Carbon, Hydrogen, and Nitrogen Isotope Fractionation Trends in N-Nitrosodimethylamine Reflect the Formation Pathway during Chloramination of Tertiary Amines

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ABSTRACT: Assessing the precursors and reactions leading to the carcinogenic N-nitrosodimethylamine (NDMA) during drinking water disinfection is a major challenge. Here, we investigate whether changes of $\delta^{13}C/\delta^{13}C$, $\delta^2H/\delta^2H$, and $\delta^{15}N/\delta^{14}N$ ratios of NDMA give rise to isotope fractionation trends that can be used to infer NDMA formation pathways. We carried out compound-specific isotope analysis (CSIA) of NDMA during chloramination of four tertiary amines that produce NDMA at high yields, namely ranitidine, S-(dimethylaminomethyl)furfuryl alcohol, N,N-dimethylthiophene-2-methylamine, and N,N-dimethylbenzylamine. Carbon and hydrogen isotope ratios of NDMA function as fingerprints of the N(CH$_3$)$_2$ moiety and exhibit only minor isotope fractionation during the disinfection process. Nitrogen isotope ratios showed that NH$_2$Cl is the source of the N atom of the nitroso group. The large enrichment of $\delta^{15}N$ in NDMA was indicative of the isotope effects pertinent to bond-cleavage and bond-formation reactions during chloramination of the tertiary amines. Correlation of $\delta^{15}N$ versus $\delta^{13}C$ values of NDMA resulted in trend lines that were not affected by the type of tertiary amine and treatment conditions, suggesting that the observed C and N isotope fractionation in NDMA may be diagnostic for NDMA precursors and formation pathways during chloramination.

INTRODUCTION

N-Nitrosamines form as drinking water disinfection byproducts (DBPs) and are of public and regulatory concern due to their mutagenicity and potential carcinogenicity. N-Nitrosodimethylamine (NDMA) is a frequently detected DBP in finished drinking waters and often exceeds guidance values of 9–100 ng/L. NDMA is produced unintentionally with typically used disinfectants, that is, chlorine, chloramine, and ozone, through reactions with organic compounds in raw waters including natural organic matter (NOM). The different molecular structures of NDMA precursors have led to the conclusion that pathways of NDMA generation differ widely among the various disinfection procedures. Nevertheless, detailed knowledge of chemical reaction mechanisms remains scarce so that a systematic prediction and prevention of NDMA formation during water treatment is currently hampered.

One promising new tool with which NDMA can be related to its precursors and formation reactions is compound-specific isotope analysis (CSIA). Previous studies have demonstrated that changes in the natural stable isotope composition of DBPs, that is the so-called stable isotope fractionation, provide evidence for reactive precursor materials and DBP formation pathways. CSIA was applied, for example, to monitor changes of $\delta^{13}C/\delta^{13}C$ ratios in chloroform produced upon chlorination of lake water. Chloroform was enriched in $\delta^{12}C$ due to a preferential reaction of light (i.e., $^{12}C$-containing) isotopologues when produced from resorcinol-like moieties of natural organic matter (NOM). Conversely, chloroform was enriched in $\delta^{13}C$ when generated from chlorination of phenolic functional groups of NOM. These contrasting isotopic preferences reflect different chloroform formation pathways. Each of these exhibits different kinetic isotope effects (KIEs), which reflect which chemical bonds are broken and formed. Because KIEs are specific features of a reaction mechanism, the stable isotope fractionation
observed in the reactants and products can serve as proxy for the transformation pathway.24−28

We have recently introduced analytical procedures for $^{13}\text{C}/^{12}\text{C}$, $^2\text{H}/^1\text{H}$, and $^{15}\text{N}/^{14}\text{N}$ isotope ratio measurements of $N$-nitrosamines.29 However, this method has not been applied to study disinfection processes and it is currently unknown how C, H, and N isotope fractionation in NDMA can be indicative of its formation pathway(s). Elucidation of reaction mechanisms with CSIA typically focuses on the analysis of substrate disappearance, in which substrate isotope fractionation reveals the isotope effects of the first elementary reaction steps leading to irreversible bond cleavage(s).24−27 Gaining such information from product isotope fractionation is less common and CSIA of reaction products is applied mostly to study the isotope effects of well-defined reactions leading to known and measurable products (e.g., refs 30−34). None of the previous applications of CSIA reflect cases similar to the one of NDMA, which forms in a sequence of (partially) unknown reactions. Each of these reactions exhibits an isotope effect and thus contributes to the final isotopic composition of NDMA.11,35 However, the identification of reactive processes from the isotope fractionation in reaction products is a common approach in (bio)geosciences. In this discipline, the elucidation of events in the past is often made through isotopic analysis of frequently occurring molecules and minerals in natural samples such as hydrocarbons, sulfur species, oxides, and carbonates.36,37 The methodology is based on observations made in multiple isotope systems, that is from the comparison of isotope fractionation from different elements in a molecule (e.g., $^2\text{H}/^1\text{H}$ versus $^{13}\text{C}/^{12}\text{C}$ in methane and $^{18}\text{O}/^{16}\text{O}$ versus $^{34}\text{S}/^{32}\text{S}$ in sulfate) and different isotopes of the same elements (e.g., $^{18}\text{O}/^{16}\text{O}$ versus $^{17}\text{O}/^{16}\text{O}$ in $\text{O}_2$ and $^{34}\text{S}/^{32}\text{S}$ versus $^{33}\text{S}/^{32}\text{S}$ in sulfates).38−43 Regardless of a quantitative understanding of isotope effects of individual reactions, evidence for reactive processes can then be obtained from correlations of isotope fractionation, which, in most cases, result in indicative isotope fractionation trendlines.

