

2018 Project Abstract

For the Period Ending June 30, 2023

PROJECT TITLE: Improving Drinking Water for Minnesotans through Pollution Prevention

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04f as extended by M.L. 2021, First Special Session, Chp. 6, Art. 6, Sec. 2, Subd. 18

APPROPRIATION AMOUNT: \$345,000

AMOUNT SPENT: \$337,534

AMOUNT REMAINING: \$7,466

Sound bite of Project Outcomes and Results

This project comprehensively studied the spatio-temporal occurrence of N-nitrosodimethylamine (NDMA, a potent carcinogen) precursors in the Crow River watershed as well as treatment approaches for NDMA precursor removal. The project results will aid in evaluation and mitigation of potential risks from NDMA formation during disinfection of drinking water with chloramines.

Overall Project Outcome and Results

NDMA, a contaminant of emerging concern in drinking water supplies, is notorious both for its potent carcinogenicity and toxicity and for its formation in drinking water following the addition of chloramines for disinfection. From a preliminary sampling campaign, the Crow River was found to be a major contributor of NDMA precursors to the Upper Mississippi River, the drinking water supply for the Twin Cities.

To protect the public from potential exposure to NDMA, spatial and temporal variations of NDMA precursors were investigated by collecting water samples from two tributaries and the main stem of the Crow River from 2020 to 2022. A non-targeted analysis approach using mass spectrometry was developed to identify NDMA precursors. Experiments evaluated the effectiveness of pre-oxidation with ozone, free chlorine, or both on removal of NDMA precursors.

NDMA precursor concentrations in raw (untreated) water exhibited seasonal variation, with resulting NDMA concentrations ranging from 7.5 to 109 ng/L. Lime-softening treatment typically increased NDMA formation during disinfection with chloramines by reducing the concentration of natural organic matter (NOM). NOM competes with the precursors for the chloramines. Animal operations and municipal wastewater discharges were identified as potential sources of NDMA precursors. A fluorescence analysis of water samples revealed a correlation between NDMA precursor levels and the protein-like fraction of NOM. Specific NDMA precursors identified in the Crow River include pharmaceutical compounds (e.g., tramadol, metformin, and venlafaxine) and 3-cyclohexyl-1,1-dimethylurea. NDMA formation could be controlled via treatment of water using ozone at doses as low as 1 mg/L or free chlorine.

This project offered valuable insights to water treatment plant operations and policy makers by providing important information about the sources of NDMA precursors into surface waters and how precursor levels vary

with seasons. Additionally, the project findings provide water utilities with potential strategies for mitigating NDMA formation when precursors are present.

Project Results Use and Dissemination

To date, this project has produced two peer-reviewed journal articles ([Noe et al. 2023](#), Li et al. 2023 (accepted)), one journal article is in review, and there one more journal article will be submitted soon. The research has been directly communicated to scientists at the Minnesota Department of Health. The findings of this project were presented at several conferences, including the annual conference of the Minnesota Section of the American Water Works Association, the National Meeting of the American Chemical Society, and the Association of Environmental Engineering and Science Professors conference. Results are also summarized in a master's thesis (Eric Noe) and doctoral dissertation (Jiaqi Li), which are available in the [UMN Digital Conservancy](#).



Environment and Natural Resources Trust Fund (ENRTF) M.L. 2019 Final Work Plan

Date of Report: August 15, 2022

Final report

Date of Work Plan Approval: June 24, 2019

Project Completion Date: June 30, 2023

PROJECT TITLE: Improving Drinking Water for Minnesotans through Pollution Prevention

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Location: Statewide

Total ENRTF Project Budget:

ENRTF Appropriation: \$345,000

Amount Spent: \$337,534

Balance: \$7,466

M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04f as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

Appropriation Language: \$345,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to reduce exposure of Minnesotans to a toxic, cancer-causing chemical by identifying key pollutant precursor sources in the upper Mississippi River watershed and assessing options to reduce the formation of this chemical during drinking water treatment.

M.L. 2022 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2023]

PROJECT TITLE: Improving Drinking Water for Minnesotans through Pollution Prevention

