins and peptides are four to five times higher than free amino acids.[8, 10] Although, free amino acids only contribute a small fraction of the total amino acids,[8, 11, 12] free amino acids are poorly removed during biological filtration,[13] and concentrations of free amino acids can even increase after sand filtration.[14] Hence, it is likely that free amino acids will be present in waters during drinking water disinfection. Amino acids are known to form organic chloramines (*N*-chloramino acids) when chlorinated,[15, 16] and are believed to be the main contributors to the formation of organic chloramines from organic matter.[17, 18] Amino acids can also form organic bromamines if brominated, or if bromine is present in the water.[19] However, organic chloramines formed from amino acids have been found to be unstable.[20, 21] In our study of 18 organic chloramines formed from amino acids, we found that 12 had a half-life of less than 90 minutes.[20] Consequently, as the time between chlorination of drinking water and final distribution to the end consumer increases, it becomes more likely for consumers to be exposed to the degradation products of organic chloramines, rather than the organic chloramines themselves. Therefore, a full assessment of the health impact of organic chloramine formation in drinking water must include the identification of stable by-products from the reaction of amino acids and chlorine.

Previous studies of organic chloramines formed from isoleucine, phenylalanine and valine have shown that many organic chloramine degradation by-products retain structural components from the original amino acid precursor,[22-24] but that DBPs like the trihalomethanes,[25] haloacetic acids[25] and haloacetonitriles[26] can also form. The main degradation product identified from organic monochloramines has been the corresponding

aldehyde, while aldehyde, nitrile and *N*-chloraldimine degradation products have all been detected from organic dichloramines.[23] A generalised formation and degradation pathway has previously been proposed to fit these experimental results (Figure 1),[23, 27, 28] however not all the species proposed have been confirmed experimentally, in part due to the lack of suitable analytical methods. In addition, rate constants for the reactions in this generalised formation and degradation pathway have not yet been determined.

In this paper, we have further investigated the chlorination of amino acids using valine as a model compound. To facilitate improved understanding of the reaction pathways of the chlorination of valine, liquid chromatography- and gas chromatography-based mass spectrometric methods were developed for the identification of organic monochloramines, and aldehyde, nitrile and *N*-chloraldimine by-products. The effect of secondary functional groups (apart from the amino acid group) on the formation of organic chloramines was also studied using lysine, tyrosine, and valine. Finally, the rates of formation of the aldehyde, nitrile and *N*-chloraldimine were also investigated from chlorination of valine using both modelling and experimental results. This is the first study to investigate the rate of reaction for every step in the reaction pathway for the chlorination of valine.

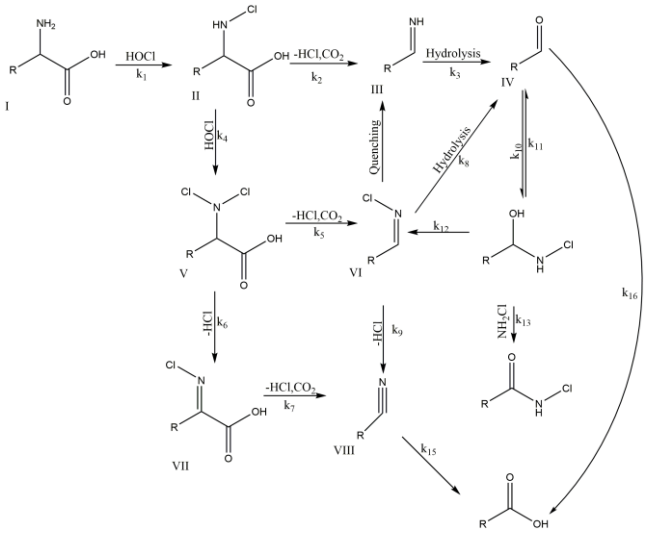

Figure 1. Reaction pathways for the chlorination of amino acids. (Adapted from Conyers and Scully, 1993[23]; Kimura, 2015[27]; Yu and Reckhow, 2015[28])