The objective of this work was to evaluate whether multielement isotope fractionation analysis is applicable to track precursor moieties and formation pathways of NDMA through analysis of C, H, or N isotope ratios of the DBP. This work builds on two of our recent studies on the NDMA formation from the chloramination of tertiary amines29,35 and information on the reaction mechanism provided by others.17,44−46 Based on the evidence of radical intermediates and reactions of molecular oxygen in the NDMA formation pathway,35 we hypothesized that tertiary amines undergo a sequence of substitution, electron transfer, oxygenation, and radical coupling reactions shown in Scheme 1. Those reactions will be the source of isotope fractionation in NDMA. In our previous work on stable isotope analysis,29 we have used the chloramination reaction of ranitidine to validate our methodology, and we found that NDMA formation processes can be studied at low $\mu\text{M}$ concentrations if NDMA yields are high. Therefore, we focused our current investigation on chloramination of tertiary amines, which can give rise to yields of NDMA that exceed 60%.11,14,25,35,47 We conducted chloramination experiments with four tertiary amines, namely ranitidine, $S$-(dimethylaminomethyl)-furfuryl alcohol (DFUR), $N,N$-dimethylthiophene-2-methylamine (DMTA), and $N,N$-dimethylbenzylamine (DMBA; see Scheme 1), and investigated C, H, and N isotope fractionation in NDMA during its formation from these precursors. First, we inferred the sources of C, H, and N atoms in NDMA by examining the initial and site-specific isotope ratios of selected precursors and by conducting experiments with isotopically distinct monochloramines. Second, we investigated to which extent the C and N isotope fractionation trends in two model tertiary amines and NDMA reflect NDMA formation pathways involving multiple isotope-sensitive reaction steps. Finally, we assessed whether the multielement isotope fractionation trends of NDMA are characteristic for chloramination of tertiary amines and could potentially be used as a proxy for the NDMA formation pathway during drinking water disinfection.

**Scheme 1. NDMA Formation Pathway during the Chloramination of Selected Tertiary Amines (Adapted from Spahr et al.35). Including the Precursor Compounds Examined in This Study (Ranitidine, DFUR, DMTA, and DMBA)"**

At pH 8.0 deprotonation of tertiary amines (1 to 2) occurs prior to nucleophilic attack on $\text{NH}_2\text{Cl}$. Reactive intermediates include substituted dimethylhydrazines (3), aminyl radicals (4), $N$-peroxyl radicals (5), and tetroxide species (6). Decomposition of 6 leads to two equivalents of NDMA (7) and carbocations (8). Based on a molar NDMA yield of <100%, precursors or intermediates 3−5 also undergo reactions to unidentified products.
salts exhibited distinctly different and well-defined N isotope ratios corresponding to N isotope signatures, $\delta^{15}N$, of $-1.4\%e$ and $+3.7\%e$ for NH$_4$Cl and (NH$_4$)$_2$SO$_4$, respectively.30

Chloramination experiments were carried out in 14 amber glass bottles containing 1 L of either 10 mM phosphate buffer (pH 8.0), 10 mM phosphate buffer (pH 7.0), 50 mM phosphate buffer (pH 8.0), or 10 mM borate buffer (pH 8.0). Each reactor was spiked with 100 $\mu$L of a methanolic stock solution to obtain initial concentrations of 3 $\mu$M ranitidine or DFUR and 40 $\mu$M DMTA or DMBA. The formation of NDMA was initiated through the addition of NH$_4$Cl in 15-fold excess corresponding to initial NH$_4$Cl concentrations of 45 or 600 $\mu$L, respectively. At predefined time points, one L reactor was sacrificed for chemical analyses. We measured the solution pH and NH$_4$Cl concentration, and quenched the chloramine reaction by adding 0.5 g of Na$_2$S$_2$O$_3$ to the reactor. To quantify the concentrations of NDMA, ranitidine, and DFUR, 1 mL of the solution was filled into an 1.5 mL amber autosampler glass vial. For analyses of DMTA and DMBA, 40 mL of the solution was transferred into 50 mL amber glass flasks, and the pH of the solution was adjusted to 11.3 through addition of 5 M NaOH. All samples were stored in the dark at 4 °C until concentration analyses and further processing for stable isotope analyses. Two control experiments were set up (i) to quantify losses of the organic amine precursor in the absence of NH$_4$Cl and (ii) to determine the self-decay rate of NH$_4$Cl in the absence of organic amines. Unless stated otherwise, reported NH$_4$Cl concentrations were corrected by the self-decay of NH$_4$Cl. The presence of methanol had no effect on the consumption of NH$_4$Cl and the formation of NDMA as shown previously.35

