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ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · SEPTEMBER 2012

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Dichloroacetonitrile and Dichloroacetamide Can Form Independently during Chlorination and Chloramination of Drinking Waters, Model Organic Matters, and Wastewater Effluents

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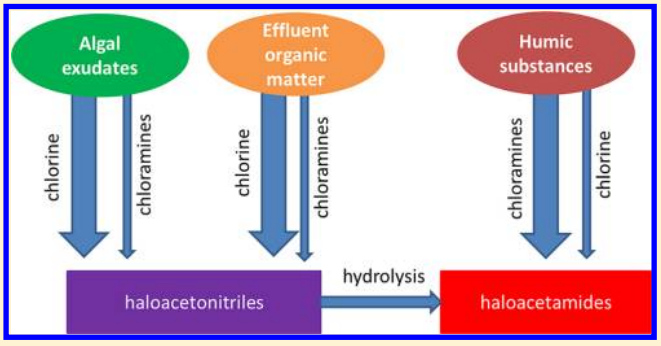
Supporting Information

ABSTRACT: The increasing usage of organic nitrogen-rich wastewater- or algal-impacted waters, and chloramines for secondary disinfection, raises concerns regarding the formation of haloacetonitriles, haloacetamides and other nitrogenous disinfection byproducts (N-DBPs). Previous research obtained contradictory results regarding the relative importance of chlorination or chloramination for promoting these by-products, but applied chlorine and chloramines at different doses and exposure periods. Additionally, mechanistic work, mostly using model precursors, suggested that haloacetonitrile and haloacetamide formation should be correlated because hydrolysis of haloacetonitriles forms haloacetamides. In this work, the formation of dichloroacetonitrile (DCAN) and dichloroacetamide (DCAcAm) were compared across a range of chlorine and chloramine exposures for drinking waters, wastewater effluents, algal extracellular polymeric substances (EPS), NOM isolates and model precursors. While chlorination favored formation of DCAN over DCAcAm, chloramination nearly always formed more DCAcAm than DCAN, suggesting the existence of haloacetamide formation pathways that are independent of the hydrolysis of haloacetonitriles. Experiments with asparagine as a model precursor also suggested DCAcAm formation without a DCAN intermediate. Application of ^{15}N-labeled monochloramine indicated initial rapid formation of both DCAN and DCAcAm by pathways where the nitrogen originated from organic nitrogen precursors. However, slower formation occurred by pathways involving chloramine incorporation into organic precursors. While wastewater effluents and algal EPS tended to be more potent precursors for DCAN during chlorination, humic materials were more potent precursors for DCAcAm during chlorination and for both DCAN and DCAcAm during chloramination. These results suggest that, rather than considering haloacetamides as haloacetonitrile hydrolysis products, they should be treated as a separate N-DBP class associated with chloramination. While use of impaired waters may promote DCAN formation during chlorination, use of chloramines may promote haloacetamide formation for a wider array of waters.



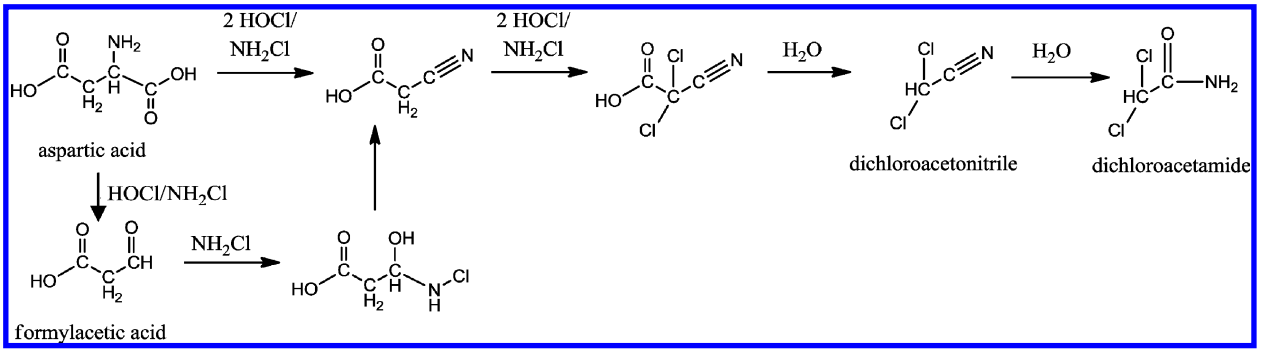
INTRODUCTION

To meet growing water demands, utilities are exploiting source waters influenced by algal blooms or municipal wastewater effluents, and, in certain cases, implementing indirect potable reuse of municipal wastewater effluents.[1,2] These types of impaired waters are characterized by high dissolved organic nitrogen (DON) content,[3] which can serve as a source for nitrogenous disinfection byproduct (N-DBP) precursors. N-DBPs, including haloacetonitriles and haloacetamides, have raised concerns due to their high toxicity compared with the regulated trihalomethanes (THMs) and haloacetic acids (HAAs). For example, dichloroacetonitrile (DCAN) and dichloroacetamide (DCAcAm), the most frequently detected members of each class,[4] were approximately 2 orders of magnitude more cytotoxic than dichloroacetic acid.[5,6]