Materials and Methods

Formation of organic chloramines. Chemicals and materials used are detailed in the Supporting Information (SI) Text S1. The amino acids used in this study (Table S1) were chosen because of data existing in previous studies (valine), or because of the presence of specific secondary functional groups (lysine and tyrosine). In addition, the organic monochloramines that form from these amino acids have half-lives of 45 min or longer,[20] which was the time required for each chromatographic separation. Organic chloramines (0.03 mM or 1.6 mM) were formed by chlorination of individual amino acids (0.04 mM or 2 mM) at chlorine to amino acid molar ratios (Cl:AA) of 0.8 and 2.8. The lower concentration (0.03 mM) was used for gas chromatography-mass spectrometric (GC-MS) experiments, while the higher concentration (1.6 mM) was used for liquid chromatography coupled with ultraviolet and high resolution mass spectrometric (LC-UV-HRMS) experiments. The Cl:AA ratios were chosen because previous studies have shown that only organic monochloramines form at Cl:AA = 0.8 and only organic dichloramines form at Cl:AA = 2.8.[20, 22] The conditions used for the reaction were: pH 7.5 at room temperature (20-25 ºC). Reaction mixtures were reacted in the dark in air tight vials.

Analytical methods. Amino acids and organic chloramines were measured using LC-UV-HRMS analysis. The chromatographic conditions were as described in our previous work.[29] Analytes were detected first by UV (λ=255 nm) using an Accela photodiode array detector (Thermo Scientific, Waltham, USA), then by high resolution mass spectrometry (HRMS) using

a LTQ Orbitrap XL (Thermo Scientific) fitted with electrospray ionization operated in either positive or negative ionization mode, as previously described.[20] HRMS screening used a full-MS scan in the range 70-300 *m/z* with a mass resolution of 15000 at 400 *m/z*. Organic chloramines were identified by comparing the measured mass and isotope pattern against the theoretical mass (< 5ppm, relative error) and the predicted isotope pattern, while amino acids were confirmed by comparison with their respective analytical standards. Data was processed using X-calibur QualBrowser 2.0.7 SP1 (Thermo Scientific). The HRMS instrumental conditions are provided in the Supporting Information (Table S2).

Volatile degradation by-products, namely the aldehyde, nitrile and *N*-chloraldimine, were extracted using an automated headspace solid-phase microextraction (HS SPME) GC-MS method previously developed by our group,[30] using a 75 µm CAR/PDMS fibre. Sodium sulphate (3 g) and the internal standard solution (1,2-dibromopropane-d_6 in methanol, 100 µg L^{-1}, 2 µL) were added to each sample (10 mL in a 20 mL glass vial). Samples were agitated at 500 rpm at 60 °C for 10 minutes before the SPME fibre was inserted into the sample headspace for 15 minutes and then transferred to the GC injector port for thermal desorption at 300 °C for 3 minutes. GC-MS analysis used an Agilent 6890N gas chromatograph coupled with an Agilent 5975 mass selective detector using conditions reported in SI Table S3. The samples were analysed in both MS scan mode (20 – 320 *m/z*) to screen for the degradation products and single ion monitoring mode to quantify selected aldehydes, nitriles and *N*-chloraldimines (quantifying and qualifying ions are listed in SI Table S4). Liquid-liquid extraction (LLE) was used for the extraction of by-products from the chlorination of lysine (organic solvent used was dichloromethane) and also from the chloramination of isobutyraldehyde in anhydrous conditions (organic solvent used was diethyl ether). The GC-MS program used for the LLE was identical to that of the HS SPME-GC-MS method (Table S3), except that the injector temperature was 250 °C and initial holding time was 3 min instead of 2 min.

Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was used to determine the positions of substitution of the chlorine atoms on the *N*-chloramino acids formed. All NMR spectra were obtained using a Bruker AVANCE III 400 MHz spectrometer (Bruker, Australia), by dissolution of amino acids or the *N*-chloramino acids in 90% water and 10% D_2O, with water signal suppression.