**Chemical Analyses.** The concentration of aqueous NH$_4$Cl stock solutions (30 mM) was quantified as described previously using a Varian Cary 100 Bio UV-visible spectrophotometer.29,50 In reaction mixtures containing tertiary amines, reactive intermediates, and NDMA, NH$_4$Cl was quantified with a colorimetric method using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diazonium salt (ABTS).29,52 A detailed comparison of chloramine quantification methods can be found in section S5 in the Supporting Information for Spahr et al.35 Concentrations of NDMA, ranitidine, and DFUR were determined by reverse-phase HPLC with UV detection (Dionex UltiMate 3000).35

Concentrations of DMTA and DMBA were measured by solid-phase microextraction (SPME) coupled to a gas chromatograph-mass spectrometer (GC/MS) (Thermo TRACE GC Ultra and Thermo TRACE DSQ II). Amber autosampler glass vials (2 mL), which contained 0.3 g NaCl, were filled with 1.3 mL of aqueous sample in 10 mM phosphate buffer (pH 11.3) and shaken on a Vortex mixer. Direct immersion SPME was carried out with a polydimethylsiloxane/divinylbenzene-coated fiber (PDMS/DVB, 65 $\mu$m, Supelco) after the fiber was conditioned daily for 30 min at 250 °C. Analytes were extracted for 45 min at 40 °C and desorbed within 3 min at 270 °C in the split/splitless injector of the GC.25 The GC was equipped with 1 m DPTMDS (methyl/phenyl) deactivated fused-silica guard column (0.53 mm i.d., BGB) and a 30 m x 0.25 mm ZB-S5s column (0.25 $\mu$m, Zebron, Phenomenex). Helium carrier gas was used at a constant pressure of 130 kPa. The temperature program was 1 min at 50 °C, 10 °C/min to 250 °C, and 5 min at 250 °C. DMTA and DMBA concentrations were quantified with an external calibration of 0.1–1.5 $\mu$L.

**Stable Isotope Analyses.** Stable C, H, and N isotope ratios of NDMA were measured using gas chromatography-isotope ratio mass spectrometry (GC/IRMS) coupled to solid-phase extraction (SPE), as reported in Spahr et al.29 C and N isotope analysis of DMTA and DMBA in aqueous samples was conducted with SPE-MC-IRMS. The SPE procedure, GC setup, and temperature program was identical to that for GC/MS analysis, but a 30 m x 0.32 mm ZB-S5s column (1 $\mu$m, Zebron, Phenomenex) was used. For all C and N isotope measurements, a Ni/Ni/ Pt reactor was operated at 1000 °C.56 Method quantification limits (MQLs) of the SPE-MC/IRMS measurements of DMTA and DMBA were determined according to the moving mean procedure of Jochmann et al.,54 and measurements were made in concentration ranges of 0.3–0.6 and 2.5–16 $\mu$L for DMTA and DMBA, respectively (Figure S1). The $^{15}$N equilibrium isotopic effect associated with the deprotonation of DMTA was investigated by SPE-MC/IRMS at pH 8.4, 8.7, 9.4, 10.4, and 11.3 in 10 mM phosphate buffer at ionic strength of 4 M using DMTA concentrations of 66, 12 (pH 8.7 and 9.4), 6.6, and 5 $\mu$L, respectively.56

Carbon, hydrogen, and nitrogen isotope ratios are reported as $\delta^{13}C$, $\delta^1H$, and $\delta^{15}N$ relative to Vienna PeeDee Belemnite, Vienna standard mean ocean water, and air, respectively.24,29 All isotope signatures are reported in permil (‰) as arithmetic mean of triplicate measurements ($\pm$σ). To ensure the accuracy of the measured isotope ratios, we used a series of isotopic standard materials purchased from Indiana University,55,56 as documented in Spahr et al.,29 as well as calibrated in-house standards in standard bracketing procedures. In-house standards of ranitidine, DFUR, DMTA, and NH$_4$Cl were obtained through C and N isotope ratio measurements with an elemental analyzer IRMS (Table S1). Isotopic analysis of NH$_4$Cl was impeded owing to its thermal instability and self-decay to ammonia. Instead, we used the $\delta^{15}N$ values of NH$_4$Cl or (NH$_4$)$_2$SO$_4$, from which NH$_2$Cl was produced, as a proxy for the initial $\delta^{15}N$ values of NH$_4$Cl. This assumption was based on high molar NH$_4$Cl yields (>94%) from the reaction of HOCl with NH$_4$Cl or (NH$_4$)$_2$SO$_4$.