Formation pathways for halonitriles and haloamides have recently been reviewed.[7] Two pathways may be relevant to the formation of haloacetonitriles and haloacetamides. First, via the "decarboxylation pathway" (Scheme 1), application of free chlorine or chloramines to free amino acids (e.g., aspartic acid) results in rapid dichlorination of the α-terminal amine group.[8]

Received: June 26, 2012
Revised: August 20, 2012
Accepted: September 5, 2012
Published: September 5, 2012

Scheme 1



Concerted decarboxylation (i.e., coupled loss of CO_2 and hydrochloric acid) forms a nitrile. With aspartic acid, the nitrile and carboxylic acid groups in the intermediate render the methylene carbon acidic, promoting its chlorination. Hydrolysis releases carbonic acid and DCAN. Hydrolysis of DCAN forms DCAcAm and then dichloroacetic acid.[9] Via the "aldehyde pathway",[10,11] attack of monochloramine's electron lone pair on an aldehyde (e.g., formylacetic acid) incorporates the monochloramine nitrogen into the aldehyde (Scheme 1). Elimination of water and hydrochloric acid forms a nitrile. Subsequent steps are analogous to those of the decarboxylation pathway.

The relative importance of these formation pathways may have practical significance. Utilities are increasingly applying chloramines for secondary disinfection of drinking waters to reduce consumer exposure to regulated THMs and HAAs.[12] Whether the increasing use of chloramination increases the formation of haloacetonitriles and haloacetamides is an important concern. While both free chlorine and chloramines can participate in the decarboxylation pathway, the aldehyde pathway applies predominantly to chloramines. Additionally, if the decarboxylation pathway dominates, its requirement for an organic nitrogen precursor suggests that the increasing reliance of utilities on impaired waters may enhance exposure to haloacetonitriles and haloacetamides.

Previous studies comparing DCAN formation during the chlorination and chloramination of model compounds and natural organic matter (NOM) isolates obtained contradictory results. Unfortunately, chlorine and chloramine exposures generally were different, hampering efforts to compare these oxidants. Dotson et al.[13] found that DCAN formation was generally higher during application of chlorine than chloramines, although the chloramination protocol involved a lower initial dose and longer contact time than did the chlorination protocol. Studies involving the application of chlorine and chloramines at the same initial concentrations and contact times to natural waters and treated waters also observed higher DCAN formation during chlorination,[14,15] although the chlorine residual decays faster than chloramines. However, another study involving dissolved organic matter (DOM) isolates observed five times higher DCAN concentrations for chloramination than chlorination, although the chloramination protocol involved a higher initial dose and longer contact time.[16] Organic nitrogen-rich isolates produced the most DCAN during chlorination, suggesting the importance of the decarboxylation pathway.[16] Using ^{15}N-NH_2Cl, Yang et al.[17,18] found that the DCAN nitrogen was provided predominantly by inorganic chloramines for Suwannee River NOM, glutamic acid, tryptophan, tyrosine, asparagine, and methylpyrrole, but by the organic precursor for cytosine and pyrrole, implying different contributions of the aldehyde and decarboxylation pathways for different compounds.

Less work has focused on haloacetamides, as they were first reported as DBPs in a 2000−2002 drinking water survey in the U.S.[19,20] Assuming the importance of the decarboxylation pathway, previous research has focused mostly on chlorination.[21,22] Furthermore, most research has assumed that DCAcAm arises predominantly from hydrolysis of DCAN (Scheme 1).[4,9,19,21,22] Using aspartic acid, a potent free amino acid precursor,[21] Chu et al.[22] found that DCAcAm formation increased with pH while DCAN formation declined. Formation of DCAcAm at pH 7 decreased with increasing chlorine doses, while formation of DCAN and dichloroacetic acid increased. However, the maximum yield of DCAcAm from aspartic acid was 0.09%,[22] raising questions about the importance of free aspartic acid as a precursor in natural waters. A study employing tyrosine as a model precursor found that a combination of chlorination and chloramination produced the most DCAcAm, followed by chlorination alone and chloramination alone.[23] Chlorination of an algal-impacted water indicated that formation of DCAN exceeded DCAcAm by an order of magnitude.[21] Furthermore, chlorination produced more DCAcAm than chloramination, although the different oxidant exposures employed render comparison of these oxidants difficult. The hydrophilic acid fraction from this water, believed to be rich in proteinaceous soluble microbial products, produced the most DCAcAm during chlorination.[21]

The first objective of this study was to compare the efficacy of chlorination and chloramination for the formation of DCAN and DCAcAm using a common exposure basis. As utilities generally strive to maintain a residual rather than a common initial dose, comparison on an exposure basis may be more relevant to treatment conditions. A second objective was to evaluate the relative importance of the decarboxylation and aldehyde pathways for DCAN and DCAcAm formation during chloramination by application of ^{15}N-labeled chloramines. If the decarboxylation pathway is more important, then the increasing use of organic nitrogen-rich impaired waters would be expected to exacerbate exposure to DCAN and DCAcAm. As byproduct yields from model precursors have been minor in previous studies (e.g., 0.09% DCAcAm yield from aspartic acid[22]), currently identified model precursors may not reflect source water precursors. Accordingly, both objectives were pursued with authentic drinking waters. To assess whether exploitation of impaired waters will promote DCAN and DCAcAm production, formation was compared from NOM isolates as models for pristine water precursors, algal extracellular polymeric substances (EPS) as a model for algal-derived