I. PROJECT STATEMENT:

The upper Mississippi River, the water supply for ~1 million Minnesota residents, is under constant assault by a variety of chemical and microbiological contaminants. A contaminant of emerging concern in Twin Cities drinking water supplies is the **highly toxic and potent cancer-causing chemical N-nitrosodimethylamine (NDMA)**. NDMA itself is not present in the river water but the chemical is formed by the reaction of so-called 'precursors' in the water with chloramines, a form of chlorine added to the water for disinfection. Although NDMA currently is not a regulated contaminant, many states are considering regulations and California has established a notification level of 10 ng/L (10 parts-per-trillion). Initial testing has shown high levels of precursors in Upper Mississippi River water, with the potential to form more than 50 ng/L of NDMA. A preliminary sampling campaign completed with the aid of St. Paul Regional Water Services personnel has identified the Crow River as a major contributor to NDMA precursor levels in the Upper Mississippi, but the sources of these precursors to the Crow River are not known. NDMA precursors are associated with municipal wastewater discharges and include some industrial chemicals and pharmaceutical compounds such as ranitidine, a medicine used to treat acid reflux. Another potential source in the Crow River watershed is animal waste from agricultural operations. To protect drinking water consumers from NDMA and other toxic nitrosamines, it is critical to first identify and then curb the main sources of precursors. **The main impact of the proposed project is to reduce exposure of Minnesotans to the toxic chemical NDMA by recommending pollution prevention strategies to the MN Pollution Control Agency (MPCA) and the MN Department of Health (MDH) and treatment options to water utilities.** This will be done by:

- Assessing seasonal variability in NDMA precursor levels in the Upper Mississippi and Crow Rivers;
- Identifying the major sources of NDMA precursors to the Crow by sampling along the length of the river from the confluence and working upstream along the North and South Forks as well as Buffalo Creek; and
- Investigating options to reduce NDMA formation during water treatment.

This research project will benefit Minnesotans by providing critically important information for policy makers regarding which waste streams to target to reduce inputs of NDMA precursors to surface waters and for water utilities regarding potential treatment strategies for reducing NDMA formation when precursors are present.

II. OVERALL PROJECT STATUS UPDATES:

AMENDMENT REQUEST August 9, 2019

We are requesting that funding for a post-doc in the amount of \$3,704.22 (salary + fringe) be shifted from graduate student support to post-doctoral researcher. The post-doctoral researcher is needed to train the graduate student to work on the project. The training concerns collecting water samples along the Crow and Mississippi Rivers and how to perform jar tests that simulate the local water treatment systems, two critical aspects of the project.

Amendment Approved by LCCMR **9/22/2019**

First Update March 1, 2020

A solid-phase extraction liquid chromatography-mass spectrometry (SPE-LC/MS) method to determine NDMA concentration was developed and tested in the laboratory. Six water samples were collected on July 18, 2019 from the North Fork of the Crow River, South Fork of the Crow River, and main stem of the Crow River and analyzed for NDMA precursors before and after lime softening. An investigation of industrial, agricultural and other activities in the Crow River watershed and associated chemical uses was done through online searching.

Second Update September 1, 2020

From July to November, a monthly sampling campaign was conducted in the North Fork of the Crow River, South Fork of the Crow River, the mainstem of the Crow River, and in the Mississippi River both upstream and downstream of the confluence with the Crow. Each campaign consists of a total of twelve sampling locations. Sixty water samples were collected during the 5 sampling events and subsequently processed in the laboratory. Water samples collected in August, September, and October have been analyzed for NDMA precursors before and after lime softening. We indirectly analyze for NDMA precursor levels by dosing the water samples with chloramines to simulate the disinfection practice of the local water treatment plants, incubating for 3 days to allow the NDMA to form, then analyzing for NDMA. Because each sample was analyzed twice (i.e., duplicate analyses), a total of 132 samples have been analyzed for NDMA after the incubation period using the SPE-LC/MS method developed previously. Analysis of the samples collected in July and November is ongoing.

Third Update March 1, 2021

From December 2020 to February 2021, the sampling campaign described above continued. Because of ice cover at some locations, we were unable to collect water at all 12 locations. In total, 24 water samples were collected and analyzed for NDMA precursors before and after lime softening during the three sampling events. Duplicate water samples were incubated for three days after dosing monochloramine to simulate the disinfection process in a water treatment plant. In this step, NDMA precursors in the water samples react with the chloramines and are transformed into NDMA. NDMA concentration was analyzed using the SPE-LC/MS method developed previously. From July 2020 to Feb 2021, a total of 264 samples were analyzed. During each sampling event, water samples before and after lime softening as well as chloraminated samples were stored in a freezer for future non-targeted analysis work in an attempt to identify NDMA precursors. We are working with the mass spectrometry facility in the UMN chemistry department on non-target analysis. Pre-oxidation of NDMA precursors with ozone or an ozone/advanced oxidation process prior to chloramination might destroy NDMA precursors and thus serve as an effective strategy to limit NDMA formation. Pre-oxidation experiments via ozone are currently being designed and will begin in March 2021.

AMENDMENT REQUEST August 24, 2021

We are requesting a 12-month no-cost extension to June 30, 2023. The reason for the request is delays resulting from the COVID-19 pandemic. Everything has proven to be more challenging since the pandemic began, from obtaining research supplies, to assembling crews for sampling, to accessing core facilities for our analyses, etc. The additional time would allow us to complete the study as close to as originally planned as possible.