**Data Evaluation.** We conducted chloramination experiments with two model compounds, namely DMTA and DMBA, to study isotope fractionation in the tertiary amines. Bulk isotope enrichment factors for carbon and nitrogen, $\varepsilon_C$ and $\varepsilon_N$, were derived from linear regression of $\delta^{13}C$ and $\delta^{15}N$ values versus fractional amount of remaining precursor ($c/c_0$) according to eq 1 (see Figure S3):

$$ln \left( \frac{\delta^E_E + 1}{\delta^E_0 + 1} \right) = \varepsilon_E \cdot ln \left( \frac{c}{c_0} \right)$$

(1)

where $\delta^E_E$ and $\delta^E_0$ are isotope ratios of an element E in the precursor at the beginning and during the reaction, respectively. Apparent kinetic isotope effects, AKIE$_E$, were calculated according to eq 2 considering the total number of atoms of an element (n), the number of atoms in reactive positions (x), and the number of atoms in intramolecular competition (z). The AKIE$_E$ values are reported as average secondary isotope effect for all C atoms in DMTA (n = x = 7, z = 1) and DMBA (n = x = 9, z = 1). AKIE$_E$ values stand for primary isotope effects and both precursors contain one N atom (n = x = z = 1). Uncertainties of $\varepsilon_E$ and AKIE$_E$ values are reported as a 95% confidence interval:

$$AKIE_E = \frac{1}{1 + (n/x) \cdot z \cdot \varepsilon_E}$$

(2)
The observable AKIE_N of DMTA or DMBA during chloramination at pH 8.0 originates from the combination of a deprotonation step (eq 3) and the subsequent reaction of the neutral tertiary amine (eq 4). As we have shown previously,\textsuperscript{35,57} the observable AKIE_N therefore consists of a combination of a \( ^{15}\text{N}\)-equilibrium isotope effect for the quaternary amine deprotonation, EIEN\(^{BH+}B\)- and an apparent kinetic isotope effect of the subsequent reaction, AKIE_N\(^{B}\) (eq 5):

\[
\text{BH}^+ \xrightarrow{k_1} \text{B} + \text{H}^+ \tag{3}
\]

\[
\text{B} \xrightarrow{k_2} \text{P} \tag{4}
\]

\[
\text{AKIE}_N = f_{BH^+} \cdot \text{EIEN}^{BH+} - B \cdot \text{AKIE}_N^B + (1 - f_{BH^+}) \cdot \text{AKIE}_N^B \tag{5}
\]

where \( k_1 \) and \( k_2 \) are rate constants of H\(^+\) exchange reaction, \( k_2 \) is the rate constant for transformation of the neutral amine, and \( f_{BH^+} \) is the fraction of the conjugate acid of the tertiary amine (see section S4 in the Supporting Information for details).

### RESULTS AND DISCUSSION

**Observable C, H, and N Isotope Fractionation Trends in NDMA.** We used 5-(dimethylaminomethyl)furfuryl alcohol (DFUR) as model compound for the chloramination of tertiary amines and studied the NDMA formation kinetics as well as the C, H, and N isotope ratios of NDMA. Figure 1a shows the formation of NDMA during the reaction of DFUR (3 \( \mu \)M) with NH\(_2\)Cl (45 \( \mu \)M) in 10 mM phosphate buffer at pH 8.0. The reaction was completed within 10 h with a molar NDMA yield of 65 ± 2%.\textsuperscript{35,47,58} Consistent with our earlier observations, we found a lag phase of approximately 1 h, in which only 0.2 \( \mu \)M DFUR was transformed to 0.1 \( \mu \)M NDMA (dashed line in Figure 1a).\textsuperscript{35} After 1 h, DFUR disappeared at a faster rate concomitant with the formation of NDMA. This kinetic behavior implies that reactive intermediates such as the \( N,N\)-dimethylhydrazine species (compound 3 in Scheme 1) and possible radical intermediates (4 and 5) are short-lived and transformed to NDMA and other unidentified products more rapidly than the initial transformation of DFUR to compound 3. The total consumption of NH\(_2\)Cl amounted to 12.0 \( \mu \)M and thus exceeded the initial concentration of DFUR by a factor of 4.1, in agreement with previous findings.\textsuperscript{35} No lag phase was observed for the disappearance of NH\(_2\)Cl (Figure 1a), indicating that side reactions, which did not lead to NDMA, likely contributed to the overstoichiometric consumption of NH\(_2\)Cl.