dx.doi.org/10.1021/es3025808 | Environ. Sci. Technol. 2012, 46, 10624−10631

Table 1. Basic Water Quality Results[a]

sample	DOC (mg/L)	DON (mg/L)	DOC/DON	NH_3-N (mg/L)	NO_2^--N (mg/L)	NO_3^--N (mg/L)	Br^- (mg/L)	UV_{254} (cm^{-1})	SUVA (L mg^{-1} cm^{-1})	pH
utility A	2.5	0.06	42	0.03	ND	ND	0.04	0.0545	2.2	7.6
utility B	2.3	0.40	6	0.05	0.02	2.80	ND	NM	NA	7.8
utility C	3.7	0.15	25	0.03	0.01	1.20	ND	0.0727	2.0	8.4
WWTP A	8.8	1.51	6	1.10	0.35	1.39	ND	0.1557	1.8	7.6
WWTP B	4.7	0.30	16	0.04	ND	ND	0.83	0.0958	2.0	7.6
Leonardite humic acid	5.0	0.13	39	ND	ND	ND	ND	0.4145	8.3	8.9
Chlorella algal EPS	3.0	0.49	6	0.01	0.19	8.82	ND	0.0201	0.7	8.3
Ettlia algal EPS	2.5	0.53	5	0.01	0.01	3.69	ND	0.0104	0.4	7.1

[a]ND = not detected. NM = not measured. NA = not applicable.

precursors, and wastewater effluents as a model for wastewater impacts. Together with model compound studies, these experiments were also employed to re-evaluate the assumption that DCAcAm forms predominantly by hydrolysis of DCAN.

MATERIALS AND METHODS

Materials. Accustandard DCAN, Alfa Aesar DCAcAm (>98%), boc-asparagine (>98%), and 1,2-dibromopropane (98%), J.T. Baker HPLC grade ethyl acetate and sodium sulfate (99%), and Sigma-Aldrich aspartic acid (>98%) and boc-aspartic acid (99%), Acros asparagine (99%) and ascorbic acid (99%), and Fisher sodium hypochlorite were used without further purification. ^{15}N-labeled monochloramine stock solutions were prepared daily by adding sodium hypochlorite to ^{15}N-labeled ammonium chloride (99%, Cambridge Isotope Laboratories) solutions at a ratio of 0.8 mol/mol. Suwannee River NOM, and Leonardite, Elliott Soil and Pahokee Peat humic acids were obtained from the International Humic Substances Society.

Water Samples. Cultures of the green algae, *Chlorella vulgaris* (UTEX #259) and *Ettlia oleoabundans* (UTEX #1185), were grown under fluorescent bulb illumination in modified Bold 3N medium and Bold 3N medium, respectively, which were nitrate-based culture media (Table SI-1 in the Supporting Information), for about 1 week; DCAN and DCAcAm formation in chlorinated or chloraminated blanks of the culturing media indicated that the culturing media contributed <0.4 nM DCAN and <0.3 nM DCAcAm. The cell suspensions were filtered with prebaked 0.7-μm nominal pore size borosilicate glass fiber filters (Environmental Express) and the filtrates were collected as algal EPS stock solutions. Drinking water samples were collected from the influent of drinking water utilities upstream of disinfectant application in EPA Region 9 (Utility A) and Region 3 (Utility B) and the recarbonation effluent of a lime clarifier at a utility in EPA Region 5 (Utility C). Wastewater effluent samples were collected from the plant effluents of two wastewater treatment plants (WWTP) in EPA Region 1. Both plants practice nitrification and separate-stage denitrification, using methanol as a carbon source, and neither plant applied disinfectants at the time of sample collection. The samples were filtered and stored at 4 °C. The samples were analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrate, nitrite, ammonia, bromide, UV absorbance and pH (Table 1). DOC and TDN were measured by a TOC analyzer (Shimadzu TOC-V_{CPH}). Ammonia was measured by the salicylate-hypochlorite method, and nitrite by the *N*-(1-naphthyl)ethylenediamine dihydrochloride colorimetric method.[24] Nitrate and bromide were measured by ion chromatography (Dionex ICS-1000). DON was calculated by subtracting nitrate, nitrite, and ammonia nitrogen from TDN.

Chlorination and chloramination experiments were conducted at room temperature under headspace-free conditions in 40 mL amber glass bottles shielded from light. Free chlorine or preformed ^{15}N-labeled monochloramine was dosed at 10 mg/L as Cl_2 to water or wastewater samples (except for WWTP A effluent chlorination, which was dosed at 20 mg/L), algal EOM solutions (diluted to 3 mg/L DOC for *Chlorella* and 2.5 mg/L DOC for *Ettlia*), or model NOM (5 mg/L DOC) or compound solutions (0.1 mM), all buffered at pH 6.9 with 10 mM phosphate buffer. The decay of the total chlorine residual was periodically monitored in additional aliquots by the DPD colorimetric method.[24] Oxidant exposures were calculated based upon integrating concentration versus time curves capturing oxidant decay. Although the total chlorine residual analysis would capture organic chloramines, these were unlikely to be important as the free chlorine dose applied (140 μM) greatly exceeded the 4−35 μM DON generally observed (Table 1). Reactions were halted by quenching residual chlorine with ascorbic acid at 1.5 times the initial chlorine or chloramine dose on a molar basis. To prevent hydrolytic loss of byproducts upon storage, samples were extracted immediately after quenching as described below.