Reaction rates for the reaction pathway of the chlorination of valine at Cl:AA = 2.8. To measure the reaction rates of the formation of *N*-chlorovaline, chlorinated valine solutions were analysed by LC-UV-HRMS. Solutions were injected 5 min after chlorination, without

quenching. Both the amino acid and the organic chloramine were measured at 45 min intervals until 185 min after chlorination. To measure the rate of formation and degradation of isobutyraldehyde, isobutyronitrile and *N*-chloroisobutyraldimine, chlorinated valine solutions were analysed by HS SPME-GC-MS, without quenching, at t = 15 min after chlorination, and then at one hour intervals until t =7 hours, and 12 hour intervals from t = 12 to 72 hours. As the samples could not be quenched for the determination of organic chloramines, each data point was determined from the average of two repeated experiments. The concentration of *N*-chloroisobutyraldimine was estimated as the difference in concentration of isobutyraldehde between quenched and unquenched experiments at 7 hours. The measurements were then used to plot the first-order linear kinetic plot, ln (At/Ao) vs time in seconds, where At was the mass abundance at a specific time and Ao was the initial mass abundance.

Reaction rates that could not be determined experimentally were predicted by iteration through modelling using Kintecus,[31] as described in the SI text S2. The accuracy of the predicted rate constants was assessed by a sensitivity analysis to investigate the effect of varying modelled rate constants from their chosen value.

Results and Discussion

Effect of a reactive secondary functional group on the formation of organic monochloramines and dichloramines. The formation of organic monochloramines and/or organic dichloramines (to be referred to as monochloramines and dichloramines) from the chlorination of amino acids is controlled by the Cl:AA ratio and by the presence of secondary functional groups that may also react with chlorine.[20, 32] Formation experiments using a Cl:AA = 0.8 were used in this study to produce monochloramines, while avoiding dichloramine formation. In our previous work, we used LC-UV-HRMS to confirm formation of both *N*-chloroisoleucine and *N*-chlorovaline after the chlorination of isoleucine and valine, respectively.[20] The same technique was used to detect and identify monochloramines formed from the chlorination of the other amino acids tested in this current study: lysine and tyrosine, which have not previously been reported to be detected by MS methods. For all amino acids, monochloramine peaks were detected in both UV spectra and MS spectra, and the mass to charge ratios of these suspected monochloramine peaks were within 5 ppm error of their theoretical values (Table 1) and the measured isotopic patterns were similar to the predicted isotopic patterns. In addition, comparison of LC-UV-HRMS chromatograms of unquenched samples and samples quenched with ascorbic acid showed that the suspected organic

monochloramine peaks were absent after quenching, indicating that the suspected peaks were the organic monochloramines (Figure S1).

Table 1. Comparison of the measured mass of suspected organic monochloramines and their theoretical mass. The difference (ppm) in the measured and theoretical mass was always less than 5 ppm.

Compounds	Measured mass	Theoretical mass	Difference (ppm)
Positive $[M+H]^+$			
N-chlorolysine	181.07369	181.07383	-0.784
N-chlorotyrosine	216.04179	216.04220	-1.886
N-chlorovaline	152.04660	152.04728	-4.491

Formation experiments using Cl:AA = 2.8 were designed to preferentially form dichloramines from the amino acid precursors,[20, 22] except for lysine where Cl:AA = 4.8 (Cl:N = 2.4) was used, as discussed in more detail below. As dichloramines are, in general, not detectable by UV,[20, 22, 23, 33] suspected dichloramine peaks were confirmed by LC-HRMS, including the exact mass and isotopic ratio. In addition, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was used to determine the positions of substitution of the chlorine atoms on the organic chloramines that formed, and LLE or HS SPME GC-MS were used to identify by-products. *N,N*-Dichloramine species were not detected by LC-HRMS for lysine or valine. This might be due to the reported instability of the *N,N*-dichloramine species formed from α-amino acids.[34] However, other dichloramine species were detected for lysine and tyrosine, which was unexpected. The exact mass to charge ratios of the detected dichloramines were within 5 ppm of the theoretical values (-2.742 ppm error for lysine and -1.886 ppm error for tyrosine). Lysine has two amine nitrogens in its structure and, when chlorinated, can produce a chloramine functional group at both of the nitrogens. At Cl:N = 0.4, two monochloramine peaks were detected from lysine (Figure S2a), suggesting that two species of monochloramine were formed. At Cl:N = 1.2, only one dichloramine peak was detected (Figure S2b), most likely the *N,N'*-dichlorolysine, given the subsequent detection of the corresponding aldehyde, 5-chloraminopentanal, by LLE and analysis with GC-MS. When the Cl:N ratio was increased to 2.4, no peaks corresponding to any dichlorolysines were detected by LC-HRMS, and the corresponding aldehyde of *N,N',N'*-trichlorolysine, 5-dichloraminopentanal, was detected by LLE GC-MS. The formation of *N,N'*-dichlorolysine at a Cl:AA ratio of 2 and *N,N',N'*-trichlorolysine at a Cl:AA ratio of 4 was also inferred by Conyers and Scully,[35] through the detection of the corresponding aldehydes.