Figure 1b shows C, H, and N isotope signatures of NDMA during its formation. The \( \delta^{13}\text{C} \) values of NDMA (depicted as upward triangles) increased within 10 h from −24.8‰ to −8.7‰. This \( N \) isotope fractionation is caused by primary kinetic isotope effects that occur when chemical bonds to \( N \) are broken or formed in rate-determining reaction steps.\textsuperscript{25,59} In contrast, \( \delta^{13}\text{C} \) and \( \delta^2\text{H} \) values of NDMA changed only slightly from −36.8‰ to −34.3‰ and from −133.5‰ to −110.7‰, respectively. This C and H isotope fractionation is small compared to reactions, in which bonds to C and H are broken (e.g., refs 60 and 61) and is likely due to secondary kinetic isotope effects of atoms that do not participate in chemical reactions. The identical \( \delta^2\text{H} \) values of the N(CH\(_3\))\(_2\) moiety in ranitidine and the \( \delta^2\text{H} \) of NDMA reported earlier confirmed the accuracy of isotope ratio measurements by GC/IRMS.\textsuperscript{29} This finding implied that the \( \delta^{13}\text{C} \) value of NDMA also corresponds to the \( \delta^{13}\text{C} \) value of the N(CH\(_3\))\(_2\) group of the tertiary amine. Similar to previous results for ranitidine, we observe here for DFUR that the final \( \delta^{13}\text{C} \) value of NDMA (−34.3‰) was 14.5‰ more negative than the average \( \delta^{13}\text{C} \) value of the 8 C atoms in the precursor molecule (−19.8‰, gray line in Figure 1b). The average \( \delta^{13}\text{C} \) value of the C atoms in the furfuryl moiety...
and methylene C was $-14.9\%\text{o}$. This result confirms not only the uneven distribution of $^{12}\text{C}$ and $^{13}\text{C}$ atoms in the tertiary amine but also that $^{12}\text{C}$ atoms are preferentially found in the $\text{N}(\text{CH}_3)_2$ group.

**Source of N Atoms in NDMA.** N isotope signatures of NDMA reflect the average isotope ratios of both N atoms of NDMA and can reveal the sources of nitrogen. For the reasons outlined above, the N atom of the $\text{N}(\text{CH}_3)_2$ group stems from the tertiary amine, whereas the N atom of the nitroso group derives from $\text{NH}_2\text{Cl}$. However, simple N isotope mass balances would fail to show the origin of the N atoms because only 65% of DFUR was transformed to NDMA so that 35% of the N atoms ended up in unidentified products. Moreover, less than 5% of the N atoms of NDMA were incorporated into NDMA (final concentration of 1.9 $\mu\text{M}$) because $\text{NH}_2\text{Cl}$ was present in excess (initial concentration of 45 $\mu\text{M}$). The final $\delta^{15}\text{N}$ value of NDMA ($\delta^{15}\text{N} = -8.7\%\text{o}$) therefore does not match the average value of the initial N isotope signatures of DFUR ($\delta^{15}\text{N}_{\text{DFUR}} = -2.2\%\text{o}$, yellow line in Figure 1b) and monochloramine ($\delta^{15}\text{N}_{\text{NH}_2\text{Cl}} = -1.4\%\text{o}$, red line). Owing to the incomplete conversion of both precursors to NDMA and a preferential reaction of $^{14}\text{N}$ to NDMA caused by a normal N kinetic isotope effect, the final $\delta^{15}\text{N}$ value of NDMA (upward triangles in Figure 1b) was 6.9% more negative than the average N isotope signature of both precursors ($-1.8\%\text{o}$).

To quantify the origin of N atoms in NDMA, we conducted a second, independent NDMA formation experiment with the same DFUR of known isotopic composition but with an isotopically distinctly different batch of monochloramine (NH$_2$Cl-B). NH$_2$Cl from batch B exhibited a $\delta^{15}\text{N}_{\text{NH}_2\text{Cl-B}}$ of $+53.7\%\text{o}$ (blue line in Figure 1b) and was thus enriched in $^{15}\text{N}$ by $+55.1\%\text{o}$ compared to NH$_2$Cl-A ($\delta^{15}\text{N}_{\text{NH}_2\text{Cl-A}} = -1.4\%\text{o}$). We observed the same extent of N isotope fractionation in NDMA regardless of whether NH$_2$Cl-A or NH$_2$Cl-B reacted with DFUR. $\delta^{15}\text{N}$ values of NDMA changed by $+16.1\%\text{o}$ and $+17.6\%\text{o}$, respectively, during its formation. Because of a $\delta^{15}\text{N}_{\text{NH}_2\text{Cl-B}}$ value of $+53.7\%\text{o}$, $\delta^{15}\text{N}$ values of NDMA shifted toward much more positive values (downward triangles in Figure 1b) compared to NDMA formed from NH$_2$Cl-A. The shift of $\delta^{15}\text{N}$ of NDMA due to the use of NH$_2$Cl-B instead of NH$_2$Cl-A amounted to $+26.9 \pm 2.2\%\text{o}$ (indicated as black arrows in Figure 1b). This offset corresponds to 50% (i.e., 27.6%\text{o}) of the difference of $\delta^{15}\text{N}$ between the two NH$_2$Cl batches ($-1.4\%\text{o}$ vs $-53.7\%\text{o}$) and, therefore, confirms that one N atom in NDMA originates from NH$_2$Cl-A and one N atom stems from the tertiary amine. Note that $\delta^{15}\text{C}$ values of NDMA produced with NH$_2$Cl-A and NH$_2$Cl-B (grey and black diamonds, respectively, in Figure 1b) were identical, which is in agreement with the fact that both C atoms of the N(CH$_3$)$_2$ group of NDMA originate from DFUR.