Analytical Methods. DCAN and DCAcAm were extracted by liquid−liquid extraction, concentrated by nitrogen blow down and then analyzed by gas chromatography/mass spectrometry (GC/MS, Varian Saturn 2200). Although brominated analogues were not measured, they were unlikely to be important in most cases, as bromide was rarely detected (Table 1). Sample aliquots (40 mL) were transferred to 60 mL glass vials, supplemented with 6 g of anhydrous sodium sulfate, and extracted twice with 5 mL of ethyl acetate containing 1,2-dibromopropane as an internal standard. The extracts were combined, blown down to 0.4 mL with nitrogen gas, and analyzed by GC/MS. Injections (2 μL) were splitless at an injection port temperature of 220 °C onto a DB-1701 30 m × 0.25 mm × 0.25 μm column. The column oven was held at 40 °C for 15 min, ramping to 100 at 5 °C/min, ramping to 200 at 20 °C/min and holding for 5 min, and ramping to 230 at 40 °C/min and holding for 2 min. DCAN was analyzed by electron impact using m/z 74 and 75 as quantification ions for unlabeled DCAN (^{14}N-DCAN) and ^{15}N-labeled DCAN (^{15}N-DCAN), respectively.[17] DCAcAm was analyzed by methanol chemical ionization using m/z 128 and 129 as quantification ions for unlabeled DCAcAm (^{14}N-DCAcAm) and ^{15}N-labeled DCAcAm (^{15}N-DCAcAm), respectively (Figure SI-1 of the SI);

no excitation amplitude was applied to minimize fragmentation of these parent ions.

Since ^{15}N-labeled DBPs were not commercially available, the concentrations of ^{15}N-labeled DBPs were quantified indirectly from the concentration of unlabeled DBPs. For example, chloramination was performed under the same conditions by dosing unlabeled and ^{15}N-labeled monochloramine at 25 mg/L as Cl_2 to Suwannee River NOM, and Leonardite, Elliott Soil, and Pahokee Peat humic acids (5 mg/L DOC) for 72 h, respectively. The concentration of ^{15}N-DCAN was determined by subtracting the ^{14}N-DCAN concentration produced after dosing ^{15}N-labeled monochloramine from the ^{14}N-DCAN concentration produced after dosing unlabeled monochloramine. The resulting concentration ratio of ^{15}N-DCAN and ^{14}N-DCAN was identical to the ratio of the MS peak areas of m/z 75 and 74 (Figure SI-2 of the SI), indicating that the MS responses were similar for ^{14}N-DCAN and ^{15}N-DCAN. Thus the concentration of ^{15}N-DCAN could be determined using the ^{14}N-DCAN standard curve. Similar comments pertain to ^{15}N-labeled DCAcAm.

RESULTS AND DISCUSSION

Comparison of DCAN and DCAcAm Formation during Chlorination and Chloramination. Initial experiments compared DCAN and DCAcAm formation from application of chlorine or chloramine formation potential tests used by previous researchers[13,21,25] to 5 mg_C/L of several NOM models. Briefly, the chlorination formation potential test involved application for 24 h of a 25 mg/L as Cl_2 chlorine dose determined as follows: Cl_2 (mg/L) = 3 × DOC (mg_C/L) + 8 × NH_3–N (mg_N/L) + 10 (mg/L). The chloramine formation potential test involved application for 72 h of a 15 mg/L as Cl_2 preformed monochloramine dose determined as follows: NH_2Cl (mg/L) = 3 × DOC (mg_C/L). For these formation potential assays, the relative importance of chlorination or chloramination for DCAN formation varied among the NOM models, being higher during chloramination for Suwannee River NOM and Leonardite humic acid, but higher during chlorination for Elliott Soil humic acid and Pahokee Peat humic acid (Figure SI-3 of the SI). Formation of DCAcAm was higher during chloramination for all four NOM isolates.

Because the formation potential tests involve application of different initial disinfectant concentrations for different time periods, it is difficult to compare the ability of chlorine and chloramines to promote DCAN and DCAcAm formation. Instead, chlorination and chloramination were compared by evaluating DCAN and DCAcAm formation as a function of oxidant exposures. Use of the exposure metric accounts for differences in oxidant decay rates, and enables comparison to exposures relevant to drinking water disinfection. Figure 1 presents DCAN and DCAcAm formation versus exposure from chlorination and chloramination of a drinking water influent, a model NOM, an algal EPS, and a municipal wastewater treatment plant effluent. Figure SI-4 of the SI presents similar data for two other drinking waters, an additional algal EPS and an additional municipal wastewater treatment plant effluent. For the three drinking water samples, DCAN formation during chlorination ranged from 12.9 nM (1.4 μg/L) at 1000 mg·min/L to 50.5 nM (5.5 μg/L) at 30,000 mg·min/L; for an average distribution system residual of 1.5 mg/L as Cl_2, these exposures correspond to 0.5 and 14 d residence times, respectively.

