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RESEARCH ARTICLE

Identification of important precursors and theoretical toxicity evaluation of byproducts driving cytotoxicity and genotoxicity in chlorination

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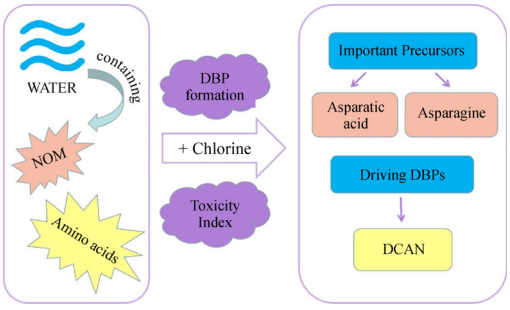
HIGHLIGHTS

- NOM formed more C-DBPs while amino acids formed more N-DBPs during chlorination
- Aspartic acid and asparagine showed the highest toxicity index during chlorination
- Dichloroacetonitrile might be a driving DBP for cytotoxicity and genotoxicity
- Dichloroacetonitrile dominated the toxicity under different chlorination conditions

GRAPHIC ABSTRACT



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ABSTRACT

Chlorination, the most widely used disinfection process for water treatment, is unfortunately always accompanied with the formation of hazardous disinfection byproducts (DBPs). Various organic matter species, like natural organic matter (NOM) and amino acids, can serve as precursors of DBPs during chlorination but it is not clear what types of organic matter have higher potential risks. Although regulation of DBPs such as trihalomethanes has received much attention, further investigation of the DBPs driving toxicity is required. This study aimed to identify the important precursors of chlorination by measuring DBP formation from NOM and amino acids, and to determine the main DBPs driving toxicity using a theoretical toxicity evaluation of contributions to the cytotoxicity index (CTI) and genotoxicity index (GTI). The results showed that NOM mainly formed carbonaceous DBPs (C-DBPs), such as trichloromethane, while amino acids mainly formed nitrogenous DBPs (N-DBPs), such as dichloroacetonitrile (DCAN). Among the DBPs, DCAN had the largest contribution to the toxicity index and might be the main driver of toxicity. Among the precursors, aspartic acid and asparagine gave the highest DCAN concentration (200 g/L) and the highest CTI and GTI. Therefore, aspartic acid and asparagine are important precursors for toxicity and their concentrations should be reduced as much as possible before chlorination to minimize the formation of DBPs. During chlorination of NOM, tryptophan, and asparagine solutions with different chlorine doses and reaction times, changes in the CTI and GTI were consistent with changes in the DCAN concentration.

1 Introduction

Water disinfection is one of the most important measures for public health progression. Chlorination is currently the most widely used disinfection process (Du et al., 2017a; Mao et al., 2018). In Canada, about 90% of drinking water

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is disinfected by chlorine (Chowdhury et al., 2011). However, with the development of analytical techniques, disinfection byproducts (DBPs) such as trihalomethane have been detected in chlorinated water (Du et al., 2017b; Richardson et al., 2007; Wu et al., 2019). DBPs are formed when organic matter in water reacts with chlorine. The organic matter in water is generally within the concentration range of several milligrams per liter, and is mainly made up of natural organic matter (NOM) and other compounds such as amino acids (Farré et al., 2013; Hu et al., 2016).

Most DBPs are cytotoxic and genotoxic (Amjad et al., 2013). During daily activities such as drinking, bathing, and swimming, humans can be exposed to DBPs through ingestion, inhalation, and dermal contact (Amjad et al., 2013; Lee et al., 2013), which leads to potential health risks. Epidemiological studies have suggested that long-term exposure to chlorinated water is correlated to increased cancer risk, especially for bladder and rectal cancers (IARC, 1995; IARC, 2004). To ensure the safety of drinking water, some DBPs are regulated by the World Health Organization and in many countries (Richardson et al., 2007). Trihalomethanes and haloacetic acids have received the most attention among the DBPs in epidemiological studies. However, many emerging and unregulated DBPs, such as haloacetonitriles, haloacetaldehydes, and haloacetamides, have been detected in water and are regarded more toxic than the regulated DBPs (Richardson et al., 2007). Therefore, unregulated DBPs should be investigated.

To date, many studies have described the formation of DBPs from NOM, such as humic acids and algal metabolites in water (Lu et al., 2009; Bougeard et al., 2010; Yang et al., 2013). Amino acids are also common in surface water and wastewater. Trehy et al. (1986) found that chloral hydrate (CH) and dichloroacetonitrile were the dominant byproducts during chlorination of amino acids. Some researchers have speculated that the chlorination of proteins during algal blooms might play an important role in the formation of trihalomethanes (THMs) in natural water (Scully et al., 1988). However, it is unclear which of the various organic matters have the highest potential risks during chlorination.

Generally, the DBPs present in the highest concentrations (i.e., trihalomethanes) have received the most attention. However, their contributions to the total DBP-associated toxicity need to be considered. For instance, THMs are found in high concentrations in chlorinated water and have received the most attention, but they are reportedly less toxic than other DBPs (Li et al., 2019). Researchers are concerned about DBPs because of their potential toxic effects (Du et al., 2018a; Du et al., 2018b). Toxicity indexes like the cytotoxicity index (CTI) and genotoxicity index (GTI) have been proposed for quantification of DBP toxicity. These are quantitative, comprehensive indexes that combine both the concentration and toxic potency of DBPs (Wagner and Plewa, 2017). In this study, the CTI and GTI were used to assess the toxicity of disinfected water and to identify the DBPs driving toxicity during chlorination.

The objectives of this study were (1) to compare DBP formation from the chlorination of NOM and typical amino acids, (2) to investigate the CTI and GTI from chlorination of NOM and typical amino acids with a theoretical evaluation method, and (3) to identify the contributions of different DBPs to the CTI and GTI to identify the DBPs that cause toxicity. To achieve the objects stated above, first DBPs concentration formed by NOM and amino acids during chlorination were determined; then CTI and GTI of DBPs were calculated to indicate potential cytotoxicity and genotoxicity; finally the contribution to the total toxicity index of each DBP were quantitatively compared, so that the driving DBP for toxicity would be identified.

2 Materials and methods

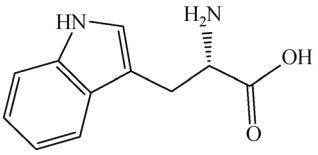
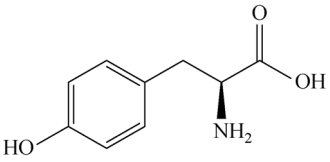
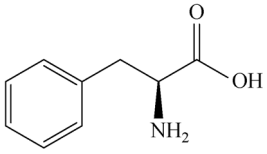
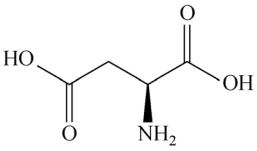
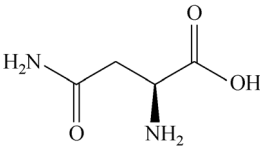
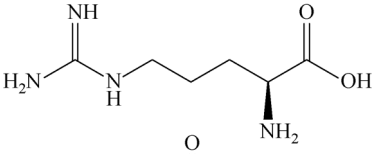
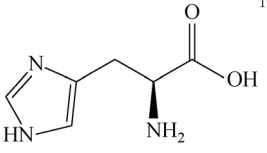
2.1 Chemicals and reagents

