Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Pollution of natural water and even source water with pharmaceuticals is problematic worldwide and raises concern about the possibility of disinfection byproduct (DBP) formation during subsequent water treatment. In this study, the formation of DBPs, especially dichloroacetamide (DCAcAm), was investigated during chlorination and chloramination of tetracyclines, which are a class of broad-spectrum antibiotics. DBPs including DCAcAm were formed during chlorination and chloramination of tetracycline (TC). Although the concentrations and theoretical cytotoxicity of the DBPs formed from TC were affected by the contact time, disinfectant dose, and pH, DCAcAm was the main contributor determining the yields and cytotoxicity of the measured DBPs. The DCAcAm yields from four tetracycline antibiotics ranged from 0.43% to 54.26% for chlorination. For chloramination, the DCAcAm yields reached 44.57%, and the nitrogen in DCAcAm mainly came from tetracyclines during chloramination. ClO₂ pre-oxidation and UV photolysis decreased DCAcAm formation during chlorination and chloramination of TC. The high yields observed in this study suggest that tetracycline antibiotics are possible precursors of DCAcAm.

© 2020 Elsevier Ltd. All rights reserved.

1. Introduction

Antibiotics are widely used as human and veterinary medications, and in aquaculture and livestock growth promotion. Because of bacterial co- or secondary infections, antibiotic treatment is also included in COVID-19 empirical antibacterial therapy and has proven to be effective and save lives (Chang and Chan, 2020; Metlay and Waterer, 2020). Antibiotics may be divided into different groups by their chemical structures such as tetracyclines, β-lactams, quinolones, macrolides, sulfonamides, and others.
Tetracycline antibiotics are a class of broad-spectrum antibiotics. Because of their favorable antimicrobial properties, tetracycline antibiotics are used extensively in human and veterinary medicine to treat bacterial infections and promote animal growth (Kumar et al., 2005). Common tetracycline antibiotics include natural products, such as tetracycline (TC), chlorotetracycline (CTC), and oxytetracycline (OTC), and semisynthetic compounds, such as doxycycline (DC) (Cetecioglu et al., 2013; Zhou et al., 2014). Since they were first detected in river water samples in the 1980s (Watts et al., 1984), tetracycline antibiotics have been frequently detected in surface water samples, especially over the last decade (Danner et al., 2019; Proia et al., 2013; Tong et al., 2014). In surface water samples, OTC, CTC, and TC have been detected at high concentrations in East China (2.20 μg/L, 2.42 μg/L, and 0.81 μg/L, respectively) and the United States (0.34 μg/L, 0.69 μg/L, and 0.11 μg/L, respectively) (Kolpin et al., 2002; Quoc Tuc et al., 2017; Wei et al., 2011). Tetracycline antibiotics have also been detected in surface water samples from some European countries, including Spain (maxima: 0.87 μg/L; T. b. France (maximum 0.68 μg/L OTC) (Pelosa-Felizzola and Chiron, 2009; Proia et al., 2013). A recent overview shows that the global mean concentrations of TC and OTC were in the range of 0.01–1 μg/L in surface waters while were up to 100 μg/L in wastewater treatment plant effluents (Kovalakova et al., 2020). High levels of TC and OTC were also detected in hospital wastewater effluents (mean 1.41 μg/L and 1.48 μg/L, respectively) (Wang et al., 2018). Many studies have focused on the occurrences of tetracycline antibiotics in the aquatic environment; however, the behavior of tetracycline antibiotics during disinfection for water and wastewater treatment has received less attention.

Disinfection is used in water treatment to effectively control pathogens. Wastewater disinfection is also important for wastewater reuse (Chen et al., 2020). Because of the COVID-19 epidemic, disinfection in all areas of life, and especially in medical sewage treatment, has greatly increased (Wang et al., 2020). Disinfectants such as chlorine, chloramine, and chlorine dioxide (ClO₂) can react with organic matter to form disinfection byproducts (DBPs) (Chaukura et al., 2020; Han and Zhang, 2018; Kimura and Ortega-Hernandez, 2019). Although DBPs are generally formed by the reaction of disinfectants with natural organic matter, antibiotics with activated benzene rings or other functional groups that can react with disinfectants are also potential DBP precursors, especially in heavily wastewater-affected waters (Chu et al., 2016), Zhou et al. (2014) found that chlorination and chloramination of tetracycline antibiotics formed trichloromethane (TCM), carbon tetrachloride, dichloroacetonitrile (DCAN), and dichloroacetonitrile. Shen and Andrews (2011) reported that TC acted as a precursor of N-nitrosodimethylamine during chlorination.