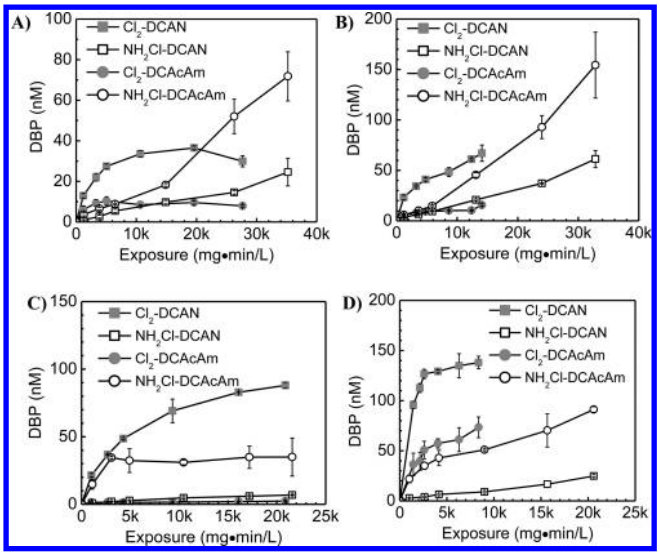


Figure 1. DCAN and DCAcAm formation versus exposure during chlorination and chloramination at pH 6.9 with 10 mM phosphate buffer of (A) drinking water Utility A influent, (B) 5 mg/L as DOC Leonardite humic acid model NOM, (C) 3 mg/L as DOC *Chlorella* algal EPS, and (D) municipal wastewater treatment plant A effluent. Error bars represent the standard deviation of replicate measurements (n = 2).

However, over similar exposures, DCAN formation during chloramination of the drinking waters was lower, ranging from 1.1 nM (0.1 μg/L) to 24.6 nM (2.7 μg/L). For a similar exposure range, DCAcAm formation during chlorination of drinking waters ranged from 5.6 nM (0.7 μg/L) to 18.3 nM (2.3 μg/L), but from 3.7 nM (0.5 μg/L) to 71.8 nM (9.1 μg/L) for chloramination.

For all samples, DCAN formation was higher during chlorination than during chloramination at exposures <10 000 mg·min/L. Similar results were obtained at higher exposures, although formation during chloramination approached that observed during chlorination for drinking water Utility A at exposures near 30 000 mg·min/L. For most waters, DCAcAm formation during chloramination exceeded that during chlorination for exposures >8000 mg·min/L, but was comparable at lower exposures. However, DCAcAm formation during chloramination exceeded that during chlorination across the range of exposures for the two algal EPS and the municipal wastewater treatment plant B effluent, while the opposite was true for the municipal wastewater treatment plant A effluent.

Previous research suggested that DCAcAm formed from hydrolysis of DCAN.[9,21,22] While the fact that DCAN formation exceeded DCAcAm formation throughout chlorine exposures in our experiments concurs with this suggestion, it does not prove the suggestion; additional results described below suggest that DCAcAm formation pathways independent of DCAN hydrolysis exist during chlorination. However, with the possible exception of chloramine exposures <6000 mg·min/L for the Leonardite humic acid, DCAcAm formation exceeded DCAN formation across the range of chloramine exposures for all waters, including for the lowest exposures corresponding to 2 h. In separate experiments, application of 10 mg/L as Cl_2 of chloramines to either DCAN or DCAcAm at pH 6.9 exhibited negligible degradation of either compound over 24 h (Figure SI-5 of the SI). Preliminary modeling using published rate constants presented in the SI demonstrates that it is not

possible to explain DCAcAm formation solely by hydrolysis of DCAN. Accordingly, these results suggest that a pathway to DCAcAm formation exists that is independent of DCAN hydrolysis.

Note that DCAN and DCAcAm formation during chlorination and chloramination tended to be highest for the municipal wastewater treatment plant A effluent (Figure 1), but this sample featured the highest concentrations of DOC and DON (Table 1). To compare the potencies of the organic matter precursors in the different sample types for forming DCAN and DCAcAm, their concentrations were normalized on a DOC basis (Figure 2 and Figure SI-9 of the SI). During