Natural organic matter (the sample number is 2R101N, which was collected at Suwannee River with RO isolation) was obtained from the International Humic Substances Society (Colorado-Denver, USA). Amino acids, including *L*-tryptophan, *L*-tyrosine, *L*-phenylalanine, *L*-aspartic acid, *L*-asparagine, *L*-arginine, and *L*-histidine were purchased from J&K Scientific (Table 1). Sodium hypochlorite (NaClO, 99% purity) was obtained from Shenzhen Tongyu Technology. Sodium thiosulfate ($Na_2S_2O_3$, 97% purity) was obtained from Tianjin Zhiyuan Chemical Reagent Company. Sodium dihydrogen phosphate (NaH_2PO_4, 98.0% purity), disodium hydrogen phosphate (Na_2HPO_4, 99.0% purity), and anhydrous sodium sulfate (Na_2SO_4, 99.0% purity) were bought from Macklin. Methyl-*t*-butyl ether (reagent grade) was obtained from BCR International Trading Ltd. Company. 1,2-Dibromopropane internal standard (reagent grade) was obtained from Sigma–Aldrich. In addition, standards of trichloromethane (TCM), bromodichloromethane, tribromomethane, and dibromochloromethane (99% purity) were purchased from o2si Smart Solutions. Trichloronitromethane (5 mg/L), bromochloroacetonitrile (99% purity), dichloroacetonitrile (DCAN, 98% purity), dibromoacetonitrile (98% purity), trichloroacetonitrile (98% purity), dichloroacetamide (99% purity), trichloroacetamide (99% purity), CH (99% purity), 1,1-dichloropropanone (1,1-DCP, 99% purity), and 1,1,1-trichloropropanone (1,1,1-TCP, 97.9% purity) were purchased from J&K Scientific (China).

2.2 Water sample preparation

A NOM stock solution was prepared by dissolving NOM in ultrapure water (UPW, Milli-Q), and then filtered

Table 1 Information of amino acids used in this study

Amino acids	CAS	Formula	Structure
L-tryptophan	73-22-3	$C_{11}H_{12}N_2O_2$	
L-tyrosine	60-18-4	$C_9H_{11}NO_3$	
L-phenylalanine	63-91-2	$C_9H_{11}NO_2$	
L-aspartic acid	56-84-8	$C_4H_7NO_4$	
L-asparagine	70-47-3	$C_4H_8N_2O_3$	
L-arginine	74-79-3	$C_6H_{14}N_4O_2$	
L-histidine	71-00-1	$C_6H_9N_3O_2$	

through a 0.45-m filter membrane. A total organic carbon analyzer (TOC-VCPH, Shimadzu, Japan) was used to determine the dissolved organic carbon content. The NOM stock solution was stored at 4°C. Stock solutions of seven amino acids were prepared by dissolving *L*-tryptophan, *L*-tyrosine, *L*-phenylalanine, *L*-aspartic acid, *L*-asparagine, *L*-arginine, and *L*-histidine in UPW. All stock solutions were stored in brown glass sealed bottles in the dark at 4°C. Before use, the stock solutions would be diluted to 3 mg/L NOM with UPW.

2.3 Chlorination

Before chlorination, the samples were buffered with 1 mM Na_2HPO_4 and NaH_2PO_4 solution (pH 7.0) in a 2-L glass bottle. The stock solution of NaClO ($[Cl_2]$ = 16.15 mg/mL, or 22.7 mM) was then added according to the required chlorine dose. In previous studies, 24 h chlorination was usually practical to detect the yields of DBPs no matter amino acids used as the precursors (Chu et al., 2015) or NOM used as the precursor (Van et al., 2011). Thus, in this study, 24 h was selected as the reaction time. Each sample was chlorinated at 25±1°C for the required contact time and kept in the dark. To stop the chlorination, free chlorine residues were detected by a chlorine analyzer (HI-93711, Hanna Instruments, Italy) and then quenched by adding 105% of the stoichiometric amount of 1.0 mol/L sodium thiosulfate.

2.4 Extraction of DBPs

Liquid–liquid extraction was used to concentrate the DBPs in the water samples. For each water sample, a 30-mL sample was quickly added by 3 mL of methyl-*t*-butyl ether, which contained 100 g/L 1,2-dibromopropane as an internal standard for chemical analysis. Then, 6.0 g of Na_2SO_4 was added. The samples were then placed in a rotary vibrator (QB-328, Qilin Bell Instrument Manufacturing) for 5 min and mixed. The mixed samples were left to stand for 10 min to allow the organic and aqueous phases to separate. Then, 1 mL of concentrated liquid from the upper organic phase was carefully transferred into a brown glass sample bottle using a glass dropper and sealed with plastic cover, then stored in a –20°C refrigerator for analysis.

2.5 Analytical methods

A gas chromatography-electron capture detector instrument (7890B, Agilent) equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 μm) was used to analyze DBPs in the concentrated sample after liquid–liquid extraction. The instrument was operated as follows: inlet temperature 230°C; pressure 60676 pa; heat-insulated purge flow rate 3 mL/min and its temperature is 250°C; tail gas flow rate 60 mL/min; column flow rate 1.2 mL/min; gas interface temperature 230°C; and injection volume, 1 μL in splitless mode. The column temperature was initially held at 3°C for 9 min, increased to 40°C at 2°C/min and held for 1 min, increased to 160°C at 10°C/min and held for 2 min, and finally increased to 220°C at 40°C/min and held for 2 min.

2.6 Theoretical calculation of the CTI and GTI

In this study, the half lethal concentration (LC_{50}, M) and 50% Tail DNA (TDNA) or midpoint of tail moment (M) (Table 2) were used to calculate the CTI and GTI, respectively (Wagner and Plewa, 2017). The LC_{50} was defined as the DBP concentration that induced a cell density of 50% of that of the negative control and was used to evaluate the cytotoxicity. The 50% TDNA or midpoint of tail moment (M) value was defined as the point where 50% of the DNA had migrated away from the nucleus in the comet assay and was used to evaluate the genotoxicity (Wagner and Plewa, 2017). Wagner and Plewa (2017) provided a mathematical method for quantitative analysis of DBP-induced cytotoxicity and genotoxicity toward Chinese hamster ovary cells. After obtaining the LC_{50} and 50% TDNA or midpoint of tail moment value, the CTI and GTI of the eight precursors were calculated using Eqs. (1) and (2) and used to evaluate the toxicity of these precursors in water after chlorination.

$$\mathrm{CTI} = \Sigma(\text{DBP concentration(M)}/\mathrm{LC_{50}}) \quad (1)$$

$$\mathrm{GTI} = \Sigma(\text{DBP concentration(M)}/\text{50\%TDNA or midpoint of tail moment}) \quad (2)$$

Smaller LC_{50} (M) and 50% TDNA or midpoint of tail moment (M) values gave larger CTI and GTI.

2.7 Quality assurance and quality control

To reduce background pollution, all glass containers were cleaned with tap water and UPW, 30 times and 15 times respectively. Three parallel samples were set in a group. Every sample was determined two times. Results were expressed as the average value±standard deviation, within a certain amount of relative error (less than 5%).

3 Results

3.1 Levels of DBP formation during chlorination of different precursors

The water samples prepared using different precursors in this study contained no bromide, and no brominated DBPs were detected. Seventeen DBPs were measured and the following five chlorinated DBPs were detected: TCM, CH, DCAN, trichloroacetamide (TCAM), and trichloroacetone (TCA). Figure 1 shows the levels of DBPs formed from the eight precursors after chlorination. Among the precursors, NOM gave the highest concentration of TCM (nearly 100 g/L), followed by tryptophan and tyrosine. Tryptophan, aspartic acid, and asparagine gave the highest concentrations of CH, followed by NOM, tyrosine, and histidine. By contrast, phenylalanine and arginine gave only low CH concentrations. Among the precursors, aspartic acid and asparagine gave the highest concentration of DCAN (nearly 200 g/L), and the DCAN concentrations from the other precursors were very low. TCAM was

Table 2 LC_{50} (M) and 50% TDNA or midpoint of Tail moment (M) of DBPs reported in the literature

DBPs	LC_{50} (M)	50% TDNA or midpoint of Tail moment (M)	Reference
TCM	9.62×10^{-3}	NA a)	Wagner and Plewa (2009)
CH	1.16×10^{-3}	NA [a)]	Jeong et al. (2015)
DCAN	5.73×10^{-5}	2.75×10^{-3}	Muellner et al. (2007)
TCAM	2.05×10^{-3}	6.54×10^{-3}	Plewa et al. (2007)
TCA	NA [a)]	NA [a]	Wagner and Plewa (2017)

Note: a) not available.