It is worth noting that tetracycline antibiotics contain connected ring systems with an amide group (Fig. S1). Previous studies have reported that aspiragmine (Asn), which has an amide group, and the amide-based organic polymer polyacrylamide and its monomer acrylamide can produce haloacetonamides (HACAmS) during chlorination and chloramination (Ding et al., 2018a; Huang et al., 2012). The cytotoxicity of HACAmS are 142 and 1.4 times higher than those of five regulated haloacetic acids and halonitromethanes, respectively (Plewa et al., 2008). Thus, the identification of HACAm precursors has attracted much attention (Chen and Westerhoff, 2010). Aromatic molecules are found to be converted into halogenated aromatic DBPs during chlorination (Jiang et al., 2020). An amide side chain in a tetracycline antibiotic consisting of four aromatic rings may be easily attacked by oxidants to form DCACAm, which is the most abundant HACAm species formed in water (Bond et al., 2011).

This research aimed to determine whether tetracycline antibiotics acted as important HACAm precursors. The formation of DBPs, including DCACAm, during chlorination and chloramination of TC, was investigated, and the theoretical cytotoxicity of the DBPs formed was estimated. DCACAm formation from three tetracycline antibiotics (DC, CTC, and OTC) was also investigated and compared with that from reported precursors of DCACAm. Finally, the effects of the common pretreatment methods of ClO₂ pre-oxidation, ultraviolet light (UV) photolysis, and the UV/chlorine advanced oxidation process on DCACAm formation during subsequent chlorination and chloramination of TC were evaluated.

2. Materials and methods

2.1. Chemicals and materials

Four tetracycline antibiotics were used: TC (98%, TCI, Tokyo, Japan), CTC (97%, J&K Chemical, Beijing, China), OTC (98%, J&K Chemical), and DC (97%, TCI). Ascorbic acid (reagent grade), Asn (98%), 15N-ammonium chloride (99%), DCAN (98%), and sodium hypochlorite (4.00%–4.95%) were obtained from Sigma-Aldrich (Munich, Germany). Methanol (HPLC grade) and methyl tert-butyl ether (HPLC grade) were purchased from Thermo Fisher Scientific (Walsham, MA). 1,2-Dibromopropane, DCACAm, dichloroacetaldehyde, and trichloroacetamide (TCACAm) were obtained from Alfa Aesar (Heysham, United Kingdom). An EPA 551B Halogenated Volatiles Mix and trihalomethanes were purchased from AccuStandard (New Haven, CT).

A free chlorine stock solution was prepared by dilution of a sodium hypochlorite solution. Chloramine disinfectant was a monochloramine solution that was prepared by adding a NaClO solution to an NH₄Cl solution at a Cl₂/N mass ratio of 4:1. A ClO₂ stock solution was prepared by adding sulfuric acid to a sodium chloride solution. All disinfectants were prepared fresh when required and the actual effective chlorine concentration was measured before use.

2.2. Experimental procedures

Chlor(am)ination experiments were performed under headspace-free conditions at 25 °C in 30-mL amber glass bottles that were shielded from light. The four tetracycline antibiotics were separately dissolved in ultrapure water to yield 0.05 mM solutions (Chu et al., 2016; Zhang et al., 2019a). These solutions were chlorinated for 2 h or chloraminated for 24 h and buffered at pH 7 with a chloride solution. All disinfectants were prepared fresh when required and the actual effective chlorine concentration was measured before use.