Although accurate chemical formula of the chlorinated products formed from tyrosine and detected by LC-HRMS could be determined from the measured accurate mass to charge ratio, LC-HRMS could not definitively indicate the position of the chlorine atoms, as chlorine was the first atom to be removed during fragmentation. Therefore, samples suspected to contain *N*-monochlorotyrosine and *N,N*-dichlorotyrosine were analysed using ^{1}H NMR (Figure S3) and ^{13}C NMR (Figure S4) spectroscopy. Complete details of the NMR results are discussed in SI Text S3, with a summary provided here. Tyrosine has two reactive functional groups (phenol and amino acid), and the NMR results confirmed that *N*-monochlorotyrosine was formed at Cl:AA = 0.8. However, at Cl:AA = 2.8, new aromatic signals consistent with chlorine substitution on the aromatic ring were observed, and a mixture of *N*-monochloro-3-chlorotyrosine and *N,N*-dichlorotyrosine in a slightly lower than 1:1 ratio was formed. Finally, when the Cl:AA ratio was increased to 12, degradation of the aromatic ring of tyrosine was observed.

Thus, while all amino acids tested demonstrated similar reaction pathways, with formation of *N*-monochloramino acids at Cl:N $\leq$ 1 and formation of dichloramino acids at Cl:N $\geq$2, results from the chlorination of lysine and tyrosine at Cl:AA = 2.8 demonstrate that the presence of other reactive groups in the amino acids can delay formation of the *N,N*-dichloramine to higher Cl:AA ratios, or lead to formation of dichloramine isomers other than the *N,N*-dichloramine species.

Investigation of the reaction pathways of degradation of *N*-chloroamino acids using valine as a model compound. In order to better understand the generalised formation and degradation pathways for *N*-chloroamino acids, the rate of reaction for each pathway was investigated using experimental and modelling methods. Valine was chosen as a model amino acid for this investigation due to the detection of its *N*-monochloroamino acid and the detection of all three degradation products and valine is reported to be one of the most abundant amino acids in surface waters.[36, 37] The reaction rates for each reaction step in the reaction of valine with chlorine were determined experimentally or predicted by iteration through modelling using Kintecus.[31]

The investigation of reaction rates for each reaction pathway in the chlorination of valine as a model amino acid (Table 2) showed that the *N,N*-dichloramine was formed from a stepwise reaction through the formation of the *N*-monochloramine, and that the initial formation (first 15 min) of the aldehyde at Cl:AA > 2 was from the degradation of the *N*-monochloramine, rather than from the *N*-chloraldimine. The investigation also confirmed that the nitrile and the

243 *N*-chloraldimine both formed from the *N,N*-dichloramine *via* competitive reaction pathways.
244 After formation, the *N*-chloraldimine continued to degrade slowly to either the aldehyde or the
245 nitrile. The nitrile and aldehyde also degraded slowly to presumably the corresponding
246 carboxylic acid; in the case of valine, isobutyric acid. The formation of isobutyric acid was
247 confirmed from the GC-MS detection and confirmation of isobutyric acid in the reaction
248 mixture from the chlorination of valine. Further discussion of these pathways is presented
249 below.
250
251 **Table 2: Proposed reaction rates for the chlorination of valine.**