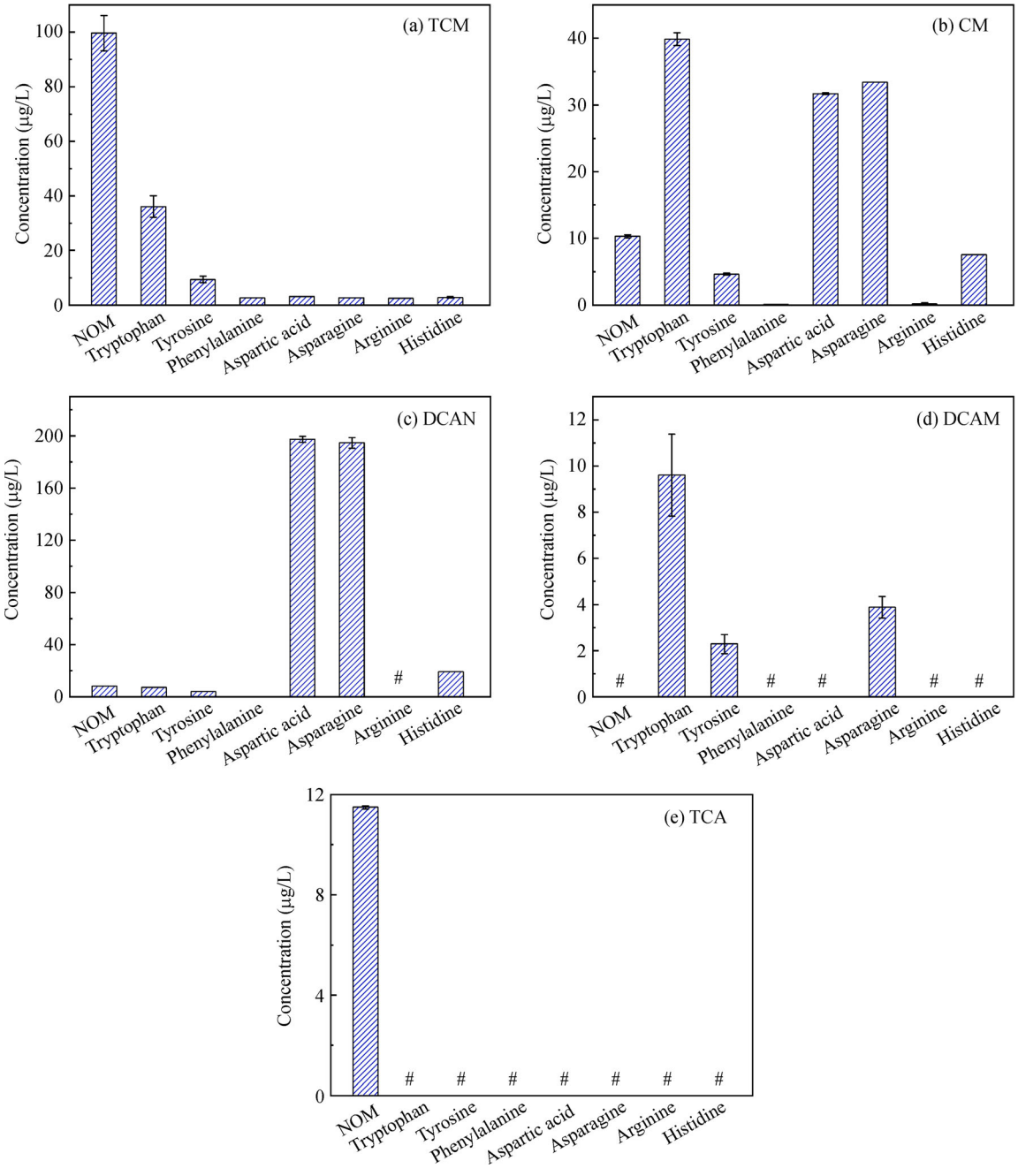


Fig. 1 Levels of DBPs formed during chlorination of different precursors: (a) TCM, (b) TCAL, (c) DCAN, (d) TCAM, and (e) TCA (chlorine dose 9 mg/L, 12.7 μM; and contact time 24 h).

detected only in tryptophan, tyrosine, and asparagine solutions. In addition, NOM formed TCA (11.5 g/L), which was not formed by any of the amino acids.

3.2 Chlorine and nitrogen contents in DBPs from different precursors

The concentrations of the DBPs were used in Eqs. (3) and (4) to calculate chlorine contents in byproducts (Cl_{BP}) and nitrogen contents in byproducts (N_{BP}) for the eight precursors to characterize their chlorine and nitrogen substitution capacity.

$$Cl_{BP} = \Sigma(\text{DBPs concentration} \times \text{percentage of Cl in molecular weight}) \quad (3)$$

$$N_{BP} = \Sigma(\text{DBPs concentration} \times \text{percentage of N in molecular weight}) \quad (4)$$

Figure 2 shows that the Cl_{BP} (nearly 150 μg/L) and N_{BP} (nearly 24 μg/L) concentrations for aspartic acid and asparagine were similar and the highest among the eight precursors. The Cl_{BP} concentrations for NOM, tryptophan, tyrosine, and histidine were higher than those formed by phenylalanine and arginine. The N_{BP} concentrations for aspartic acid and asparagine were almost 25 times those formed by tryptophan and histidine. The Cl_{BP} concentrations for phenylalanine and arginine were only several micrograms per liter, and arginine did not form any N_{BP}.

3.3 CTI and GTI from chlorination of different precursors

The CTI and GTI of the DBPs produced by aspartic acid and asparagine were similar and the highest among all the precursors (Fig. 3). The CTI of these two amino acids were 3.1×10^{-2} and the GTI were nearly 6.5×10^{-4}. Histidine, tryptophan, NOM and tyrosine followed aspartic acids and asparagine. The CTI of these four precursors were 3.1×10^{-3}, 1.4×10^{-3}, 1.4×10^{-3} and 6.9×10^{-4}, respectively. And the GTI of these four precursors were 6.4×10^{-5}, 3.3×10^{-5}, 2.7×10^{-5} and 1.6×10^{-5}, respectively. The CTI and GTI of phenylalanine and arginine were lower than those of the other precursors, which agreed with the Cl_{BP} and N_{BP} results. In addition, among the DBPs, DCAN had the largest contributions to the CTI and GTI, the proportion of DCAN in CTI arranged at 81%–90% and that in GTI arranged 72%–100%, which was followed by TCAM. The other DBPs had lower contributions.