**Isotope Fractionation Trends of Tertiary Amines and NDMA Reflect the Multistep NDMA Formation Pathway.** As illustrated in Scheme 1, NDMA formation is a multistep process, in which reactions of N atoms play a key role. Deprotonation of tertiary amines (compound 1 in Scheme 1) occurs prior to the initial reaction of 2 with NH$_2$Cl leading to a hydrazine-type intermediate (3). Subsequently, the NH$_2$-group of 3 is oxidized to a N-centered radical 4 that reacts with aqueous O$_2$. The final release of NDMA from compound 6 requires the formation of a nitroso moiety as well as the cleavage of a C–N bond to the methylene group of the tertiary amine. Because molar NDMA yields were smaller than 100%, tertiary amines or intermediate species also react to products other than NDMA. Every elementary reaction step depicted in Scheme 1 is associated with an isotope effect and can thus cause isotope fractionation in the precursors (tertiary amine and NH$_2$Cl) as well as in NDMA. In the following sections, we evaluate how these reactions contribute to the observable isotope fractionation in the tertiary amine and in NDMA.

C and N Isotope Fractionation in Tertiary Amines. We studied C and N isotope fractionation in two tertiary amine model compounds, namely $N,N$-dimethylthiophene-2-methylamine (DMTA) and $N,N$-dimethylbenzylamine (DMBA) during chloramination. DMTA and DMBA are structurally similar to DFUR (Scheme 1), known to produce high NDMA yields, and are, in contrast to DFUR, amenable to isotope analysis by GC/IRMS. Figure 2a shows the NDMA formation during the reaction of DMTA (40 $\mu\text{M}$) with NH$_2$Cl (600 $\mu\text{M}$) in 10 mM phosphate buffer at pH 8.0. Panel (a) shows DMTA degradation, NH$_2$Cl consumption, and NDMA formation over time. The symbols in panel (b) demonstrate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of DMTA and NH$_2$Cl. Solid lines represent the initial $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of DMTA and NH$_2$Cl. Standard deviations of triplicate $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements of DMTA and NDMA were $<0.2\%\text{o}$ and $<0.8\%\text{o}$, respectively, and smaller than the depicted symbols.
fractionation in the remaining tertiary amine (Figure 2b). The δ13C and δ15N values of DMTA changed by +8.6‰ and +52.9‰, respectively, demonstrating that precursor molecules containing 13C and 15N reacted preferentially. This isotopic preference correlates to apparent 13C and 15N kinetic isotope effects of 1.0021 ± 0.0003 (AKIE13C) and 1.0127 ± 0.0007 (AKIE15N) derived from eqs 2 and 5 (see details in section S4 and Table S2).

The notable extent of N isotope fractionation in DMTA reflects the N isotope effects of all reactions of the reactant and its transient products up to and including the first irreversible step in the reaction sequence. However, only few isotope effects of the reactions shown in Scheme 1 have been studied in independent experiments and the rate-limiting steps of the chlorination of tertiary amines to NDMA are not known. The reaction kinetics shown in Figures 1a and 2a suggest that the disappearance of the tertiary amine and formation of NDMA occur simultaneously without the formation of long-lived intermediates. Therefore, we hypothesize that one rate-determining step governs the kinetics of tertiary amine disappearance and formation of NDMA. This step could be the formation of the hydrazine intermediate (2 → 3) or the formation of the N-centered radical (3 → 4). Reactions 4 → 5 and 5 → 6 involve radical species and are likely irreversible and faster than the preceding reaction steps. Given that approximately 98% of the DMTA molecules are protonated at pH 8.0, the H+ exchange reaction 1 → 2 may also contribute to the observable N isotope fractionation in DMTA.