Chlor(am)ination experiments were performed under headspace-free conditions at 25 °C in 30-mL amber glass bottles that were shielded from light. The four tetracycline antibiotics were separately dissolved in ultrapure water to yield 0.05 mM solutions (Chu et al., 2016; Zhang et al., 2019a). These solutions were chlorinated for 2 h or chloraminated for 24 h and buffered at pH 7 with a disinfectant dose of 0.5 mM as the baseline condition to allow high enough concentrations of DBPs to form. An orthogonal matrix experimental design was used in which the disinfectant dose, contact time, and pH were individually varied from the baseline condition. The disinfectant dose was varied from 0.25 to 1.25 mM, the contact time from 0.5 to 24 h, and the pH from 5 to 9. Buffers were prepared with phosphate. After preselected intervals, the chloride residual of the solution was determined and the reaction was halted by quenching the chlorine residual with ascorbic acid at a molar excess of 1.5 times the initial chlor(am)ine dose. Ascorbic acid was used as the quenching agent because that the common quenching agents, including sodium sulfite, sodium thiosulfate, and ascorbic acid were demonstrated to expedite DCACAm degradation, whereas ascorbic acid was the least in effective (Ding et al., 2018c).

The effects of various pretreatment methods, including ClO₂ oxidation, UV photolysis, and the UV/chlorine process, on DCACAm formation during chlor(am)ination of TC were investigated. In the ClO₂ pretreatment experiment, ClO₂ (0.25 mM, 0.5 mM, or...
0.75 mM) was added to the TC solutions. After 30 min of incubation in the dark at 25 °C without headspace, the samples were purged with nitrogen gas to remove residual ClO2 and then chlor(am)ination was conducted (Gan et al., 2019). For the UV experiments, 700 mL of the solution to be tested was placed in a sealed quartz cylindrical reactor with a quartz tube in the center that contained a low-pressure mercury lamp (254 nm, GPH212T5L/4H11). The temperature was maintained at 25 ± 0.2 °C using a water bath. The effective path length was 2.43 cm and iodide/iodate chemical actinometry showed that the UV photon flux entering the solution was 0.471 μEinstein/(L s). The solution was continuously mixed using a magnetic stirrer during the reaction. After preselected intervals (5–30 min), the solution was sampled and chlor(am)ination was conducted. The UV/chlorine experiments were conducted in a similar manner with 0.5 mM free chlorine added at the beginning (Zhang et al., 2019b). All experiments were conducted in duplicate.

2.3. Analytical methods

Free and total chlorine residuals were determined using a colorimetric analyzer (HI96711, Hanna Instruments). DBPs were extracted using methyl tert-butyl ether and analyzed using a gas chromatograph (GC9720, FuLi Instruments) with an electron capture detector. TCM, dichloroacetaldehyde, chloral hydrate (CH), DCAN, trichloroacetonitrile, trichloronitromethane, DCACAm, and TCACAm were determined in this study. Chromatographic separation of the DBPs was performed using a DB-5 column (30 m × 0.25 mm i.d., 0.25 μm, Agilent, Santa Clara, CA). The analysis methods were consistent with those of Huang et al. (2017). The theoretical cytotoxicity was calculated by dividing the measured concentration of each DBP by the corresponding published median lethal concentration (LC50) (Table S1), which is the dose required to induce 50% viability of Chinese hamster ovary cells.
compared with untreated controls (Chuang and Mitch, 2017; Gao et al., 2019; Li et al., 2020; Xiang et al., 2020).

3. Results and discussion

3.1. DBP formation from TC

As TC has the simplest structure among the three natural tetracycline antibiotics which were frequently detected in aquatic water, TC was used to investigate DBP formation during chlorination and chloramination. TCM and DCAN formed during chlorination and chloramination of TC (Fig. 1), and this was consistent with the results of a previous study (Zhou et al., 2014). In addition, CH, DCAcAm, and TCAcAm formation was observed. The yield of each of these DBPs, displayed as a percentage shown in Fig. 1, was defined as a molar ratio of the produced DBP concentration to the initial precursor concentration. The maximum yields of dichloroacetalddehyde, trichloroacetonitrile, and trichloronitromethane were all less than 0.1%, so they are not shown in Fig. 1 but the concentrations of them are provided in Fig. S2.