3.4 Effects of the chlorine dose and reaction time on DBP formation and the toxicity index

As described in Section 3.1, the concentrations of DBPs formed by NOM, tryptophan, and asparagine were relatively high, this was especially true for DCAN formed from asparagine, which gave a much higher concentration than the other precursors. Therefore, NOM, tryptophan,

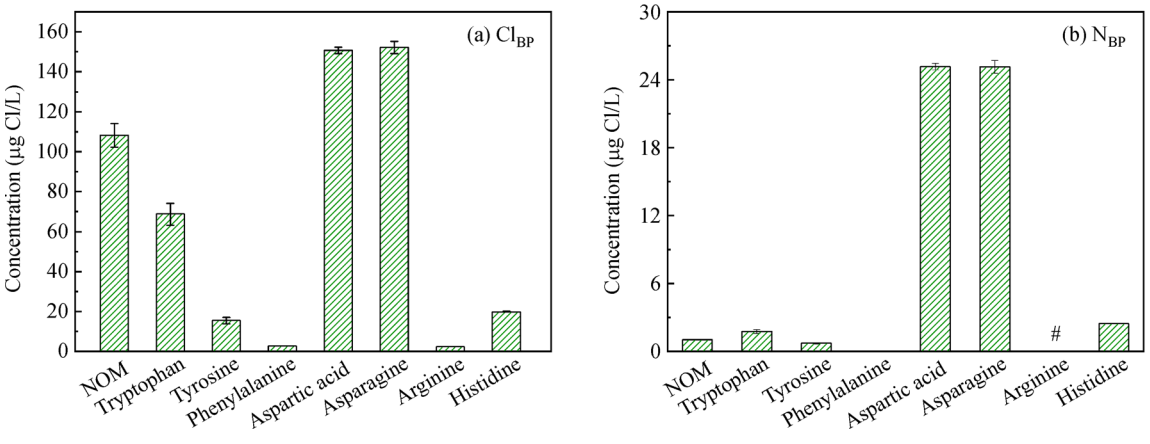


Fig. 2 (a) Chlorine byproduct (Cl_{BP}) and (b) nitrogen byproduct (N_{BP}) concentrations produced by different precursors during chlorination (chlorine dose 9 mg/L, 12.7 μM; and contact time 24 h).

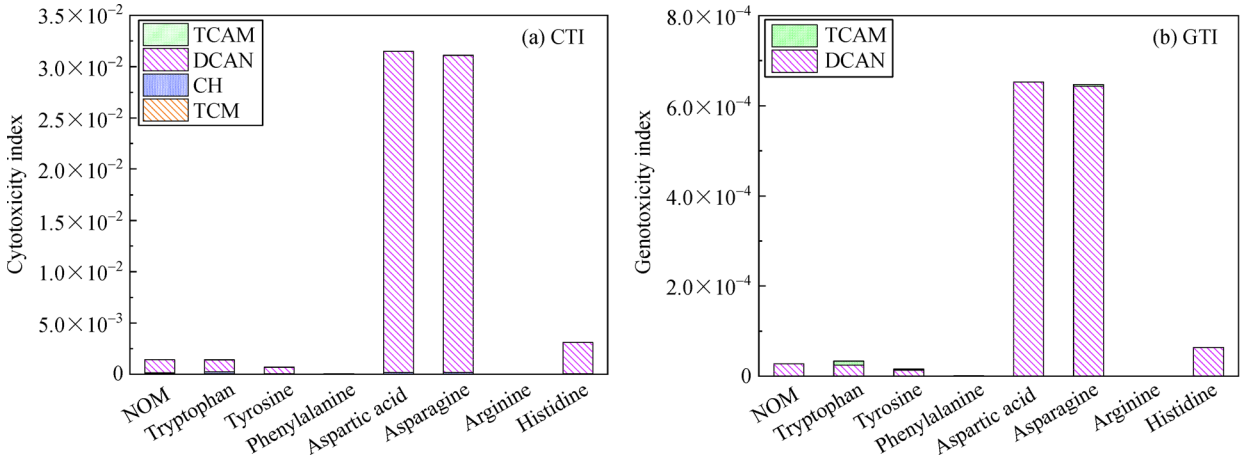


Fig. 3 (a) Cytotoxicity index and (b) genotoxicity index results for the chlorination of different precursors (chlorine dose 9 mg/L, 12.7 μM; and contact time 24 h).

and asparagine were selected as precursors to study the effects of the chlorine dose and reaction time on DBPs formation and the toxicity index.

3.4.1 Effects of the chlorine dose on DBPs formation and toxicity index

Figures 4(a)–4(c) shows the formation of DBPs after 1 h chlorination of NOM, tryptophan, and asparagine solutions with various chlorine doses (0–9 mg/L, or 0–12.7 μM), and Figs. 4(d) and 4(e) shows how the CTI and GTI were affected by the chlorine dose. When NOM was the precursor, the concentrations of TCM, CH, DCAN, and TCA increased with the chlorine dose, and TCM was the dominant DBP. When tryptophan was the precursor, the concentrations of all DBPs except DCAN increased almost linearly with the chlorine dose and CH was the dominant DBP. When asparagine was the precursor, DCAN was the main DBP formed, and its concentration first increased to a maximum (about 40 μg/L) and then decreased. The concentration of CH increased stably but there were no obvious changes in the concentration of TCM and TCA. In

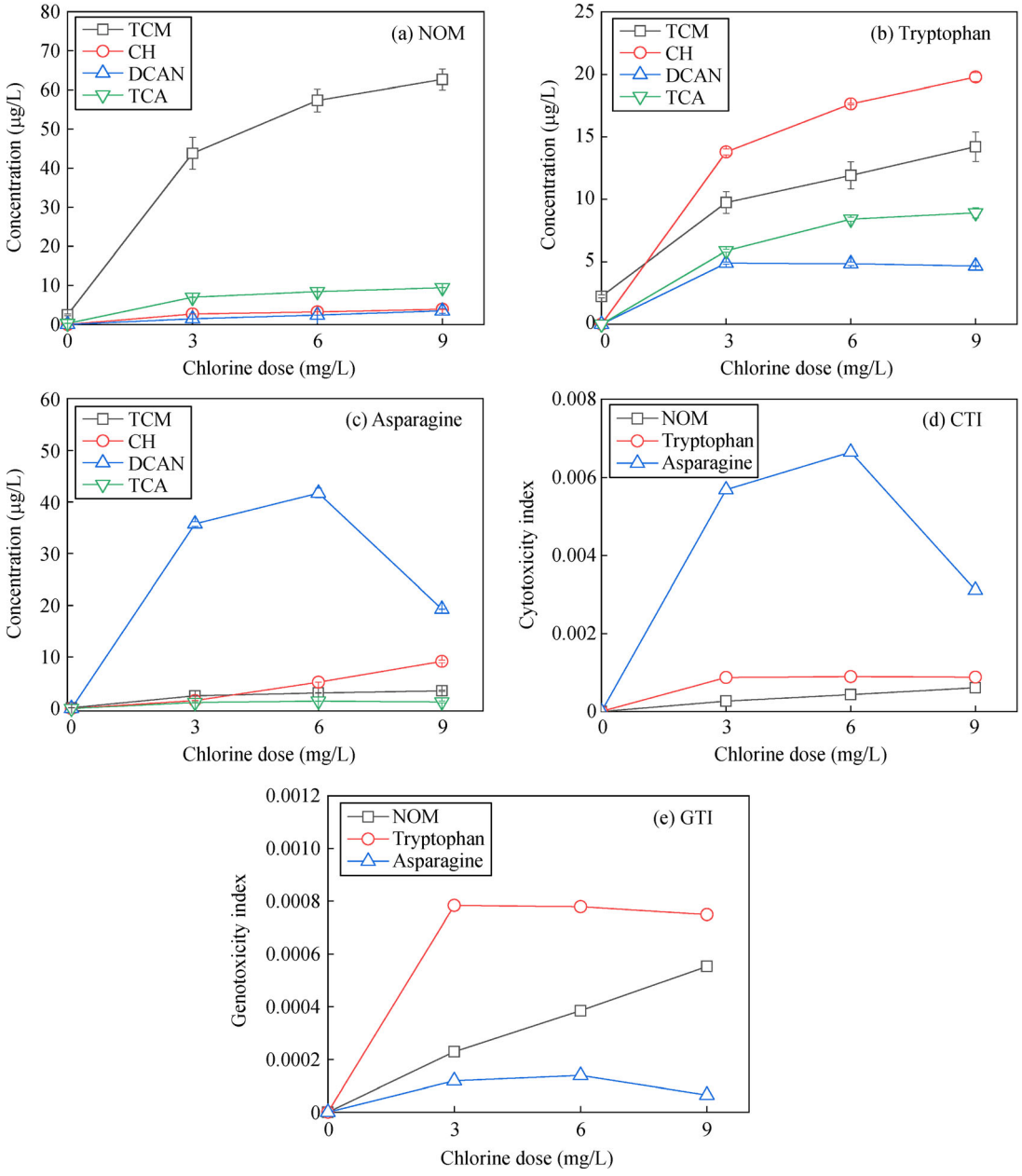


Fig. 4 Effects of chlorine dose (chlorine dose 0, 3, 6, 9 mg/L (or 0, 4.2, 8.5, 12.7 μM); and reaction time 1 h) on DBP formation and the toxicity index: (a) NOM as the precursor, (b) tryptophan as the precursor, (c) asparagine as the precursor, (d) cytotoxicity index (CTI), and (e) genotoxicity index (GTI).