It is thus likely that reactions 1 → 2 → 3 or 1 → 2 → 3 → 4 are the source of N isotope fractionation in the reactant. Indeed, the deprotonation of conjugate acids of N,N-dimethylaniline and substituted anilines gives rise to a 15N equilibrium isotope effect, EIE15N – B, of 1.014 to 1.0203.53,63,64 For the deprotonation of DMTA, we determined an EIE15N – B of 1.0103 ± 0.0004 (eq 5 and Table S2). Taking into account this contribution of the deprotonation step to the overall observable N isotope fractionation in DMTA, an apparent kinetic N isotope effect of 1.0025 ± 0.0011 (AKIE15N; eqs 5 and S2) can be assigned to the reaction of the deprotonated DMTA species. This AKIE15N can originate from the nucleophilic substitution reaction of the tertiary amine with NH2Cl (2 → 3), the oxidation of the hydrazine intermediate (3 → 4), or a combination thereof (2 → 3 → 4). A more rigorous assignment of N isotope fractionation is currently speculative but data for similar reactions such as the N atom oxidation of aromatic N-alkylamines, which could mimick reaction 3 → 4, confirms that N isotope effects following the deprotonation step are indeed small.57 N60 Note that we neglect unknown reactions causing the NDMA yield to be <100%. These side reactions that did not lead to NDMA are more likely caused from reactions of radical intermediates 4 and 5, which do not affect the N isotope fractionation determined in the reactant.

The results obtained from the chlorination of DMBA were almost identical to those with DMTA despite a smaller molar NDMA yield (58 ± 0.6‰, Figure S2 and Table S2). Using a pK1 value of 9.0 for DMBA66 and the EIE15N – B obtained for DMTA, the AKIE15N associated with the transformation of the deprotonated DMBA (2) was 1.0056 ± 0.0007. This value is only slightly larger than the one for DMTA (1.0024 ± 0.0011) and supports the assumption that the chlorination of different tertiary amines proceeds through the same reaction mechanism.

Carbon isotope fractionation in DMTA and DMBA was substantially smaller than that for N (Figures 2b and S2b). The smaller AKIECs of 1.0021 ± 0.0003 and 1.0014 ± 0.0001 (Table S2) of DMTA and DMBA, respectively, are consistent with the assumptions made for the origins of N isotope fractionation. EIE15N – B for the deprotonation of –RN–H bonds are small (<1.001).53 Moreover, we found that the methylthiophene-moiety of DMTA did not react with NH2Cl (Figure S7). Consequently, the reaction with NH2Cl (2 → 3) occurs exclusively at the N(CH3)2 group of DMTA and DMBA and only involves N atoms. Small changes in δ13C values of DMTA and DMBA are due to secondary 13C isotope effects of C atoms that are not located at the reactive sites of the tertiary amines.

Isotope Fractionation Trends in NDMA as a Proxy for Its Formation Pathway. Effects of the Molecular Structure of Tertiary Amines. We hypothesize that the 13C and 15N kinetic isotope effects of the many reactions shown in Scheme 1 result in C and N isotope fractionation trends in NDMA that are characteristic for the NDMA formation through chlorination of tertiary amines. Correlations of isotope fractionation are used frequently to identify how a reactant (i.e., an organic contaminant) is transformed (e.g., refs 59 and 68–70). Conversely, the methodology of multielement isotope fractionation is applied only rarely to fingerprint the processes leading to contaminants. Here, we evaluated the linear correlation of C and N isotope fractionation in NDMA from four different precursors, namely ranitidine, DFUR, DMTA, and DMBA.

Figure 3 shows how δ13C and δ15N values of NDMA increase during its formation from different precursors and correlations with slopes of Δ13CNDMA/Δ15CNDMA. The differences of initial δ13C and δ15N values of NDMA are due to the different C and N isotope compositions of the precursor compounds (Table S1). The 15N AKIEs are much larger than the 13C AKIEs because only the N atoms of NDMA undergo bond cleavage and bond formation reactions. Consequently, N isotope fractionation in NDMA is substantially larger than C isotope fractionation. The Δ13CNDMA/Δ15CNDMA slopes are compiled in Table 1 and...
span from 5.1 ± 2.1 to 13 ± 5.0 under identical experimental conditions (that is, pH 8.0 in 10 mM phosphate buffer). Neither the type of precursor molecule nor the molar yield of NDMA (from 58 ± 1% to 97 ± 4%) Table 1 and Figure S5) affects the observed C and N isotope fractionation trend systematically. Except for ranitidine (Δ15NNDMA/Δ13CNDMA = 13 ± 0.5), all slopes are identical within uncertainty. Note, however, that these correlations are very sensitive to the extent of C isotope fractionation affecting the N(CH3)2 and methylene groups. Such secondary C isotope effects of nonreactive moieties are not well understood and we cannot rule out that C isotope fractionation in the N(CH3)2 moiety of ranitidine differs from that of the other, smaller precursor molecules (Scheme 1).

Impact of Buffer Type and pH. Similar observations regarding the correlation of C and N isotope fractionation in NDMA were made when NDMA was formed from DFUR and ranitidine at pH 7.0 and 8.0 in the presence of different buffers and buffer concentrations (Figure 3, Table 1, and sections S6 and S8). All Δ15NNDMA/Δ13CNDMA values derived from experiments in phosphate buffers were confined to a small range (5.0 ± 0.9 to 6.9 ± 0.5) as for NDMA produced from DMTA and DMBA.