DBP formation during chlorination of TC was plotted against the contact time (Fig. 1a), chlorine dose (Fig. 1c), and pH (Fig. 1e). The yields of TCM, CH, DCAcAm, TCAcAm, and DCAN from the chlorination of TC were 4.87%–30.87%, <0.01%–0.49%, 4.39%–34.77%, 0.07%–4.02%, and 0.77%–2.37%, respectively. At a Cl2/TC molar ratio of 10:1, the chlorination time had little effect on the amounts of the DBPs formed during chlorination (Fig. 1a). Interestingly, the yields of DCAcAm were all higher than those of the other DBPs, including TCM during chlorination of TC for different times. As the chlorine dose increased from 0.25 mM to 1.25 mM, the yields of TCM, CH, and TCAcAm from TC after chlorination for 2 hours gradually increased, the DCAN yield increased and then decreased, and that of DCAcAm decreased (Fig. 1c). The tendencies of the TCM and DCAN yields were consistent with previous research (Zhou et al., 2014). The decreases in the DCAN and DCAcAm yields were probably caused by accelerated decomposition of DCAN and DCAcAm with excess chlorine (Ding et al., 2018c; Yu and Reckhow, 2015). It has been reported that hypochlorite can react rapidly with DCAcAm to form N-chloro-dichloroacetamide (N–Cl–DCAcAm) as a major reaction intermediate and DCAA as an end product, and chlorination of DCAN formed DCAcAm and N–Cl–DCAcAm as major reaction intermediates and DCAA as an end product (Ding et al., 2018c; Yu and Reckhow, 2015). During chlorination of TC, hypochlorite can react with TC to form DCAN and DCAcAm and simultaneously react with formed DCAN and DCAcAm to cause DCAN and DCAcAm decomposition. The decomposition rate of DCAN and DCAcAm increased with increasing chlorine concentration (Ding et al., 2018c; Yu and Reckhow, 2015). Therefore, when the decrease in the DCAN or DCAcAm concentration caused by DCAN or DCAcAm decomposition in the presence of increasing residual chlorine exceeded the increase in the DCAN or DCAcAm concentration caused by the increasing chlorine dose, DCAN or DCAcAm concentration decreased as the chlorine dose increased. During chlorination of TC, the DCAcAm concentration decreased as the chlorine dose ratio increased from 5 to 25, while DCAN concentration started to decrease while increasing chlorine dose when the chlorine dose ratio reached 15. This was probably related to that the DCAcAm chlorination rate was higher than the DCAN chlorination rate (Ding et al., 2018c). The yield of TCM gradually increased as the pH increased from 5 to 9 during chlorination of TC (Fig. 1e), which agreed with previous research (Chu et al., 2015; Zhou et al., 2014). However, the DCAcAm yield decreased from 23.58% to 4.39% as the pH increased. This was because of base-catalyzed hydrolytic decomposition of DCAcAm with higher hydrolysis rates observed at higher pH (Chu et al., 2009; Ding et al., 2018b).

TCM, DCAcAm, TCAcAm, and DCAN formed during chlorination of TC and their yields were 0.01%–21.8%, 1.82%–44.57%, <0.01%–0.12%, and <0.01%–0.80%, respectively (Fig. 1b, d, and 1f). The DBP yields gradually increased as the contact time and chloramine dose increased during chloramination (Fig. 1b and d). This result was related to the relative stabilities of the DBPs, including DCAcAm and DCAN, in the presence of monochloramine (Ding et al., 2018b). Unlike chlorine, excess monochloramine did not greatly promote DCAN or DCAcAm hydrolysis to reduce their concentrations. The DCACAm yield first increased and then decreased with increasing pH, and the maximum yield was obtained at pH 6 (Fig. 1f). The low DCACAm yields at alkaline pH values were attributed to base-catalyzed hydrolysis of DCAcAm (Chu et al., 2009), while the low DCACAm yield at pH 5 was probably caused by NH3Cl decomposition to NHCl2 through the acid-catalyzed NH3Cl disproportionation reaction (Vikesland et al., 2001; Zhou et al., 2014). The TCM yield from chloramination was much lower than that from chlorination, which was consistent with previous studies (Bond et al., 2011; Hong et al., 2015). However, the DCACAm yield from chloramination was comparable to that from chlorination, and the maximum yield of DCACAm during chloramination was slightly higher than that during chlorination. The yields of DCACAm were much higher than those of other DBPs formed during chloramination of TC. Notably, during chlorination TC, TCM formed with a maximum yield of around 30% but DCACAm formed with an even higher yield of 35%. Moreover, the DCACAm yields were far higher than those of DCAN and other DBPs during chloramination of TC. These results suggest that TC is an important DCACAm precursor and might form DCACAm through a pathway not involving DCAN hydrolysis.