Reaction (number)	Rate	Units
Valine + HOCl → *N*-monochlorovaline (k_1)	5.4×10^{4}	$M^{-1} s^{-1}$
N-Monochlorovaline → isobutyrimine (k_2)	5.0×10^{-4}	s^{-1}
Isobutyrimine → isobutyraldehyde (k_3)	5.0×10^{4}	s^{-1}
N-Monochlorovaline + HOCl → *N,N*-dichlorovaline (k_4)	4.9×10^{2}	$M^{-1} s^{-1}$
N,N-Dichlorovaline → *N*-chloroisobutyraldimine (k_5)	7.0×10^{-5}	s^{-1}
N,N-Dichlorovaline → 2-(chlorimino)-3-methylbutanoic acid (k_6)	1.0×10^{-5}	s^{-1}
2-(Chlorimino)-3-methylbutanoic acid → isobutyronitrile (k_7)	1.0×10^{-2}	s^{-1}
N-Chloroisobutyraldimine → isobutyraldehyde (k_8)	4.0×10^{-6}	s^{-1}
N-Chloroisobutyraldimine → isobutyronitrile (k_9)	1.0×10^{-6}	s^{-1}
Isobutyraldehyde + NH_2Cl → 1-(Chloroamino)-2-methylpropan-1-ol (k_{10})	24.3	$M^{-1} s^{-1}$
1-(Chloroamino)-2-methylpropan-1-ol → isobutyraldehyde (k_{11})	0.247	s^{-1}
1-(Chloroamino)-2-methylpropan-1-ol → *N*-chloroisobutyraldimine (k_{12})	9.75	s^{-1}
1-(Chloroamino)-2-methylpropan-1-ol + NH_2Cl → *N*-Chlorisobutyramide (k_{13})	2.7×10^{-4}	$M^{-1} s^{-1}$
Isobutyraldehyde → isobutyric acid (k_{14})	3.0×10^{-6}	s^{-1}
Isobutyronitrile → isobutyric acid (k_{15})	3.0×10^{-5}	s^{-1}

252
253 When the Cl:AA ratio is less than 1, the aldehyde is reported to be formed from the
254 monochloramine through an imine intermediate (structure III in Figure 1).[23] Although the
255 detection of this imine was not possible by LC-MS or GC-MS, the rate of formation of *N*-
256 monochlorovaline ($k_1 = 5.4 \times 10^{4}$ $M^{-1} s^{-1}$ or 2.4×10^{2} s^{-1} for a HOCl concentration of 4.48 mM
257 as Cl_2) and the rate of formation of isobutyraldehyde (1.5×10^{-4} s^{-1}) were both determined
258 experimentally. Comparison of these rates showed that the formation of *N*-monochlorovaline
259 from the chlorination of valine is much faster than the rate of isobutyraldehyde formation.
260 Therefore, the rate limiting step for the formation of isobutyraldehyde is either the degradation
261 of *N*-chlorovaline into isobutyrimine (k_2) or the degradation of isobutyrimine into
262 isobutyraldehyde (k_3). We previously found the rate of degradation of *N*-chlorovaline to
263 isobutyrimine, k_2, to be 1.0×10^{-4} s^{-1},[20] which is similar to the rate of formation of
264 isobutyraldehyde determined in the current study. This suggests that the rate of degradation of

isobutyrimine into isobutyraldehyde, k_3, is faster than the degradation of *N*-monochlorovaline into isobutyrimine (k_2), and that formation of isobutyrimine is the rate determining step for formation of isobutyraldehyde from valine. Modelling using the Kintecus software indicated that $k_3 = 5.0 \times 10^4\ s^{-1}$, and this suggests the imine very quickly underwent hydrolysis to the aldehyde after formation. All reaction rates related to the formation of isobutyraldehyde were investigated using a sensitivity analysis (SI Figure S7), and results of this were used to optimise the final reaction rates, reported in Table 2, and SI Table S6.