Amendment approved June 30, 2022 (see below)

Fourth Update September 1, 2021

The monthly sampling campaign in the Crow river and its tributaries continued from March 2021 to August 2021. Besides the twelve locations described in the previous updates, we also collected samples from the headwaters of the North Fork and South Fork of the Crow river to further investigate spatial variation in NDMA precursor levels in the watershed. A total of 89 samples were collected and analyzed for NDMA precursors before and after lime softening. NDMA precursors were processed by simulated disinfection processes described previously. NDMA in the samples were then concentrated by solid phase extraction (SPE) using activated carbon cartridges, then analyzed via UHPLC-MS/MS. In total, 392 samples were analyzed by the SPE-LC-MS/MS method. The method development for non-targeted NDMA precursor identification is ongoing. Currently, we are working on developing a sample preparation method for extracting a variety of NDMA precursors from water samples with a suitable concentration factor that will permit detection on our analytical instrumentation. Finally, we have initiated experiments to determine if ozone, a common water treatment chemical, can be used to destroy NDMA precursor via chemical oxidation. Specifically, initial bench-scale experiments are being performed to measure ozone decay rates in raw and lime softened water samples obtained from the Saint Paul Regional Water Services water treatment facility. The ozone decay rate varies with

the water temperature and ozone dose applied. The ozone decay rate is important because the longer the ozone stay around in the water, the more effectively NDMA precursors can be degraded.

Fifth Update March 1, 2022

The method for non-targeted NDMA precursor identification has been further developed. The recovery efficiency of the mix-bed cartridges for NDMA precursor extraction was determined using samples collected over time at selected locations. Beginning in January 2022, water samples were collected from the Upstream Crow River sampling location every Monday for six consecutive weeks to identify NDMA precursors in the Crow River. The collected samples will be processed for both NDMA determination by the SPE-LC-MS/MS method described before and precursor identification by the SPE-LC-QTOF-MS method we developed. In addition, bench-scale experiments were performed to investigate the effect of chemical oxidation of NDMA precursors with ozone or an ozone/advanced oxidation process prior to chloramination to destroy NDMA precursors and thus limit NDMA formation. Experiments were conducted on water samples collected during the summer of 2021 from the Saint Paul Regional Water Services (SPRWS) water treatment facility. In total, 63 chemical oxidation experiments were conducted, exploring a range of treatment conditions by varying oxidant dose and water temperature. Compared to NDMA concentrations of samples prior to chemical oxidation treatment (8.5 to 26.3 ng/L), all pre-oxidation treatments reduced NDMA formation to low levels (< 4 ng/L). Pre-oxidation experiments are currently being repeated with water samples collected from SPRWS during the winter of 2022.

Update as of June 30, 2022

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022

The samples collected from Jan to March in 2022 were processed for NDMA formation potential using the SPE-LC-MS/MS method and the mass spectrometry data has been analyzed. To continuously monitor seasonal dynamics and year-to-year difference of NDMA precursors in Crow river region, a sampling campaign was conducted in May 2022. The NDMA precursor level of these sample could indicate NDMA precursor concentrations at the spring season in 2022. The data processing and analysis work about non-targeted identification of NDMA precursors in winter Crow River samples is ongoing. Moreover, the bench-scale oxidation experiments described in the last update were repeated using winter samples collected from Saint Paul Regional Water Services (SPRWS) water treatment facility to investigate the seasonal effect on the precursor removal efficiency by various oxidants. In addition, river samples collected from Crow River were treated with ozone to explore the effectiveness of ozone in reducing NDMA precursors specific in Crow River.

Seventh Update as of March 1, 2023

Sampling campaigns were conducted in the fall of 2022 and winter of 2023 to continue to assess the seasonal variability and annual differences of NDMA precursors in the Crow river region. For fall 2022, filtered raw and lime-softened samples were prepared following our previous protocol, and the NDMA formation using the uniform formation conditions test was determined. The samples collected in winter 2023 are being processed. As for the non-targeted analysis of NDMA precursors in the Crow River, a list of candidates was discovered, and several were identified and confirmed by MS/MS analysis. More identification work via MS/MS analysis to confirm the identities of the compounds is ongoing. This work will help identify which chemicals and human activities may be responsible for discharging NDMA precursors into the watershed. Moreover, the effectiveness of different oxidation practices on a known NDMA precursor, ranitidine, was examined, including 1 mg/L ozone, 2 mg/L ozone, 5 mg/L ozone, 5 mg/L free chlorine, and 2mg/L ozone + 5 mg/L free chlorine.

AMENDMENT REQUEST March 1, 2023

We are requesting that funds be shifted from the Analytical Services and Travel allocations to Laboratory Supplies and Personnel. Analytical services would decrease from \$20,000 total to \$15,010 and Travel from

\$15,000 to \$5,900. Laboratory Supplies would increase from \$33,000 to \$35,325 and Personnel would increase from \$272,000 to \$283,764. These changes are being requested to reflect differences between budgeted costs and actual expenditures.