addition, both the CTI and GTI of the NOM water sample increased monotonously with the chlorine dose. For the water sample containing tryptophan, the CTI and GTI increased rapidly when the chlorine dose was increased from 0 to 3 mg/L (4.2 μM). Then, when the chlorine dose was increased from 3 to 9 mg/L (12.7 μM), the CTI increased slowly and the GTI decreased slowly. The CTI and GTI of the water sample containing asparagine first increased to maxima (6.65×10^{-3} and 1.39×10^{-4}, respectively) and then decreased.

3.4.2 Effects of the reaction time on DBPs production and toxicity index

The effects of various reaction times (0.5–24 h) on the concentrations of DBPs formed during chlorination of NOM, tryptophan, and asparagine (Figs. 5(a)–5(c)) and the CTI and GTI (Figs. 5(d) and 5(e)) were investigated. When NOM was the precursor, the concentrations of all DBPs increased with the reaction time, and among the DBPs, the concentration of TCM increased the fastest. With

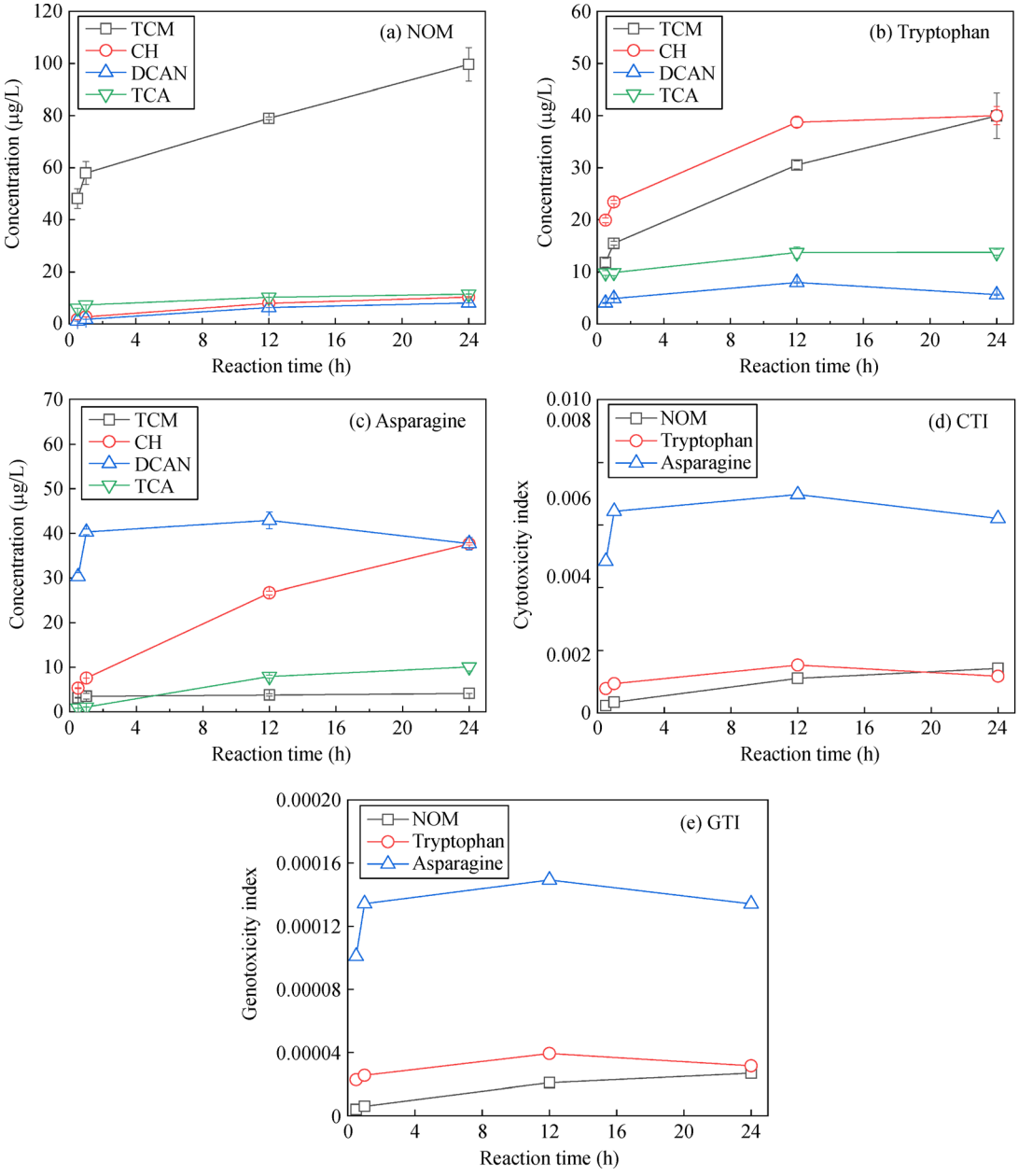


Fig. 5 Effects of reaction time (chlorine dose 9 mg/L (12.7 μM); and reaction time 0.5, 1, 12, or 24 h) on DBP formation and toxicity index: (a) NOM as the precursor, (b) tryptophan as the precursor, (c) asparagine as the precursor, (d) cytotoxicity index (CTI), and (e) genotoxicity index (GTI).

tryptophan as the precursor and a chlorine dose of 9 mg/L (12.7 μM), the concentration of DCAN first increased and then decreased as the reaction time increased from 0.5 to 24 h, while the concentration of the other DBPs just increased. When asparagine was the precursor, the concentration of TCM almost showed no growth, whereas the concentration of CH and TCAM increased with the reaction time. By contrast, the concentration DCAN increased rapidly in the first hour, slowly from 1 to 12 h, and then decreased from 12 to 24 h. The CTI and GTI of the water sample containing NOM increased with reaction time, whereas those of the water samples containing tryptophan and asparagine first increased, reaching maxima at a reaction time of 12 h, and then decreased.

4 Discussion

4.1 Formation of DBPs, Cl_{BP}, and N_{BP} from different precursors

The formation of DBPs shown in Fig. 1 was in agreement with other literatures. Bond et al. (2012) found that DCAN and other haloacetonitriles were produced from the chlorination of selected free amino acids. Yang et al. (2012) determined that asparagine, tyrosine and tryptophan generated the greatest amount of DCAN, at concentrations greater than 0.85 M DCAN/mM amino acid (pH = 7.2, 3 mM chlorine applied to 0.1 mM amino acids). Later, Jia et al. (2016) found that the yields of DCAN during chlorination by eight amino acids were much higher than that of other DBPs: Aspartic acid generated DCAN at the concentration more than 1.5 μM DCAN/mM amino acid (pH = 7.2, 3 mM chlorine applied to 0.1 mM amino acids).