3.2. Theoretical cytotoxicity of DBPs formed from TC

The theoretical cytotoxicity of the DBPs formed during chlorination and chloramination of TC were estimated (Fig. 2), and the contribution of theoretical cytotoxicity contributed by each DBP was calculated (Fig. S3). During chlorination of TC, the theoretical cytotoxicity of the DBPs formed were relatively stable over time, and the cytotoxicity contributed by DCAN was 42.2%–53.8% of the total theoretical cytotoxicity (Fig. 2a and S3a). As the chlorine dose increased, the theoretical cytotoxicity of the DBPs formed from TC increased and then decreased (Fig. 2b), which was consistent with the trend for the DCAN yield (Fig. 1c). DCACAm accounted for most of the cytotoxicity when the ratio of the chlorine dose to precursor was 5:1 (Fig. 2b and S3b). With further increases in the chlorine dose, DCAN became the main contributor to the cytotoxicity because the yield of DCACAm decreased. The theoretical cytotoxicity of the DBPs formed from the chlorination of TC at pH 6–8 was higher than at pH 5 or 9. These differences were mainly attributed to the low TCM yield at pH 5 and the low DCAN and DCACAm yields at pH 9.

The theoretical cytotoxicity of the DBPs formed from the chloramination of TC gradually increased as the contact time or chloramine dose increased, and increased first then decreased as the pH increased. These results were consistent with the trends observed for the DCACAm yield during chloramination. Among the DBPs formed during chloramination, DCACAm had the highest contributions to the concentration and cytotoxicity. For example, 77.1%–92.6% of the total cytotoxicity was attributed to DCACAm formed at different contact times (Fig. S3a). The cytotoxicity estimation indicated that DCACAm produced from TC during chlorination or chloramination contributed greatly to the cytotoxicity, which again suggested that TC was an important DCACAm precursor.
3.3. DCAcAm formation from tetracycline antibiotics

In our initial experiments, we found that TC gave very high DCAcAm yields. Next, we investigated DCAcAm formation during chlorination and chloramination of three other tetracycline antibiotics (DC, CTC, and OTC) and compared the results with those for TC (Fig. 3). TCM and DCAN formation during chlorination and chloramination of the tetracycline antibiotics was also investigated (Figs. 54 and 55). During chlorination, DCAcAm formation was higher than DCAN formation for all four tetracycline antibiotics, and it was higher than TCM formation for CTC and OTC. The DCAcAm yields were much higher than the TCM and DCAN yields during chloramination of DC, CTC, and OTC, which was similar to the results for TC.

The changes in the DCAcAm yield during chlorination of DC, CTC, and OTC with changes in the chlorine dose and contact time were similar to those observed for TC (Fig. 3a and b). Less DCAcAm formed from DC than from TC during chlorination. The DCAcAm yields from chlorination of CTC and OTC were 0.84%–54.26% and 3.51%–48.59%, respectively, and were generally higher than that of DCAcAm from chlorination of TC under the same conditions. The significant differences of DCAcAm formation from four tetracycline antibiotics may be caused by their structural characteristics, which needs further study. Because it is reportedly an important precursor of DCAcAm (Chu et al., 2010; Huang et al., 2012), we used Asn to investigate DCAcAm formation. The DCAcAm yield from Asn ranged from 0.84% to 16.26% with changes in the chlorine dose for a contact time of 2 h, and from 0.14% to 3.51% with a contact time of 24 h (Table S2). These results were consistent with those of previous studies (Chu et al., 2016; Huang et al., 2012). In a recent report, chlorination of six amide precursors (acetamide, acetoacetamide, polyacrylamide, acrylamide, β-alaminamide, and 4-hydroxybenzamide) generated DCAcAm with yields ranging from 0.3% to 0.16%. In this study, each amide was added at 10 μM and buffered at pH 7 or 8, and disinfectant was added at 20 M/M for 4 or 24 h (Sfynia et al., 2020). Chu et al. (2016) found that the antibiotic chloramphenicol and two of its analogs formed DCAcAm in yields as high as 8%–10% when the Cl₂/precursor molar ratio was 5:1 and contact time was 3 or 24 h. In the present study, the DCAcAm yields from tetracycline antibiotics were higher than those reported for amide or chloramphenicol precursors. In particular, the DCAcAm yields from TC, CTC, and OTC were even higher than that from Asn under the same chlorination conditions. These results suggest that tetracycline antibiotics might be important precursors of DCAcAm during chlorination.