Amendment Approved by LCCMR 5/16/2023

AMENDMENT REQUEST as of June 30, 2023

We request four budget adjustments to reflect final accounting of the project:

- Increase personnel by 11,764 from 272,000 to 283,764
- Increase laboratory supplies by 2,538 from 33,000 to 35,538
- Decrease travel by 9,523 from 15,000 to 5,477
- Decrease publication by 4,779 from 5,000 to 221

Amendment Approved by LCCMR 1/15/2024

Final Report as of June 30, 2023 (to be submitted before August 15, 2023)

NDMA, a contaminant of emerging concern in drinking water supplies, is notorious both for its potent carcinogenicity and toxicity and for its formation in drinking water following the addition of chloramines for disinfection. From a preliminary sampling campaign, the Crow River was found to be a major contributor of NDMA precursors to the Upper Mississippi River, the drinking water supply for the Twin Cities.

To protect the public from potential exposure to NDMA, spatial and temporal variations of NDMA precursors were investigated by collecting water samples from two tributaries and the main stem of the Crow River from 2020 to 2022. A non-targeted analysis approach using mass spectrometry was developed to identify NDMA precursors. Experiments evaluated the effectiveness of pre-oxidation with ozone, free chlorine, or both on removal of NDMA precursors.

NDMA precursor concentrations in raw (untreated) water exhibited seasonal variation, with resulting NDMA concentrations ranging from 7.5 to 109 ng/L. Lime-softening treatment typically increased NDMA formation during disinfection with chloramines by reducing the concentration of natural organic matter (NOM). NOM competes with the precursors for the chloramines. Animal operations and municipal wastewater discharges were identified as potential sources of NDMA precursors. A fluorescence analysis of water samples revealed a correlation between NDMA precursor levels and the protein-like fraction of NOM. Specific NDMA precursors identified in the Crow River include pharmaceutical compounds (e.g., tramadol, metformin, and venlafaxine) and 3-cyclohexyl-1,1-dimethylurea. NDMA formation could be controlled via treatment of water using ozone at doses as low as 1 mg/L or free chlorine.

This project offered valuable insights to water treatment plant operations and policy makers by providing important information about the sources of NDMA precursors into surface waters and how precursor levels vary with seasons. Additionally, the project findings provide water utilities with potential strategies for mitigating NDMA formation when precursors are present.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Assess seasonal variability in the quantities of NDMA precursors in the Crow River and in the Mississippi River both upstream and downstream of the Crow.

Description: Known NDMA precursors include nitrogen-containing organic compounds with specific structures called secondary and tertiary amines. Such precursors are often associated with municipal wastewater discharges, but the main sources to the Crow River are not known and may include human waste, animal waste,

or both. Additional work is needed to assess seasonal variability in precursor levels in the Crow River and the Upper Mississippi River. We will collect water from 3 sampling locations on at least 4 sampling dates from the summer of 2019 through summer of 2020. As duplicate samples will be collected from each location on every sampling date, this results in a total of at least 24 water samples. Water samples will be collected from the middle of the river just below the surface. All water samples will be lime softened using a jar test apparatus to mimic the treatment that occurs in the two Twin Cities treatment facilities. We have discovered through previous work that lime softening is critical for ‘activating’ the precursors. After lime softening, the samples will be analyzed for NDMA precursor levels (formation potential test) as well as for organic amine concentrations. Finally, the samples will be analyzed for selected known NDMA precursor chemicals in an attempt to identify the dominant precursors and possible sources (e.g., human waste, animal waste, industrial activities).

ACTIVITY 1 ENRTF Budget: \$ 112,724

Outcome	Completion Date
1. <i>Collect water samples from the Crow River and Mississippi River upstream and downstream of the Crow at least 4 times over a one-year period.</i>	August 31, 2020
2. <i>Analyze the river water samples for NDMA precursor levels using the uniform formation conditions (UFC) test.</i>	September 31, 2020
3. <i>Analyze the river water samples for total organic amine levels and specific known precursor compounds (e.g., ranitidine, selected antibiotics).</i>	December 31, 2020