From the results presented in Fig. 1, the concentrations of three classes of DBPs, namely carbonaceous DBPs (C-DBPs), nitrogenous DBPs (N-DBPs), and total DBPs, formed from each precursor were calculated using Eqs. (5), (6), and (7) (Table 3).

$$\text{N-DBPs}(\mu g/L) = \text{DCAN} + \text{TCAM} \quad (5)$$

$$\text{C-DBPs}(\mu g/L) = \text{TCM} + \text{CH} + \text{TCA} \quad (6)$$

$$\text{Toal DBPs}(\mu g/L) = \text{N-DBPs} + \text{C-DBPs} \quad (7)$$

NOM mainly formed C-DBPs, and the concentration of N-DBPs formed by NOM was much lower than that of N-DBPs formed by amino acids. This result was consistent with other research (Bond et al., 2012; Bond et al., 2014; Chu et al., 2015; Trehy et al., 1986). For instance, Trehy et al. (1986) found that aspartic acid could form both DCAN and CH during chlorination, whereas tryptophan generated TCM, DCAN, and CH. Bond et al. (2012) found that free amino acids such as aspartic acid and tryptophan were important precursors of N-DBPs like haloacetonitriles and haloacetamides. This is because NOM has a high carbon content and low nitrogen content, whereas amino acids have high nitrogen contents (Scully et al., 1988; Van Huy et al., 2011). In addition, among the precursors, aspartic acid and asparagine gave the highest concentrations of N-DBPs, total DBPs, Cl_{BP}, and N_{BP}. These results could be explained by the structures of the amino acids (Table 1). Reactive side groups such as amino nitrogen groups and activated aromatic rings in amino acids are the main chlorine substitution sites (Hureiki et al., 1994). Table 1 shows the structures of the amino acids used in this study. Aspartic acid and asparagine have similar structures. Tryptophan, tyrosine, and phenylalanine all contain a benzene ring. According to the reactivities of the compounds, the most active substitution site in each of asparagine and aspartic acid is the amino nitrogen group located in the middle of the carbon chain (Li et al., 2018), which is a flexible chain without a benzene ring. Therefore, asparagine and aspartic acid have low steric hindrance, and the amino nitrogen groups will be exposed to chlorine and readily available for reaction (Liu and Zhang, 2014; Yoon and Tanaka, 2014). This may explain why asparagine and aspartic acid gave high DBP concentrations.

4.2 Contributions of the DBPs to the toxicity index

The main contributor to the toxicity index of the water samples was DCAN (Fig. 3), even though it was present in

Table 3 DBP formation from the chlorination of different precursors

Precursors	N-DBPs (μg/L)	C-DBPs (μg/L)	Total DBPs (μg/L)
NOM	8.13	121.46	129.59
Tryptophan	16.90	75.90	92.81
Tyrosine	6.39	14.04	20.43
Phenylalanine	0.28	2.77	3.05
Aspartic acid	197.49	34.87	232.36
Asparagine	198.67	36.10	234.77
Arginine	ND [a)]	2.76	2.76
Histidine	19.26	10.40	29.65

Note: a) not detected.

a low concentration in the water samples. To more intuitively express the contributions of the DBPs to the toxicity index, the contribution of every individual DBP to the concentration, CTI, and GTI was evaluated. This method is widely used to evaluate the toxicity contributions of individual DBPs (Chuang et al., 2019; Jeong et al., 2012; Park et al., 2016). For NOM, TCM was the main DBP formed on a mass concentration basis, and it accounted for 77% of the total measured DBPs concentration. However, TCM had low contributions to the calculated CTI and GTI. DCAN had the largest contribution to the DBP-associated CTI, accounting for 90% of the total, although its contribution to the mass concentration of the total DBPs concentration was only 6%. These results are consistent with those reported for real water. For example, Watson et al. (2012) used an in vivo model (*Artemia franciscana*) to evaluate the toxicity of DBPs. Their results showed that the observed toxicity was not related to the concentrations of THMs and nitrosamines present in water samples. Hansen et al. (2012) estimated the GTI and CTI of DBPs in a treated particle suspension and found that HANs had the largest contribution to the toxicity among the measured DBPs.

4.3 Relationship between DCAN and N_{BP} concentration and the toxicity index

The results in Figs. 1–3 were used to analyze the correlations between the DCAN and N_{BP} concentration and the CTI and GTI from chlorination of different precursors (Fig. 6, Table 4). Previous studies have shown significant positive correlations between the nitrogen contents in byproducts and the toxicity of chlorinated water (Lee et al., 2007; Lin et al., 2016). The results of this study showed that DCAN was significantly positively correlated to the CTI and GTI (Pearson correlation coefficients of 1.000 and 1.000, respectively, $p < 0.01$). Therefore, DCAN might cause the toxicity of chlorinated water. The DCAN and N_{BP} concentrations could reflect the ability of the precursor to form N-DBP. The stronger the N-DBP formation ability of the precursor, the more DCAN and N_{BP} it forms and the more toxic the chlorinated water (Lin et al., 2016). From the results of this study, aspartic acid and asparagine have the strongest N-DBP formation abilities, and they form the most N_{BPs}. Therefore, to reduce the formation of chlorinated DBPs and toxicity, emerging N-DBPs such as DCAN should be investigated and

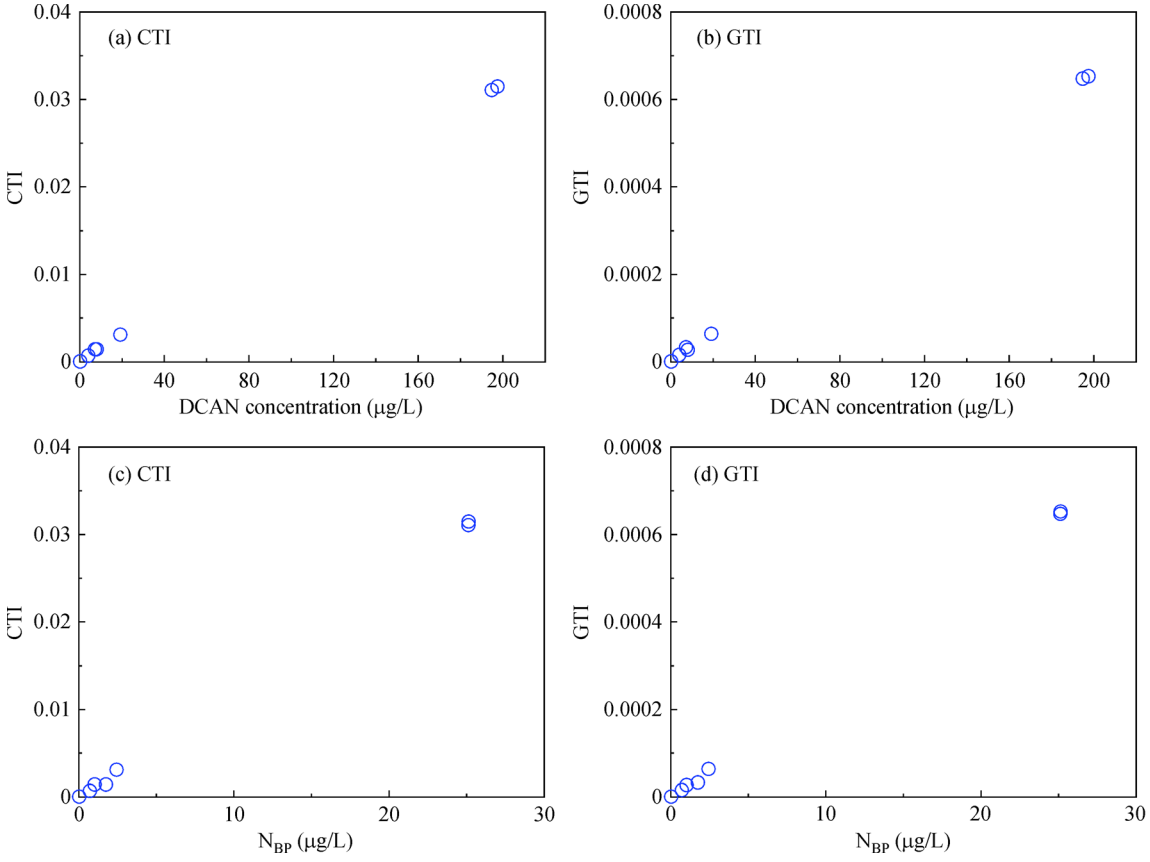


Fig. 6 Correlation between DCAN and N_{BP} concentrations and the toxicity index: (a) DCAN–CTI, (b) DCAN–GTI, (c) N_{BPs}–CTI, and (d) N_{BPs}–GTI. Data are the concentrations of DCAN and N_{BPs}, CTI, and GTI for different precursors (chlorine dose 9 mg/L (12.7 μM); and reaction time 24 h).