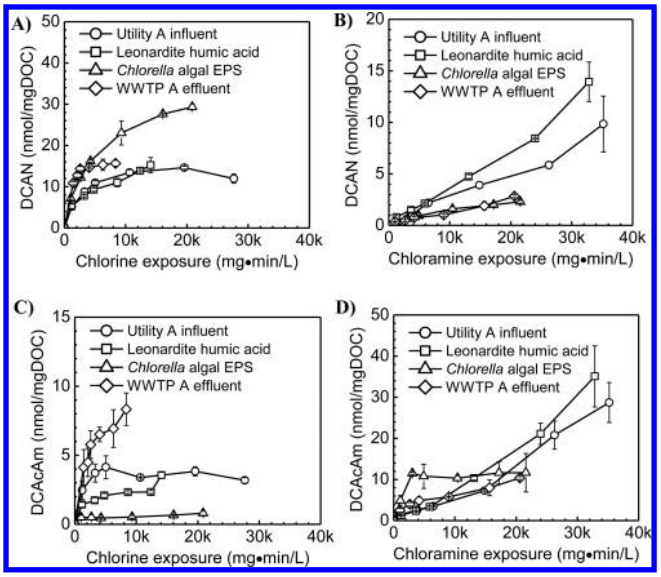


Figure 2. Comparison of drinking water Utility A influent, 5 mg/L as DOC Leonardite humic acid model NOM, 3 mg/L as DOC *Chlorella* algal EPS, and municipal wastewater treatment plant A effluent as precursors for (A) DCAN formation from chlorination, (B) DCAN formation from chloramination, (C) DCAcAm formation from chlorination, and (D) DCAcAm formation from chloramination. Byproduct concentrations are normalized by DOC. Error bars represent the standard deviation of replicate experiments (n = 2).

chlorination, the DOC-normalized DCAN concentration tended to be higher for the municipal wastewater treatment plant effluents and the *Chlorella* algal EPS, than for drinking waters and NOM models, while the opposite was observed for DCAcAm excluding the municipal wastewater treatment plant A effluent. The different potencies for DCAN and DCAcAm formation exhibited by these organic precursor varieties suggest that DCAcAm formation pathways during chlorination exist that are independent of DCAN hydrolysis. However, generalizations are difficult, because DOC-normalized DCAN formation was lowest for the *Ettlia* algal EPS and DCAcAm formation was high for municipal wastewater treatment plant A effluent.

During chloramination, DOC-normalized formation of DCAN and DCAcAm tended to be higher for drinking waters and NOM models than from algal EPS or wastewater treatment plant effluents. Due to the low organic nitrogen content of humic materials (Table 1), these results suggest that humic materials can serve as significant precursors for pathways where chloramines serve as the source of nitrogen in the byproduct, a suggestion further evaluated with ^{15}N-labeled chloramination experiments below. Because byproduct formation differs despite the similar specific UV absorbances at 254 nm (SUVA) for the drinking water Utility A influent and for the wastewater treatment plant A effluent, precursors can not be attributed to aromatic structures with this data.

Pathways for DCAN and DCAcAm Formation during Chloramination. During chloramination, previous research suggests that DCAN and DCAcAm can form via the decarboxylation pathway, where the nitrogen originates from the organic nitrogen precursor, or by the aldehyde pathway, where the nitrogen originates from incorporation of the inorganic chloramines (Scheme 1).[7] ^{15}N-labeled chloramines were applied to distinguish these pathways. During application of the chloramine formation potential assay to NOM models, the percentage of ^{15}N in DCAN and DCAcAm was always >70% (Figure SI-10 of the SI), consistent with previous research for DCAN.[17] Figure 3 displays the percentages of

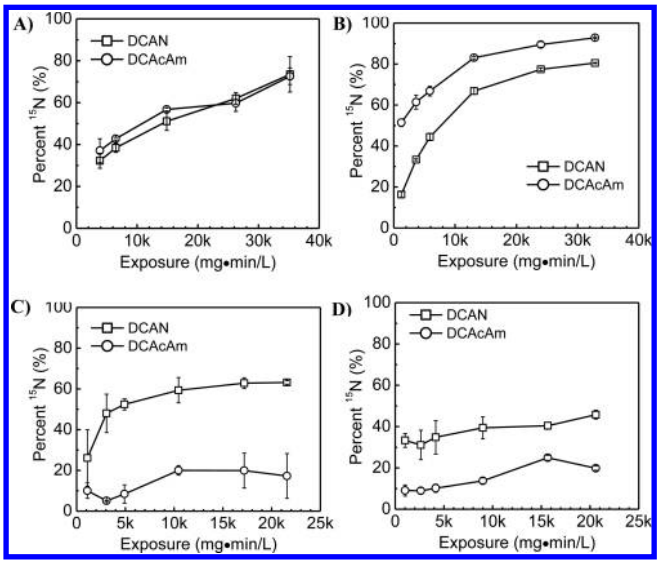


Figure 3. ^{15}N percentage for DCAN and DCAcAm vs chloramine exposure at pH 6.9 for (A) drinking water Utility A influent, (B) 5 mg/L as DOC Leonardite humic acid model NOM, (C) 3 mg/L as DOC *Chlorella* algal EPS, and (D) municipal wastewater treatment plant A effluent. Error bars represent the standard deviation of replicate experiments (n = 2).

DCAN and DCAcAm that were ^{15}N-labeled versus chloramine exposure for drinking water Utility A influent, Leonardite humic acid, *Chlorella* algal EPS, and municipal wastewater treatment plant A effluent. Similar data for samples from drinking water Utilities B and C, *Ettlia* algal EPS and municipal wastewater treatment plant B are provided in Figure SI-11 of the SI. With the exception of wastewater treatment plant A effluent, for authentic waters containing background ^{14}N-labeled ammonia, any ^{14}N-NH_2Cl formed by chlorine transfer would constitute a maximum of only 5−25% of the total chloramines.