The DCAcAm yield during chloramination of DC, CTC, and OTC increased as the contact time increased, which was similar to what we observed for DCAcAm formation from TC (Fig. 3c and d). The DCAcAm yields after chloramination of DC and CTC were 0.99%–31.68% and 2.42%–38.92%, respectively, which were comparable to the DCAcAm yields from TC (3.36%–44.57%). However, the DCAcAm yield from OTC was lower than that from TC during chloramination. The DCAcAm yields from tetracycline antibiotics were much higher than those reported elsewhere for six amide precursors, which had a maximum DCAcAm yield of only 0.15% during chloramination (Sfynia et al. (2020). In addition, our yields were comparable to DCAcAm yields during chloramination of Asn (Huang et al., 2012). This suggests that tetracycline antibiotics should be regarded as important DCAcAm precursors during chloramination.

As mentioned earlier, the concentrations of OTC, CTC, and TC in river water in China have been measured at 2.20, 2.42, and 0.81 μg/L, respectively (Wei et al., 2011). If this water is used as source water and all the tetracyclines are present during the disinfection process, we can calculate the formation potential of DCAcAm from the three tetracyclines. For these calculations we used DCAcAm yields of 22.63%, 27.37%, and 16.40% for OTC, CTC, and TC, respectively, for 2 h of chlorination at a disinfectant/precursor molar ratio of 15:1. For chloramination under the same conditions, we used DCAcAm yields of 1.73%, 6.24%, and 9.49% for OTC, CTC, and TC, respectively. Our calculations showed the total formation potentials of DCAcAm from the three tetracyclines could reach 1.26 μg/L for chlorination.
and 0.27 μg/L for chloramination. According to previous reports, DCAcAm is formed at several microgram per liter levels after chlorination and chloramination of drinking water (Bond et al., 2015; Huang et al., 2017; Kosaka et al., 2016). Although our calculations were not very accurate because they did not take into consideration factors such as the effect of tetracycline concentration on DCAcAm formation rate, the dissolved organic matter concentration or disinfection conditions, the results support the importance of tetracyclines as DCAcAm precursors.

3.4. Nitrogen sources of DCAcAm formed from tetracycline antibiotics

Because the nitrogen of nitrogenous DBPs formed during chloramination can be provided by organic nitrogen in precursors as well as inorganic nitrogen in chloramines, 15N-labeled monochloramine (15N–NH2Cl) was used to analyze the source of nitrogen in DCAcAm formed from tetracycline antibiotics. Unlabeled and 15N-labeled DCAcAm were detected, and the 15N percentages in DCAcAm produced from these compounds were measured (Table 1). The 15N percentages in DCAcAm formed during chloramination of the four tetracycline antibiotics increased as the chloramine dose increased. For all four tetracycline antibiotics, the largest 15N percentages in DCAcAm formed at the highest chloramine dose ranged from 6.31% to 18.12%. Thus, most of the nitrogen in DCAcAm was 14N, which means that the nitrogen in DCAcAm is mainly derived from tetracycline antibiotics instead of chloramines. Similar results have been found for Asn (Huang et al., 2012).