Table 4 Correlation analysis between DCAN, N_{BP} and toxicity index

DBP index	Toxicity index	Pearson correlation coefficient	Significance (p)
DCAN	CTI	1.000	< 0.01
	GTI	1.000	< 0.01
N_{BP}	CTI	1.000	< 0.01
	GTI	1.000	< 0.01

aspartic acid and asparagine concentrations should be reduced before chlorination.

4.4 Effects of the chlorine dose and reaction time on DBP formation and toxicity index

When NOM was the precursor, the formation of TCM showed the largest increases with increasing chlorine dose and reaction time. When tryptophan was the precursor, the concentration of CH increased rapidly and it was the dominant DBP. When asparagine was the precursor, despite hydrolysis, DCAN was the dominant DBP. These results could be explained by the chemical structures and reactivities of the precursors (Liu and Zhang, 2014; Yoon and Tanaka, 2014), as discussed in Section 4.1. When tryptophan and asparagine were the precursors, the concentration of DCAN first increased and then decreased with increases in the chlorine dose and reaction time. In other studies on DBP formation, it has been reported that when the chlorine dose/dissolved organic carbon exceeds a certain value, as the reaction time increases, the concentration of DCAN reaches a maximum and then decreases (Reckhow et al., 2001; Sun et al., 2013). This is because DCAN is an unstable DBP (Huang et al., 2012) and its concentration is related to both its formation and hydrolysis rates. DCAN is first hydrolyzed to dichloroacetamide and then hydrolyzed to dichloroacetic acid (Yang et al., 2018).

The results (Figs. 4(d, e) and 5(d, e)) showed the growth of CTI and GTI of NOM, tryptophan, and asparagine were similar to the growth of DCAN with increases in the chlorine dose and reaction time. Although the concentrations of DBPs in NOM, tryptophan, and asparagine solutions were different, the changes in the CTI and GTI were similar. As discussed in Section 4.3, the concentration of DCAN was positively correlated with the CTI and GTI. Therefore, within a certain concentration range, the toxicity of chlorinated water mainly depended on DBPs with higher toxicity index, such as DCAN, rather than the DBPs with higher concentrations, such as THMs, which have previously been given the most attention (Wagner and Plewa, 2017).

5 Conclusions

The formation of DBPs, Cl_{BPs}, N_{BPs}, and toxicity index were investigated during the chlorination of various precursors (NOM and amino acids). Changes in the DBPs, CTI, and GTI with the chlorine dose and reaction time were measured. The relationships between the DCAN and N_{BP} concentrations and the CTI and GTI were also analyzed. We drew the following main conclusions:

1) Under the same chlorination conditions, NOM mainly formed C-DBPs such as TCM, whereas amino acids mainly formed N-DBPs such as DCAN.

2) Among the precursors, aspartic acid and asparagine gave the highest Cl_{BP} and N_{BP} concentrations and CTI and GTI values. Therefore, aspartic acid and asparagine are important precursors driving toxicity and their levels should be reduced as much as possible before chlorination.

3) There were significant positive correlations between the DCAN and N_{BP} concentrations and the CTI and GTI; that is, the higher the DCAN and N_{BP} concentrations, the more toxic the chlorinated water.

4) DCAN might be an important DBP for both the CTI and GTI. CTI and GTI changes under different chlorine dose and contact time showed the same trend as DCAN changes. In future, attention should be focused on DBPs such as DCAN that might drive toxicity rather than those only with high concentrations.

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References

Amjad H, Hashmi I, Rehman M S U, Ali Awan M, Ghaffar S, Khan Z (2013). Cancer and non-cancer risk assessment of trihalomethanes in urban drinking water supplies of Pakistan. Ecotoxicology and Environmental Safety, 91: 25–31

Bond T, Templeton M R, Graham N (2012). Precursors of nitrogenous disinfection by-products in drinking water–a critical review and analysis. Journal of Hazardous Materials, 235-236: 1–16

Bond T, Templeton M R, Rifai O, Ali H, Graham N J D (2014). Chlorinated and nitrogenous disinfection by-product formation from ozonation and post-chlorination of natural organic matter surrogates. Chemosphere, 111: 218–224

Bougeard C M M, Goslan E H, Jefferson B, Parsons S A (2010). Comparison of the disinfection by-product formation potential of treated waters exposed to chlorine and monochloramine. Water Research, 44(3): 729–740

Chowdhury S, Rodriguez M J, Sadiq R (2011). Disinfection byproducts in Canadian provinces: associated cancer risks and medical expenses. Journal of Hazardous Materials, 187(1-3): 574–584

Chu W, Li D, Gao N, Yin D, Zhang Y, Zhu Y (2015). Comparison of free amino acids and short oligopeptides for the formation of trihalomethanes and haloacetonitriles during chlorination: Effect of peptide bond and pre-oxidation. Chemical Engineering Journal, 281:

623–631

Chuang Y H, Szczuka A, Mitch W A (2019). Comparison of toxicity-weighted disinfection byproduct concentrations in potable reuse waters and conventional drinking waters as a new approach to assessing the quality of advanced treatment train waters. Environmental Science & Technology, 53(7): 3729–3738

Du Y, Lv X T, Wu Q Y, Zhang D Y, Zhou Y T, Peng L, Hu H Y (2017a). Formation and control of disinfection byproducts and toxicity during reclaimed water chlorination: A review. Journal of Environmental Sciences (China), 58: 51–63

Du Y, Wu Q Y, Lu Y, Hu H Y, Yang Y, Liu R, Liu F (2017b). Increase of cytotoxicity during wastewater chlorination: Impact factors and surrogates. Journal of Hazardous Materials, 324(Pt B): 681–690

Du Y, Wu Q Y, Lv X T, Ye B, Zhan X M, Lu Y, Hu H Y (2018a). Electron donating capacity reduction of dissolved organic matter by solar irradiation reduces the cytotoxicity formation potential during wastewater chlorination. Water Research, 145: 94–102

Du Y, Wu Q Y, Lv X T, Wang Q P, Lu Y, Hu H Y (2018b). Exposure to solar light reduces cytotoxicity of sewage effluents to mammalian cells: Roles of reactive oxygen and nitrogen species. Water Research, 143: 570–578

Farré M J, Day S, Neale P A, Stalter D, Tang J Y, Escher B I (2013). Bioanalytical and chemical assessment of the disinfection by-product formation potential: Role of organic matter. Water Research, 47(14): 5409–5421

Hansen K M S, Willach S, Mosbæk H, Andersen H R (2012). Particles in swimming pool filters—Does pH determine the DBP formation? Chemosphere, 87(3): 241–247

Hu H Y, Du Y, Wu Q Y, Zhao X, Tang X, Chen Z (2016). Differences in dissolved organic matter between reclaimed water source and drinking water source. Science of the Total Environment, 551-552: 133–142