With the possible exception of DCAcAm formation from Leonardite humic acid and DCAN formation from *Chlorella* algal EPS, the ^{15}N percentages for DCAN and DCAcAm were <50% for all waters at chloramine exposures <4,000 mg•min/L, suggesting that the decarboxylation pathway dominates at low chloramine exposures. This exposure level is comparable to 2 days of transit time in distribution systems featuring an average chloramine residual of 1.5 mg/L as Cl_2. However, except for municipal wastewater treatment plant effluent A, the ^{15}N

dx.doi.org/10.1021/es3025808 | *Environ. Sci. Technol.* 2012, 46, 10624−10631

percentage for DCAN and DCAcAm tended to increase with chloramine exposure. These results suggest that formation during chloramination is more rapid by the decarboxylation pathway, but indicate that the aldehyde pathway involving incorporation of the chloramine nitrogen gains in importance with chloramine exposure. At the highest chloramine exposures, the ^{15}N percentages were ≤50% for the algal EPS and wastewater effluents, except for DCAN in the *Chlorella* algal EPS and municipal wastewater treatment plant B effluent. Except for DCAcAm in drinking water Utility C recarbonation effluent, the ^{15}N percentage was ≥50% for Leonardite humic acid and the drinking waters. Generally, the algal EPS and wastewater effluent samples featured higher DON concentrations (Table 1), which would suppress ^{15}N percentages by providing precursors for the decarboxylation pathway.

DCAN and DCAcAm Formation from Model Precursors. Contrary to previous suggestions that haloacetamides arise predominantly from hydrolysis of haloacetonitriles, results from our experiments suggested that pathways to haloacetamide formation exist which are independent of haloacetonitrile hydrolysis. First, during chlorination, wastewater and algal EPS samples served as potent precursors of DCAN, but not DCAcAm (Figure 2). If DCAcAm arose solely by DCAN hydrolysis, then these samples should have similarly promoted DCAcAm formation. Second, DCAcAm concentrations generally exceeded those of DCAN throughout chloramine exposures (Figure 1), and modeling indicated that DCAN hydrolysis could not explain the observed excess of DCAcAm at low chloramine exposures. Third, for several samples, the ^{15}N percentages differed for DCAN and DCAcAm (Figure 3). If DCAcAm arose solely by hydrolysis of DCAN, then the ^{15}N percentages should be similar for the two compounds. For example, the ^{15}N percentage for DCAcAm was always larger than for DCAN during chloramination of Leonardite humic acids, indicating a pathway for DCAcAm formation by chloramine incorporation into the humic acid without a DCAN intermediate. Similarly, the ^{15}N percentage for DCAcAm was always lower than for DCAN for municipal wastewater effluent A, suggesting that chloramines may reaction with organic nitrogen precursors to form DCAcAm directly, rather than through DCAN hydrolysis.

Lastly, we evaluated model precursors to provide an example for the direct formation of DCAcAm without hydrolysis of a DCAN intermediate. The specific precursors responsible for DCAN and DCAcAm formation in water samples remain uncharacterized. However, previous research has evaluated amino acids as model precursors for DCAN and DCAcAm formation.[8,17,18,22,23] We applied 10 mg/L as Cl_2 (140 μM) free chlorine or monochloramine to 100 μM of two free amino acids, aspartic acid (Asp) and asparagine (Asn), as model precursors. Since free amino acids constitute only ~5% of total amino acids,[26] boc-Asp and boc-Asn were evaluated as N-protected amino acids (i.e., protection with a tert-butoxycarbonyl group) mimicking peptide bonds (Figure 4).

For Asp, boc-Asn and boc-Asp, DCAN was the predominant product of chlorination and DCAcAm was the dominant product during chloramination (Figure 5), consistent with the results from authentic waters (Figure 1). For these compounds, the maximum yield for DCAN was 2.2% from chlorination of aspartic acid. However, for Asn, yields of DCAcAm were 14.8% and 17.6% from chlorination and chloramination, respectively, and DCAN formation was negligible, a trend observed across a range of chlorine or chloramine exposures (Figure 6); similar

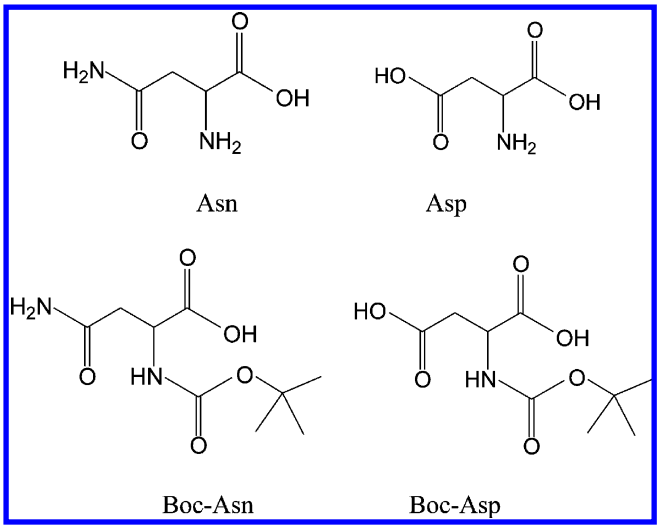


Figure 4. Structures of the model compounds.