It has previously been proposed that, when the Cl:AA ratio is > 2, aldehydes can form through hydrolysis of *N*-chloraldimine, a by-product of dichloramine degradation,[22] rather than from degradation of the monochloramine (Figure 1). However, the same study[22] and subsequent studies[23, 33] also suggested the *N*-chloraldimines are stable. To assess the importance of *N*-chloroisobutyraldimine in the formation of isobutyraldehyde, Kintecus was used to predict isobutyraldehyde concentrations in both the presence and absence of monochloramine degradation into isobutyraldehyde (Figure 2). Experimentally, isobutyraldehyde showed an instantaneous formation, followed a gradual degradation. In comparison, modelling without a contribution from monochloramine degradation showed delayed formation of isobutyraldehyde compared to the experiment. This suggested that isobutyraldehyde was not formed solely from the hydrolysis of *N*-chloroisobutyraldimine (VI→IV) in experimental studies, but also from the degradation of the monochloramine (II→III→IV). Thus, at Cl:AA > 1, *N*-monochlorovaline may either react with HOCl to form *N,N*-dichlorovaline (II→V) or be degraded into isobutyraldehyde (II→III→IV). We believe this difference might be due to the hydrolysis of N-chloroisobutyraldimine to isobutyraldehyde being catalysed by a reagent present in the system (likely to be HOCl), but not included in the modelling.

Figure 3 shows that, experimentally, the concentrations of isobutyronitrile and *N*-chloroisobutyraldimine, both by-products of *N,N*-dichlorovaline, were much higher than the concentration of isobutyraldehyde. Thus, most *N*-monochlorovaline must react with chlorine to form *N,N*-dichlorovaline (k_4) rather than degrading into isobutyraldehyde. Therefore, the reaction rate k_4 must be faster than the degradation of *N*-monochlorovaline. Again, a sensitivity analysis of the reaction rates related to the formation of isobutyronitrile (SI Figure S8) and *N*-chloroisobutyraldimine (SI Figure S9) were undertaken to optimise the final reaction rates, reported in Table 2, and SI Table S6. When the final proposed reaction rates presented in Table 2 were used to simulate the trends of formation of isobutyraldehyde, isobutyronitrile and *N*-chloroisobutyraldimine, results were very similar to the experimental trends of formation

(Figure 3), indicating that the proposed rate constants were reasonable estimations for the reaction steps.

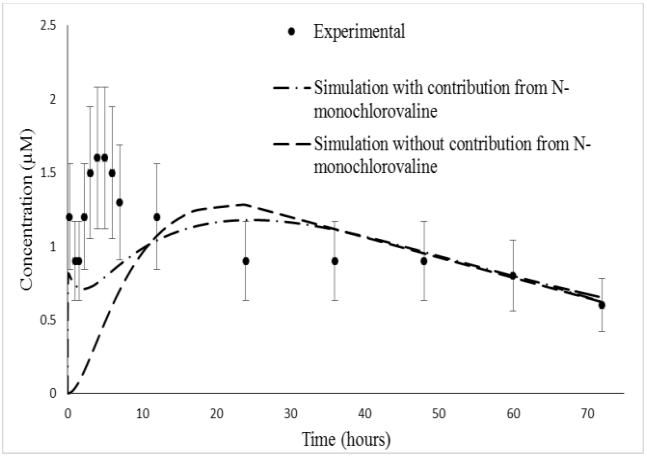

Figure 2. Concentrations of isobutyraldehyde from experimental chlorination of valine (30 µM), and from simulation of isobutyraldehyde formation with and without a contribution from the degradation of *N*-monochlorovaline (Cl-Val).

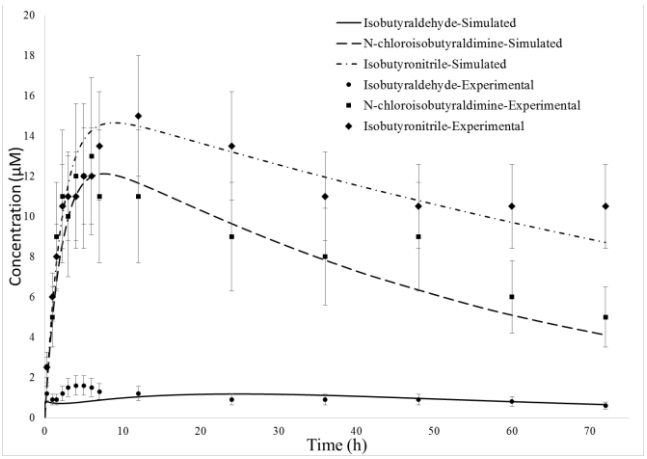

Figure 3. Concentrations of isobutyraldehyde, isobutyronitrile and *N*-chloroisobutyraldimine formed over 72 hours from both experimental and simulated chlorination of valine (30 µM). The experimental concentration of *N*-chloroisobutyraldimine was estimated from the difference in the isobutyraldehyde concentrations in quenched and unquenched samples.