First Update March 1, 2020

A SPE-LC/MS method to determine NDMA concentration was developed and verified. The limit of quantification (LOD) is 5 ng/L with at least 70% recovery efficiency for NDMA in spiked river water samples after method optimization. These LOD and recovery values are deemed sufficient for our study. Duplicate water samples were collected from three locations on July 18, 2019. Each of these six water samples was split with approximately half of the sample analyzed for NDMA precursors directly and the other half subjected to lime softening prior to precursor analysis to simulate how water is treated in Minneapolis, St. Paul, and St. Cloud. NDMA precursor testing was done using the uniform formation conditions (UFC) method. The UFC test is a standard test that was developed to simulate typical or average disinfection conditions used at water treatment plants. Use of such standardized test conditions, rather than conditions specific to a given water treatment facility, allows us to compare results for all facilities and sites in our investigation and to compare our results with those from other studies done elsewhere. The average NDMA concentrations in North fork of the Crow River, South fork of the Crow River, and the Crow River samples were 22.6, 17.4, and 22.7 ng/L, respectively. After lime softening, NDMA concentrations increased substantially to averages of 101, 57.2, and 60.8 ng/L, respectively. There were substantial differences between the duplicate softened samples suggesting that we need to refine our lime softening methods in terms of lime dosing and pH control.

Second Update September 1, 2020

Monthly sampling campaigns from July to November were conducted at twelve locations. The samples were processed and analyzed using the SPE-LC/MS method. Each sample was split into two parts: half of the sample was analyzed for NDMA formation directly and the other half was first lime softened and then analyzed for NDMA formation. Both lime-softened and raw subsamples were analyzed in duplicate. For the months of August, September, and October, the average NDMA precursor concentrations in the North Fork of the Crow river were 29.58, 18.47 and 20.33 ng/L, respectively. In the South Fork of the Crow river, the concentrations were consistently lower at 16.48, 15.67, and 16.57 ng/L, respectively, and in the mainstem of the Crow river, concentrations were 26.97, 28.44 and 15.44 ng/L, respectively. After lime softening treatment, NDMA concentrations increased by an average of 18.31%, 17.34% and 64.19% for samples collected in August, September, and October, respectively. Although there currently are no Federal or State of Minnesota regulations concerning NDMA, these NDMA concentrations are of concern because they all exceed the State of California’s 10 ng/L notification level for NDMA in drinking water. No major or obvious seasonal trends are

apparent from the limited data we have available thus far. A full year or more of data will be needed to identify any seasonality in NDMA precursor concentrations.

Third Update March 1, 2021

Monthly sampling campaigns were carried out from December 2020 to February 2021 at twelve sites. Because the river surface was completely frozen at several sites, we only collected seven samples in January and five samples in February. The monthly raw and lime softened water samples were analyzed for NDMA precursor concentration using the SPE-LC/MS method. The average NDMA precursor concentrations in the Crow River watershed including the mainstem and its two tributaries were 121, 22, 17.4, 17, 32.5, 40.2, 61.8, 109 ng/L from July 2020 to Feb 2021. The NDMA precursor concentration plummeted from July to August in 2020. After this, there was a gradual increase in NDMA precursor concentration from August 2020 to February 2021. After lime-softening treatment, the average NDMA precursor concentrations in the samples collected from the Crow River watershed were 57.2, 26.1, 18.6, 26.3, 64, 53.4, 812, 330 ng/L from July 2020 to Feb 2021. For most of the water samples, lime softening treatment “activated” the NDMA precursors and substantially increased NDMA formation. For example, in January and February the NDMA precursors concentration after lime softening were extremely high. These NDMA concentrations are of concern because they all exceeded, and in some cases far exceeded the State of California’s 10 ng/L notification level for NDMA in drinking water. It is important to note, however, that the chloramination step was always done at room temperature (~ 20 °C) with preformed chloramines according to UFC test conditions. It is likely that the NDMA formation in the local water systems would be much lower for two main reasons, one is the much lower water temperatures (3-5 °C) during chloramination at the water treatment plants and another is the use of a short free chlorine contact time prior to adding ammonia to form chloramines. Colder water is expected to result in slower NDMA formation rates (i.e., slower kinetics) and free chlorine can destroy precursors in a similar, albeit much less effective, manner as ozone. For the March sampling event, we will evaluate the effect of temperature by comparing NDMA formation from chloramination at room temperature with chloramination at ~ 5°C.

Fourth Update September 1, 2021

Monthly sampling campaigns were carried out from March 2021 to Aug 2021 in the Crow river. Raw and lime softened samples were chloraminated via Uniform Formation Condition test, which enables NDMA precursors to be transformed into NDMA. From March 2021 to July 2021, there was a gradual increase in NDMA precursor concentration. For the five months, the average NDMA precursor concentrations in the Crow River including the mainstem and its two tributaries were 14.2, 20.6, 17.0, 7.1, and 15.8 ng/L, respectively. We found above-average precursor concentrations in the headwater of North fork Crow river, which were up to 40, 35, 12.5, and 21 ng/L from April to July 2021. Compared to the high NDMA precursors (> 100 ng/L) found in the Crow River in July 2020, concentrations of precursors were much lower in July this year. We propose that the decrease in precursor concentrations may have resulted from this the exceedingly low discharge, which was about seven times lower than the discharge last year based on data from the Minnesota Department of Natural Resources Cooperative Gauge system. After lime softening, the average NDMA precursor concentrations in the water samples collected from the Crow River were 22.4, 34.2, 25.0, 9.7, 14.8 ng/L from March 2021 to July 2021. Except samples collected in July 2021, lime softening treatment generally “activated” the NDMA precursors and increased NDMA formation, but not as substantially as lime softening did for samples collected in Jan and Feb 2021.