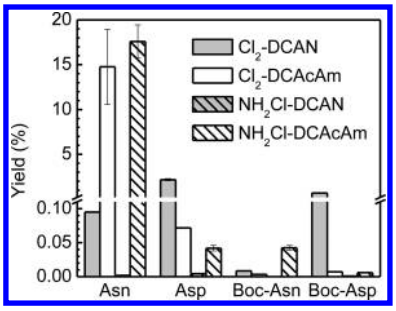


Figure 5. Molar yields of DCAN and DCAcAm compared to the organic precursor after 2 h chlorination or chloramination (10 mg/L as Cl_2) of model amino acids (0.1 mM) at pH 6.9 (10 mM phosphate buffer). Error bars represent the standard deviation of replicate experiments (n = 2).

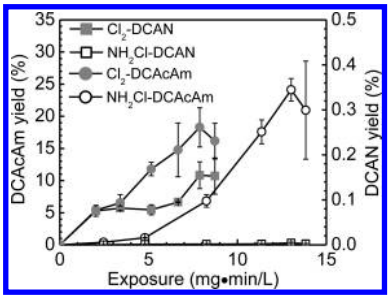
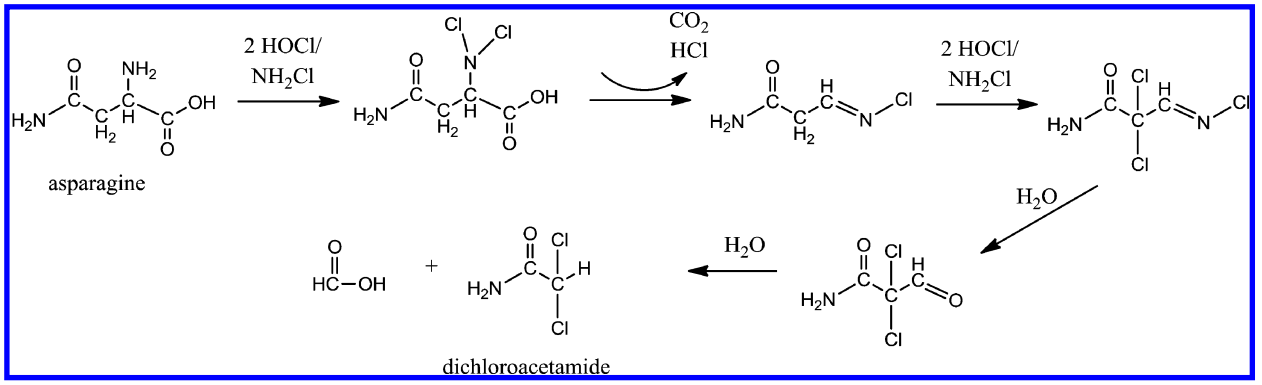


Figure 6. DCAN and DCAcAm formation from 0.1 mM asparagine vs oxidant exposure during chlorination and chloramination at 10 mg/L as Cl_2 and pH 6.9. Error bars represent the standard deviation of replicate experiments (n = 2).

results were obtained for application of chlorine or chloramines to Asn at molar ratios ranging from 1 to 4 (Figure SI-12 of the SI). A previous study employing a 30:1 oxidant to amino acid molar ratio found low levels of DCAN formation (<0.5% yield) from chlorination or chloramination of aspartic acid and asparagine.[18] Our results suggest that DCAcAm can form from chlorination and chloramination of Asn without a DCAN intermediate. During chloramination, the ^{15}N percentage was always <3%, suggesting a key role for the amide nitrogen in the Asn side chain for DCAcAm formation. Scheme 2 presents a suggested DCAcAm formation pathway from chlorination or chloramination of Asn. During chloramination, an intermediate

Scheme 2



consistent with the proposed monochloroimine (H_2N-CO–CH_2–CH=NCl) was detected using LC/MS (Figure SI-13 of the SI). Although DCAcAm yields from Asn were significant, yields from boc-Asn, as a model for Asn bound within peptides, were <0.05%. Unfortunately, a recent study evaluating amino acid concentrations in drinking water supplies did not quantify Asn,[26] and it is unclear whether concentrations of free Asn in drinking waters are sufficient to account for DCAcAm formation.

Environmental Relevance. Our results indicate that haloacetonitriles and haloacetamides should be considered as two separate N-DBP families, because, although hydrolysis of haloacetonitriles forms haloacetamides, [9] there are pathways to haloacetamide formation that are independent of haloacetonitriles. As noted previously,[7] haloacetonitriles were associated with chlorination. However, haloacetamides join nitrosamines,[27] cyanogen halides,[28] and iodinated DBPs[29,30] as byproduct families associated with chloramination. While NDMA has been associated with wastewater-impacted source waters,[31] humic materials served as potent precursors for DCAcAm. Accordingly, haloacetamides may be expected to impact a wide array of chloraminating utilities.

■ ASSOCIATED CONTENT

Supporting Information

Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by the National Science Fund of China (Key Program No. 51138006), the Chinese National Science Fund for Distinguished Young Scholars (No. 50825801), and the Tsinghua University—China Scholarship Council Postgraduate Scholarship for overseas joint-training programs. Additional support was provided by the Water Research Foundation. We thank Mr. Bryan Yoon for performing the DOC and TDN analyses, and Dr. Berat Haznedaroglu for help with the algal cultures.

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