Both isobutyronitrile and *N*-chloroisobutyraldimine are degradation products of *N,N*-dichlorovaline (Figure 1). The rates of formation of isobutyronitrile and *N*-chloroisobutyraldimine were experimentally determined from the increase in their concentrations over seven hours (Figure 3), assuming a pseudo first order reaction, and were very similar, at 1.0×10^{-4} s^{-1} and 9.2×10^{-5} s^{-1}, respectively, suggesting that both of these products are formed from *N,N*-dichlorovaline in competitive pathways. Previous work[23, 33] with other amino acids has also shown nitriles and *N*-chloraldimines both formed from *N,N*-dichloramines *via* competitive pathways. While it is proposed that isobutyronitrile can also

arise from the degradation of *N*-chloroisobutyraldimine,[23] the hydrolysis of *N*-chloroisobutyraldimine into isobutyraldehyde was found to be faster than the dechlorination into isobutyronitrile (discussed in detail later in this section).

Finally, *N*-chloroisobutyraldimine degradation into isobutyraldehyde and isobutyronitrile was also investigated. Accurate measurements of the rate of degradation of *N*-chloroisobutyraldimine would require a pure *N*-chloroisobutyraldimine solution. However, the synthesis of pure *N*-chloroisobutyraldimine proved challenging. While *N*-chloroisobutyraldimine (V) can theoretically be formed through chloramination of isobutyraldehyde (IV) (Figure 1), there was no reaction observed between isobutyraldehyde and inorganic monochloramine in an aqueous solution, even when inorganic monochloramine was present in 20 times molar excess. This result is consistent with previous reports that the formation of *N*-chloraldimines from the chloramination of aldehydes in aqueous solutions is very slow.[27, 38] Therefore, *N*-chloroisobutyraldimine was produced in anhydrous conditions; inorganic monochloramine was first produced in water and then extracted using diethyl ether. The concentration of inorganic monochloramine in the diethyl ether was assumed to be the difference in inorganic monochloramine concentration in the aqueous layer before and after the extraction. The isobutyraldehyde standard in methanol was then added to the inorganic monochloramine/diethyl ether extract at a monochloramine:isobutyraldehyde molar ratio of 10. The solution was then analysed by GC-MS for the formation of *N*-chloroisobutyraldimine every 60 minutes (time for each chromatographic separation) for 12 hours using liquid injection. Both *N*-chloroisobutyraldimine and isobutyronitrile were detected in the reaction mixture, with the concentration of isobutyronitrile increasing over time (Figure S5). In this experiment, the formation of isobutyronitrile must be from the degradation of *N*-chloroisobutyraldimine as no other pathway exists. This result confirmed that *N*-chloroisobutyraldimine can degrade into isobutyronitrile. However, further investigation into the degradation of *N*-chloroisobutyraldimine using Kintecus modelling (Table 2) indicated that the hydrolysis of *N*-chloroisobutyraldimine into isobutyraldehyde ($k_8 = 4.0 \times 10^{-6}$ s^{-1}) was faster than the dechlorination into isobutyronitrile ($k_9 = 1.0 \times 10^{-6}$ s^{-1}). Both experimental and modelled results showed a decrease in the concentration of isobutyraldehyde and isobutyronitrile after formation. This is likely due to the degradation of isobutyraldehyde and isobutyronitrile into isobutyric acid. Finally, calculation using the rates of formation of *N*-chloroisobutyraldimine and isobutyronitrile indicated that the half-life of *N,N*-dichlorovaline was around 100 min. This suggests that *N,N*-dichlorovaline was not detected because of the