Each of the tested tetracycline antibiotics contained two nitrogen atoms, one of which belonged to an amide group. Amide precursors function as precursors for HAcAms (Sfynia et al., 2020), and the amide nitrogen in the Asn side chain plays a key role in DCAcAm formation (Huang et al., 2012). Therefore, it is possible that the nitrogen in DCAcAm originates from the amide groups of tetracycline antibiotics. Because the amide-containing ring in tetracycline antibiotics has an amide group in the ortho position of the hydroxyl group, we used the structural analog salicylamide (Fig. S6) to investigate DCAcAm formation (Fig. S7). Chloramination of salicylamide produced more DCAcAm than chlorination did. Like with the tetracycline antibiotics, most of the nitrogen in DCAcAm was derived from the precursor rather than chloramine during chloramination. These results suggest that the amide group in tetracycline antibiotics contributes to the formation of DCAcAm. However, the DCAcAm yields from salicylamide were much lower than those from the tetracycline antibiotics. Unlike salicylamide, the amide-containing ring in tetracycline antibiotics has other substituents and connects to other rings. We speculated that the complex structure of tetracycline antibiotics may promote DCAcAm formation from the amide group, or another nitrogen group—the dimethylamino group—may contribute to DCAcAm formation, and further research is required to clarify this.

3.5. Effect of pretreatment on DCAcAm formation

The DCAcAm yields we observed from tetracycline antibiotics highlighted that this pathway should receive attention. Therefore, we investigated whether common pretreatments methods could

![Graph 3](image-url)

**Fig. 3.** DCAcAm formation versus contact time and dose during chlorination (a, b) and chloramination (c, d) of tetracycline antibiotics. Conditions: precursor concentration = 0.05 mM, and pH = 7.0 ± 0.2.

| 15N–NH2Cl/precursor molar ratio | 15N percentage in DCAcAm (%) |
|---------------------------------|-----------------------------|
|                                 | DC  | CTC | OTC | TC  |
| 5                               | 2.87| 2.07| 4.92| 1.82|
| 10                              | 7.07| 3.62| 10.92| 3.08|
| 15                              | 10.77| 4.89| 14.40| 4.33|
| 20                              | 13.88| 5.88| 16.52| 5.46|
| 25                              | 17.66| 7.17| 18.12| 6.31|

*Table 1* 15N percentage in DCAcAm versus the disinfection dose for chloramination of tetracycline antibiotics. Conditions: precursor concentration = 0.05 mM, contact time = 24 h, and pH = 7.0 ± 0.2.
reduce DCACAm formation from tetracyclines. Pretreatment by ClO2 pre-oxidation, UV photolysis, or the UV/chlorine advanced oxidation process is often used in water treatment to eliminate algae or micropollutants, and these methods affect DBP formation during subsequent chlor(am)ination because of transformation of DBP precursors (Jiang et al., 2019; Zhang et al., 2019b). Thus, we investigated the effects of these three pretreatment methods on DCACAm formation during chlor(am)ination of TC.

ClO2 pre-oxidation decreased the DCACAm yields from the chlorination of TC by 81.47%–96.62% and the DCACAm yields from the chloramination of TC by 35.42%–74.70% (Fig. 4). Similar results have previously been found for tyrosine and tyrosine tert-butyl ester during chlorination with a Cl2/precursor ratio of 10:1 (Yao et al., 2018). The high removal efficiency of ClO2 pre-oxidation might be related to the extremely rapidly reaction of tetracycline antibiotics with ClO2 and the oxidation of tetracycline antibiotics by ClO2 leading to (hydr)oxylation and degradation of the molecules (Wang et al., 2011). UV photolysis reduced the yield of DCACAm from chlorination of TC by 4.62%–48.98%, which was consistent with the results for pyrimidines and purines (Zhang et al., 2019b). However, the reduction in DCACAm formation for chloramination after UV photolysis was less than that for chlorination, and the maximum reduction was only 6.50%. Tian et al. (2017) found that UV photolysis was less effective for reducing 1-tribalomethane yields from chlorination than from chlorination. Compared with ClO2 pre-oxidation, the reduction in DCACAm yield with UV photolysis was lower, probably because UV irradiation only removes small quantities of TC (Xu et al., 2020). The UV/chlorine advanced oxidation process differed from ClO2 pre-oxidation and UV photolysis in that it increased the DCACAm yield from chlorination of TC by 73.58%–138.07%. A similar tendency was reported for the chlorination of cytosine and acrylamide after the UV/chlorine advanced oxidation process, and a potential explanation for this might be the generation of radicals during UV/chlorination because of transformation of tetracycline precursors in chlorination (Gao et al., 2017; Zhang et al., 2019b). The NC is the predominant contributor determining the yields and cytotoxicity of the DBPs. The DCACAm yields from the four tetracycline antibiotics (TC, CTC, OTC, and DC) ranged from 0.43% to 54.26% for chlorination and 0.65%–44.57% for chloramination, suggesting that tetracycline antibiotics are possible precursors of DCACAm. The 15N percentages in DCACAm formed by chloramination of the four tetracycline antibiotics with 15N–NH2Cl-labeling were all <19%, which suggests that the nitrogen in DCACAm dominantly arises from tetracycline antibiotics rather than chloramines. ClO2 pre-oxidation and UV photolysis decreased DCACAm formation during chloramination and chlorination of TC, and the former showed better removal.