Fifth Update March 1, 2022

Sampling campaigns were conducted bimonthly from August 2021 to Dec 2021 to monitor NDMA precursor concentrations throughout the Crow river watershed. Raw water and lime-softened samples were chloraminated via the uniform formation condition test and the resulting NDMA concentrations were analyzed by the SPE LC-MS/MS method. In August, October, and December, the average NDMA precursor concentrations in the Crow River watershed were 27.82 ng/L, 28.77 ng/L, and 15.74 ng/L, respectively. With lime softening treatment, the average NDMA precursor concentrations were 28.90 ng/L, 8.34 ng/L, 18.43 ng/L, respectively. Thus, lime softening treatment did not significantly affect NDMA formation for these three sampling dates. The

average NDMA precursor concentration in the raw samples collected in Dec 2020 was 40.2 ng/L, which is much higher than for December 2021. Samples collected in Jan and Feb 2022 have been processed and will be analyzed in March and compared with results from last year.

Update as of June 30, 2022

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022

We have sampled throughout the Crow River watershed once per month for about 1.5 years to assess how NDMA precursor concentrations change over space and time. To more intensively assess temporal variability, river water samples were collected weekly from the Upstream Crow River Site from late Jan to March for six consecutive weeks. The river water samples were processed according to the procedure mentioned in the previous update to prepare the raw water and lime-softened water for subsequent NDMA formation potential testing. The NDMA formation potential was measured using the uniform formation conditions (UFC) protocol with the resulting NDMA analyzed using the SPE-LC/MS/MS method we developed. The NDMA concentrations in raw water samples collected in the first, second, fourth, fifth and sixth weeks were 14.3, 16.9, 42.4, 54.0, and 35.7 ng/L respectively. The NDMA precursor concentration tripled from the first week to the fourth week and then declined, suggesting that variability in NDMA loading to the Crow River over the short term can be important. NDMA concentrations in lime softened samples were 19.6, 13.3, 36.6, 55.5, and 31.26 ng/L from the first week to the sixth week (week 3 excluded). Unlike last year when a dramatic increase in NDMA precursors was observed after lime softening for winter samples, NDMA precursor concentrations were essentially unaffected by lime softening during this sampling campaign.

In addition to this intensive temporal investigation, another sampling campaign was conducted throughout the Crow River watershed in May 2022. A total of 10 sampling sites were selected. Relatively low concentrations of NDMA precursors (i.e., average concentration of 9.85 ng/L) were observed in raw water samples collected from three sites in the South Fork of the Crow River. After lime softening treatment, NDMA precursor concentrations increased slightly to an average of 15.98 ng/L. The average NDMA precursor concentration in samples collected from the North Fork of the Crow River was 27.79 ng/L before lime softening and 51.51 ng/L after lime softening. For samples collected in the main stem of the Crow River, average precursor concentrations of 19.71 and 27.35 ng/L were detected before and after lime-softening treatment, respectively. Thus, lime softening increased NDMA formation potential more significantly for samples collected from the North Fork of the Crow River and the main stem of the Crow River.

Seventh Update as of March 1, 2023

The river water samples collected in the Fall of 2022 were processed to prepare the filtered raw water and lime-softened samples. The NDMA formation potential was measured via the uniform formation conditions (UFC) protocol. The NDMA concentration was determined using the SPE-LC/MS/MS method we developed previously. The mean NDMA formation was 17 ± 6.1 ng/L in raw water samples and 34.6 ± 11 ng/L in lime-softened water samples. Among all locations, a water sample collected in the headwaters of the North Fork of the Crow River had the highest NDMA formation (26 ± 1.2 ng/L) among raw water samples, followed by the main stem of the Crow River (22 ± 0.2 ng/L). The sample collected from the Mississippi River had the lowest NDMA formation among raw samples (6.4 ng/L). The percent increase in NDMA formation after lime softening treatment ranged from 0.41% to 238%, with a mean of 114.7%. In summary, the headwaters of the North Fork of the Crow River and the main stem of the Crow River are abundant in NDMA precursors and lime softening typically increases NDMA formation.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023)

The Crow River, a tributary of the Mississippi River in Minnesota, was sampled approximately monthly from 2020 to 2022. Samples were collected from twelve locations along the North Fork of the Crow River, the South