**Table 1. Molar NDMA Yield (%) as Well as Dual-Isotope Slopes (Δ15NNDMA/Δ13CNDMA) for the Reaction of DFUR (3 μM), Ranitidine (3 μM), DMTA (40 μM), and DMBA (40 μM) with NH2Cl (45μM or 600 μM, Respectively) in 10 mM Phosphate Buffer at pH 8.0**

<table>
<thead>
<tr>
<th>precursor</th>
<th>type</th>
<th>mM</th>
<th>pH</th>
<th>molar NDMA yield (%)</th>
<th>Δ15NNDMA/Δ13CNDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFUR</td>
<td>phosphate</td>
<td>10</td>
<td>8.0</td>
<td>65 ± 2</td>
<td>6.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>phosphate</td>
<td>10</td>
<td>7.0</td>
<td>66 ± 1</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>phosphate</td>
<td>50</td>
<td>8.0</td>
<td>68 ± 1</td>
<td>5.0 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>borate</td>
<td>10</td>
<td>8.0</td>
<td>88 ± 1</td>
<td>12 ± 3.5</td>
</tr>
<tr>
<td>ranitidine</td>
<td>phosphate</td>
<td>10</td>
<td>8.0</td>
<td>97 ± 4</td>
<td>13 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>phosphate</td>
<td>10</td>
<td>7.0</td>
<td>84 ± 0.4</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>DMTA</td>
<td>phosphate</td>
<td>10</td>
<td>8.0</td>
<td>75 ± 0.1</td>
<td>5.1 ± 2.1</td>
</tr>
<tr>
<td>DMBA</td>
<td>phosphate</td>
<td>10</td>
<td>8.0</td>
<td>58 ± 1</td>
<td>6.3 ± 0.6</td>
</tr>
</tbody>
</table>

*Chloramination experiments with DFUR and ranitidine were conducted using different buffer types and concentrations and pH values. †Slope of a linear regression analysis of δ15N vs δ13C; uncertainties denote 95% confidence intervals.

Δ15NNDMA/Δ13CNDMA obtained from experiments in borate buffer, however, were larger and more uncertain (12 ± 3.5).

As shown in Figure S6, we observed an increase of the rates of NDMA formation when increasing the phosphate buffer concentration from 10 to 50 mM at pH 8.0. Conversely, NDMA formation was slower at pH 7.0 (10 mM phosphate buffer; Figures S8 and S9). However, none of these variations of reaction rates affected the Δ15NNDMA/Δ13CNDMA value systematically. This finding is consistent with previous studies that showed that water matrix components, presumably natural organic matter, can slow down the formation of NDMA during chloramination of ranitidine without affecting the molar NDMA yield.17 Even though we cannot explain some of the variations, our data suggests that the combined C and N isotope fractionation trends in NDMA can serve as an indicator for the NDMA formation pathway during chloramination.

**IMPLICATIONS FOR STABLE ISOTOPE ANALYSIS OF NDMA DURING WATER DISINFECTION**

Our study provides the first evidence that isotope fractionation trends from several elements in NDMA may reflect its formation pathway. Even though the isotope effects of some of the reactions leading to NDMA are not yet known, the chloramination of a series of tertiary amines under slightly different reaction conditions resulted in a consistent pattern of C and N isotope fractionation in NDMA. Further work is warranted to confirm correlations of C and N isotope fractionation in NDMA for chloramination reactions shown here, for example, by taking into account effects of water constituents and water quality. While our work focused on the comparison of isotope fractionation of elements at reactive versus nonreactive positions, that is, primary N versus secondary C isotope effects, additional information on the mechanisms of NDMA formation may be obtained from oxygen isotopes in NDMA. The oxygenation of N atoms of the precursor compounds is a critical step in the NDMA formation pathway55 and oxygen isotope fractionation may be larger than what we showed here for C and H isotopes. Given that NDMA formation mechanisms have been shown to vary depending on the precursor and disinfectant,17 systematic investigations of the isotope fractionation behavior of NDMA formed through different pathways, with other disinfectants,
and over the entire range of observed NDMA yields and concentration ranges may lead to a new tool for the identification of NDMA formation during water treatment processes.

ASSOCIATED CONTENT

+ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b03919.

Additional details on safety considerations, a list of all chemicals, reference isotope signatures, and a list and detailed description of the determination of isotope enrichment factors and kinetic isotope effects. Figures illustrating method quantification limits, isotope fractionation, the determination of bulk isotope enrichment factors and ¹⁵N equilibrium isotope effects, reaction kinetics, the impact of buffer type and concentration as well as pH, and the chloramination of 2-thiophenemethanol. (PDF)

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REFERENCES