CRediT author statement

Zhao-Xi Ye: Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft. Kai-Li Shao: Methodology, Investigation. Huang Huang: Conceptualization, Resources, Writing - Review & Editing, Supervision, Project administration. Xin Yang: Resources, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank the support of the sponsors: Guangdong Basic and Applied Basic Research Foundation (No. 2020A1515011047) and Science and Technology Program of Guangzhou, China (No.201904010125).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.128628.

References

Bond, T., Huang, J., Templeton, M.R., Graham, N., 2011. Occurrence and control of nitrogenous disinfection by-products in drinking water - a review. Water Res. 45 (15), 4341–4354. https://doi.org/10.1016/j.watres.2011.05.034.
Bond, T., Templeton, M.R., Mohitar Kamal, N.H., Graham, N., Kanda, R., 2015. Nitrogenous disinfection byproducts in English drinking water supply systems: occurrence, bromine substitution and correlation analysis. Water Res. 85, 85–94. https://doi.org/10.1016/j.watres.2015.08.015.
Cetcioğlu, Z., Ince, B., Gros, M., Rodriguez-Mozaz, S., Barcelo, D., Orhon, D., Ince, O., 2013. Chronic impact of tetracycline on the biodegradation of an organic substrate mixture under anaerobic conditions. Water Res. 47 (9), 2959–2969. https://doi.org/10.1016/j.watres.2013.02.053.
Chang, C.Y., Chan, R.C., 2020. Underestimation of co-infections in COVID-19 due to non-discriminatory use of antibiotics. J. Infect. https://doi.org/10.1016/
and UVC/persulfate processes for tetracycline removal in water. Chem. Eng. J. 384, 123320. https://doi.org/10.1016/j.cej.2019.123320.

Yao, D.C., Chu, W.H., Bond, T., Ding, S.K., Chen, S.H., 2018. Impact of ClO2 pre-oxidation on the formation of CX3R-type DBPs from tyrosine-based amino acid precursors during chlorination and chloramination. Chemosphere 196, 25–34. https://doi.org/10.1016/j.chemosphere.2017.12.143.

Yu, Y., Reckhow, D.A., 2015. Kinetic analysis of haloacetonitrile stability in drinking waters. Environ. Sci. Technol. 49 (18), 11028–11036. https://doi.org/10.1021/acs.est.5b02772.

Zhang, S.S., Lin, T., Chen, W., Xu, H., Tao, H., 2019a. Degradation kinetics, byproducts formation and estimated toxicity of metronidazole (MNZ) during chlor(am)ination. Chemosphere 235, 21–31. https://doi.org/10.1016/j.chemosphere.2019.06.150.

Zhang, X.R., Zhai, J.X., Zhong, Y., Yang, X., 2019b. Degradation and DBP formations from pyrimidines and purines bases during sequential or simultaneous use of UV and chlorine. Water Res. 165 https://doi.org/10.1016/j.watres.2019.115023.

Zhou, S.Q., Shao, Y.S., Gan, N.Y., Zhu, S.M., Ma, Y., Deng, J., 2014. Chlorination and chloramination of tetracycline antibiotics: disinfection by-products formation and influential factors. Ecotoxicol. Environ. Saf. 107, 30–35. https://doi.org/10.1016/j.ecoenv.2014.05.008.