Fork of the Crow River, and the main stem of Crow River, and occasionally from three locations in the headwaters regions of the North and South Forks. Each of these water samples was split with approximately half of the sample analyzed for NDMA precursors directly and the other half subjected to lime softening prior to precursor analysis to explore the effect of softening treatment on NDMA formation. It is important to note that the concentration of NDMA precursors was assessed using uniform formation conditions (UFC) testing, an experimental protocol that mimics average chloramination conditions used by U.S. water utilities (room temperature, dose of 5 mg Cl₂/L of preformed monochloramine, 3-day incubation time). Important water quality parameters including dissolved organic carbon (DOC), dissolved nitrogen (DN), ammonia, nitrate, and nitrite were also quantified from July 2020 to June 2021.

Average NDMA precursor concentrations in the Crow River determined via UFC testing varied over time from 7.5 ± 4.4 ng/L in June 2021 to 109.4 ± 14.0 ng/L in Jan 2021. Lime-softening treatment, where lime was added together with alum and ferric chloride, typically resulted in an increase in NDMA formation using the UFC protocol, which was attributed to the removal of natural organic matter as indicated by the DOC concentration. NDMA_{UFC} concentrations positively correlated with DN when excluding the spring snowmelt period and negatively correlated with DOC concentration. Concentrated animal feeding operations (CAFOs) and municipal wastewater discharges are likely the major sources of NDMA precursors in the Crow River based on a combined analysis of the spatial variations of NDMA_{UFC} and the geographical distribution of anthropogenic activities in the Crow River watershed. Cropland appeared to be a less important contributor of NDMA precursors.

ACTIVITY 2: Identify NDMA precursor sources by sampling along the length of the Crow River during the season where precursor levels are highest as determined from Activity 1.

Description: Initially, a background investigation will be performed to identify likely NDMA precursor chemicals used in the Crow River watersheds. Internet searches will be performed to identify industrial, agricultural, and other pertinent activities in the Crow River region and the types of chemicals used in these activities. We will also contact representatives at the MPCA, MDH, and USGS who would have knowledge on chemical usage and occurrence in the Crow and Mississippi Rivers. We will collect samples at roughly ten-mile intervals working upstream along the Crow, North and South Forks of the Crow, and Buffalo Creek to identify where precursor levels increase significantly. Subsequent trips will involve sampling with increasing spatial resolution in river reaches with large precursor increases to hone in on specific sources. Sampling and testing of specific waste streams will be used to confirm precursor sources. We will take approximately 10 trips and collect 10 water samples per trip for a total of approximately 100 samples. Water samples will be collected from the middle of the river just below the surface. All water samples will be lime softened using a jar test apparatus to mimic the treatment that occurs in the two Twin Cities treatment facilities. After lime softening, the samples will be analyzed for NDMA precursor levels (formation potential test) as well as for organic amine concentrations. Finally, the samples will be analyzed for selected known NDMA precursor chemicals in an attempt to identify the dominant precursors and possible sources (e.g., human waste, animal waste, industrial activities).

ACTIVITY 2 ENRTF Budget: \$ 96,541

Outcome	Completion Date
1. <i>Collect river water and wastewater samples along the Crow River, North and South Forks of the Crow, and Buffalo Creek during the season with highest precursor levels.</i>	August 31, 2021
2. <i>Analyze the river and wastewater samples for NDMA precursor levels using UFC test.</i>	September 31, 2021
3. <i>Analyze the samples for total organic amine levels and specific known precursors (e.g., ranitidine, selected antibiotics).</i>	December 31, 2021

First Update March 1, 2020

An investigation of the distribution of animal feedlots, agricultural operations, and wastewater treatment plants in the Crow River watershed was done using internet searching. Antibiotics used for animal care and pesticides

used for agriculture were summarized and analyzed for their potential to form NDMA. A combined analysis on distribution of feedlots (cattle, turkey, chicken, cow, swine) and preliminary results concerning NDMA precursors in the Crow River indicates that some antibiotics used by the turkey and swine industries may be potential NDMA precursors. The herbicide diuron and other phenylurea compounds may serve as NDMA precursors because they potentially can release dimethylamine during lime softening treatment through a mechanism known as base-catalyzed hydrolysis. Dimethylamine is a well-known NDMA precursor compound. The results of NDMA precursor testing to date, including those from this study and from preliminary work in May and July of 2018, have shown that the North Fork of the Crow is consistently greater (30-50%) than the South Fork and that the Crow River is consistently greater than the Mississippi River just upstream of the confluence with the Crow. Additional sampling campaigns along the lengths of the North and South Forks of the Crow River will be performed this summer to determine “hot spots” where NDMA precursor concentrations increase suddenly suggesting nearby sources.