353 absence of a suitable analytical technique for its detection, rather than *N,N*-dichlorovaline being
354 inherently too unstable.
355
356 **Implications of this work for drinking water disinfection.** This study of the formation and
357 degradation pathways of the chlorination of valine indicates that, when Cl:AA > 2, chlorine
358 will react quickly with valine to form *N*-monochlorovaline (5.4×10^4 M^{-1} s^{-1}), with a slower
359 formation of *N,N*-dichlorovaline from *N*-monochlorovaline (4.9×10^2 M^{-1} s^{-1}). Some *N*-
360 monochlorovaline will degrade to isobutyraldehyde (5.0×10^{-4} s^{-1}), while *N,N*-dichlorovaline
361 will competitively degrade to either isobutyronitrile (1.3×10^{-4} s^{-1}) or *N*-chloroisobutyraldimine
362 (1.2×10^{-4} s^{-1}). As the reaction pathways of most amino acids with chlorine are similar, the
363 speciation of degradation products (aldehydes, nitriles and *N*-chloraldimines) from other amino
364 acids is expected be similar to that of valine, although the reaction rates of the various reactions
365 will be dependent on each individual amino acid. Amino acids with a reactive side chain, like
366 tyrosine and lysine, may not favour the formation of the *N,N*-dichloramine, depending on the
367 reactivity of the side chain functional group, and may potentially produce different degradation
368 products.
369 While organic chloramine formation is of interest due to the potential toxicity of these
370 compounds, this study has shown that the aldehyde, nitrile and *N*-chloraldimine by-products
371 are more likely to be found in finished drinking water, with potential issues for odours in
372 drinking water and as yet unknown health implications. The formation of stable aldehydes and
373 *N*-chloraldimines from the chlorination of amino acids could result in odour problems in
374 chlorinated waters.[37, 39] For example, the odour threshold concentration of isobutyraldehyde is
375 reported to range from 0.9 to 4 μg L^{-1},[39, 40] while the odour threshold concentration of *N*-
376 chloroisobutyraldimine is reported to be 0.20 μg L^{-1}.[40] The maximum molar concentration of
377 valine that has been found in a drinking source water is 63 nM.[11, 37] If this raw water was
378 chlorinated under a conventional drinking water disinfection regime, with a minimum chlorine
379 residual of 0.5 mg L^{-1} (Cl:AA $\geq$ 2), and without treatment to remove free amino acids, 0.2 μg
380 L^{-1} (3 nM) of isobutyraldehyde and 2.0 μg L^{-1} (19 nM) of *N*-chloroisobutyraldimine would be
381 formed, based on the respective molar conversions of 5% and 30% found in the current study.
382 While the potential concentration of isobutyraldehyde is below the previously reported odour
383 thresholds, the potential concentration of *N*-chloroisobutyraldimine of 2.0 μg L^{-1} is 10 times
384 higher than its odour threshold concentration (0.2 μg L^{-1}), suggesting that formation of *N*-
385 chloroisobutyraldimine during chlorination of waters containing valine could result in an odour
386 in the drinking water, especially in distribution systems with contact times less than 24 hours,

but also potentially result in odour problems in longer distribution systems (contact times longer than 24 hours) as *N*-chloroisobutyraldimine was found to be stable for more than 8 days. Additionally, no toxicity information is available for aldehydes, nitriles and *N*-chloraldimines in drinking water, and thus their presence would result in an unknown health risk. Therefore, improved knowledge of the occurrence of free amino acids and the odorous DBPs formed from the chlorination of amino acids would be beneficial for the water industry. For drinking water systems where long retention times in the distribution system (contact times longer than 24 hours) are expected, the degradation pathways of aldehydes and nitriles (e.g. to the corresponding carboxylic acids) should also be considered.

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Supporting Information Available

The Supporting Information includes: Chemicals and materials (Text S1); structures for amino acids used (Table S1); LC-HRMS and GC-MS conditions (Table S2-S4); modelling parameters and sensitivity test data (Text S2, Tables S5-S6 and Figures S6-S8); tyrosine NMR results (Text S3 and Figure S3-S4); LC chromatograms for chlorination of valine and lysine (Figure S1-S2); concentrations of iosbutyraldehyde, *N*-chloroisobutyraldimine and isobutyronitrile over time (Figure S5). This information is available free of charge via the Internet at http://pubs.acs.org.

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