Huang H, Wu Q Y, Hu H Y, Mitch W A (2012). Dichloroacetonitrile and dichloroacetamide can form independently during chlorination and chloramination of drinking waters, model organic matters, and wastewater effluents. Environmental Science & Technology, 46(19): 10624–10631

Hureiki L, Croué J P, Legube B (1994). Chlorination studies of free and combined amino acids. Water Research, 28(12): 2521–2531

IARC (1995). Monographs on the evaluation of carcinogenic risks to humans: Dry cleaning, some chlorinated solvents and other industrial chemicals. Lyon: International Agency for Research on Cancer

IARC (2004). Monographs on the evaluation of carcinogenic risks to humans: Some drinking water disinfectants and contaminants, including Arsenic. Lyon: International Agency for Research on Cancer

Jeong C H, Postigo C, Richardson S D, Simmons J E, Kimura S Y, Mariñas B J, Barcelo D, Liang P, Wagner E D, Plewa M J (2015). Occurrence and comparative toxicity of haloacetaldehyde disinfection byproducts in drinking water. Environmental Science & Technology, 49(23): 13749–13759

Jeong C H, Wagner E D, Siebert V R, Anduri S, Richardson S D, Daiber E J, McKague A B, Kogevinas M, Villanueva C M, Goslan E H, Luo W, Isabelle L M, Pankow J F, Grazuleviciene R, Cordier S, Edwards S C, Righi E, Nieuwenhuijsen M J, Plewa M J (2012). Occurrence and toxicity of disinfection byproducts in European drinking waters in relation with the HIWATE epidemiology study. Environmental Science & Technology, 46(21): 12120–12128

Jia A, Wu C, Duan Y (2016). Precursors and factors affecting formation of haloacetonitriles and chloropicrin during chlor(am)ination of nitrogenous organic compounds in drinking water. Journal of Hazardous Materials, 308: 411–418

Lee J, Kim E S, Roh B S, Eom S W, Zoh K D (2013). Occurrence of disinfection by-products in tap water distribution systems and their associated health risk. Environmental Monitoring and Assessment, 185(9): 7675–7691

Lee W, Westerhoff P, Croué J P (2007). Dissolved organic nitrogen as a precursor for chloroform, dichloroacetonitrile, N-nitrosodimethylamine, and trichloronitromethane. Environmental Science & Technology, 41(15): 5485–5490

Li Y N, He K, Wang T, Zhao B, Yue H X Z, Lin Y C (2018). Current situation and research progress of disinfection by-products and their precursors in Japan. Environmental Sciences (Ruse), 37(8): 1820–1830

Li Z, Liu X, Huang Z, Hu S, Wang J, Qian Z, Feng J, Xian Q, Gong T (2019). Occurrence and ecological risk assessment of disinfection byproducts from chlorination of wastewater effluents in East China. Water Research, 157: 247–257

Lin T, Zhou D, Dong J, Jiang F, Chen W (2016). Acute toxicity of dichloroacetonitrile (DCAN), a typical nitrogenous disinfection by-product (N-DBP), on zebrafish (Danio rerio). Ecotoxicology and Environmental Safety, 133: 97–104

Liu J, Zhang X (2014). Comparative toxicity of new halophenolic DBPs in chlorinated saline wastewater effluents against a marine alga: Halophenolic DBPs are generally more toxic than haloaliphatic ones. Water Research, 65: 64–72

Lu J, Zhang T, Ma J, Chen Z (2009). Evaluation of disinfection by-products formation during chlorination and chloramination of dissolved natural organic matter fractions isolated from a filtered river water. Journal of Hazardous Materials, 162(1): 140–145

Mao Y, Guo D, Yao W, Wang X, Yang H, Xie Y F, Komarneni S, Yu G, Wang Y (2018). Effects of conventional ozonation and electro-peroxone pretreatment of surface water on disinfection by-product formation during subsequent chlorination. Water Research, 130: 322–332

Muellner M G, Wagner E D, McCalla K, Richardson S D, Woo Y T, Plewa M J (2007). Haloacetonitriles vs. regulated haloacetic acids: Are nitrogen-containing DBPs more toxic? Environmental Science & Technology, 41(2): 645–651

Park K Y, Choi S Y, Lee S H, Kweon J H, Song J H (2016). Comparison of formation of disinfection by-products by chlorination and ozonation of wastewater effluents and their toxicity to Daphnia magna. Environmental Pollution, 215(215): 314–321

Plewa M J, Muellner M G, Richardson S D, Fasano F, Buettner K M, Woo Y T, McKague A B, Wagner E D (2007). Occurrence, synthesis, and mammalian cell cytotoxicity and genotoxicity of haloacetamides: An emerging class of nitrogenous drinking water disinfection byproducts. Environmental Science & Technology, 42(3): 955–961

Reckhow D A, Platt T L, MacNeill A L, McClellan J N (2001). Formation and degradation of dichloroacetonitrile in drinking waters. Journal of Water Supply: Research and Technology-Aqua, 50(1): 1–13

Richardson S D, Plewa M J, Wagner E D, Schoeny R, Demarini D M (2007). Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. Mutation Research, 636(1-3): 178–242

Scully F E, Howell G D, Kravitz R, Jewell J T, Hahn V, Speed M (1988). Proteins in natural waters and their relation to the formation of chlorinated organics during water disinfection. Environmental Science & Technology, 22(5): 537–542

Sun X B, Sun L, Lu Y, Jiang Y F (2013). Factors affecting formation of disinfection by-products during chlorination of Cyclops. Journal of Water Supply: Research and Technology-Aqua, 62(3): 169–175

Trehy M L, Yost R A, Miles C J (1986). Chlorination byproducts of amino acids in natural waters. Environmental Science & Technology, 20(11): 1117–1122

Van Huy N, Murakami M, Sakai H, Oguma K, Kosaka K, Asami M, Takizawa S (2011). Occurrence and formation potential of N-nitrosodimethylamine in ground water and river water in Tokyo. Water Research, 45(11): 3369–3377

Wagner E D, Plewa M J (2009). Microplate-based comet assay. In: Dhawan A, Anderson D, eds. The Comet Assay in Toxicology. London: Royal Society of Chemistry, 79–97

Wagner E D, Plewa M J (2017). CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: An updated review. Journal of Environmental Sciences (China), 58: 64–76

Watson K, Shaw G, Leusch F D L, Knight N L (2012). Chlorine disinfection by-products in wastewater effluent: Bioassay-based assessment of toxicological impact. Water Research, 46(18): 6069–6083

Wu Q Y, Zhou Y T, Du Y, Li W X, Zhang X R, Hu H Y (2019). Underestimated risk from ozonation of wastewater containing bromide: Both organic byproducts and bromate contributed to the toxicity increase. Water Research, 162: 43– 52 https://doi.org/10.1016/j.watres. 2019.06.054.

Yang F, Yang Z G, Li H P, Jia F F, Yang Y (2018). Occurrence and factors affecting the formation of trihalomethanes, haloacetonitriles and halonitromethanes in outdoor swimming pools treated with trichloroisocyanuric acid. Environmental Science: Water Research & Technology, 2(4): 218–225

Yang X, Guo W, Lee W (2013). Formation of disinfection byproducts upon chlorine dioxide preoxidation followed by chlorination or chloramination of natural organic matter. Chemosphere, 91(11): 1477–1485

Yang X, Shen Q, Guo W, Peng J, Liang Y (2012). Precursors and nitrogen origins of trichloronitromethane and dichloroacetonitrile during chlorination/chloramination. Chemosphere, 88(1): 25–32

Yoon S, Tanaka H (2014). Formation of N-nitrosamines by chloramination or ozonation of amines listed in Pollutant Release and Transfer Registers (PRTRs). Chemosphere, 95: 88–95