Second Update September 1, 2020

NDMA formation analysis of samples collected at different locations and times shows a generally high NDMA precursor concentration throughout the Crow River, indicating the sources of NDMA precursors are dispersed throughout the watershed and not concentrated in a few “hot spots”. Even samples collected from the uppermost parts of the North and South Forks of the Crow river exhibited high NDMA precursor concentrations and the concentrations did not change much along the river. Sources of precursors could include antibiotics, pesticides, or other anthropogenic compounds used in the Crow River watershed. These compounds could be transported into the Crow River via surface runoff or via shallow groundwater. Lab experiments are underway to explore NDMA formation from selected antibiotics used in the turkey and swine industries, which are prevalent throughout the Crow River watershed. Antibiotics such as chlortetracycline may be susceptible to base-catalyzed hydrolysis, a chemical reaction that occurs during the high pH conditions of lime softening. Based-catalyzed hydrolysis of chlortetracycline can release dimethylamine, a known NDMA precursor. Therefore, water samples containing a selected antibiotic will be analyzed for NDMA formation before and after base-catalyzed hydrolysis to assess the risks posed by specific chemicals used in the watershed.

Third Update March 1, 2021

For each monthly sampling event, NDMA precursor concentrations in the samples collected throughout the Crow River Watershed and in the Mississippi River were typically high for all locations, which suggests that the sources of NDMA precursors are dispersed throughout the watershed and not concentrated in a few “hot spots”. In an attempt to identify the important NDMA precursors in the Crow River, non-targeted analysis will be performed on selected raw water samples, lime softened samples, and disinfected (i.e., chloraminated) samples. Currently, we are developing a method for the non-targeted analysis which will use a new state-of-the-art QTOF mass spectrometer. We selected a few water samples with high NDMA precursor concentrations collected in October 2020 for initial testing. Samples were concentrated by a factor of ~15 using a vacufuge device and filtered in preparation for analysis by the QTOF instrument. The results are pending.

Fourth Update September 1, 2021

Sub-samples from the North Fork of the Crow River, mainstem of the Crow River and upstream Mississippi River were concentrated by a factor of 15 using a vacufuge device, and then analyzed by High-Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (HPLC-QTOF-MS). The preliminary data showed that ranitidine, a known NDMA precursor, was found in the lime softened samples collected from the mainstem and the North Fork of the Crow River but was not found in the corresponding raw water for these locations. This may imply that lime softening could release bound ranitidine, making the compound more available for transformation to NDMA upon disinfection. We are also currently trying to improve the sample preparation method for a higher concentration factor and better precursor extraction efficiency. Mix-bed cartridges packed with five different sorbents targeted at extracting various micropollutants, and PPL cartridges that are efficient in extracting natural organic matter will be used to extract NDMA precursors and concentrate the samples by a factor of approximately 500. The solid phase extraction effluent will be collected, and its NDMA

precursor concentration will be measured to evaluate the extraction efficiency. Specifically, if the NDMA precursor concentrations in the effluent is much lower than what is absorbed by the cartridges, this would indicate a satisfactory extraction efficiency.

Fifth Update March 1, 2022

Water samples were collected from three sites in the Crow and Mississippi Rivers: Downstream North Fork of the Crow (DNF), Upstream Crow (UC), and Upstream Mississippi (UM) in August, October and December. The mix-bed cartridges containing five different sorbents were used to extract potential NDMA precursors from the water samples. Both raw and lime softened samples were loaded onto the mixed-bed cartridges. To assess the precursor extraction efficiency, the SPE effluents of each sample from the mix-bed cartridges were collected in vacuum flasks. The NDMA formation of the original water samples prior to cartridge loading (SPE influents) and the SPE effluents were determined. The NDMA formation for the original water sample represents the total amount of precursors and the NDMA formation of the SPE effluent represents the precursors not retained by the cartridge. Therefore, the precursor extraction efficiency or recovery is computed as:

$$\text{SPE extraction efficiency} = (\text{SPE influent} - \text{SPE effluent}) / \text{SPE influent} \times 100\%.$$

For the samples collected from the Crow River (DNF and UC), the precursor extraction efficiencies on average were 81%, 46%, and 76% in August, October and December, respectively. For the Upstream Mississippi River samples, the extraction efficiencies were 40%, 11%, and 22% in August, October, and December, respectively. The mix-bed cartridge was more effective at recovering precursors from the Crow River samples (DNF and UC samples) than from the Upstream Mississippi River samples. This may be due to the different water matrices and the overall higher concentration of precursors in the Crow River. More importantly, the recoveries for the samples collected from the Crow River (46% ~ 81%) are sufficient for enriching precursors for non-target analysis. Therefore, solid phase extraction via mixed-bed cartridges coupled with LC-QTOF-MS analysis will be applied to identify NDMA precursors in the Crow River. Beginning in early January 2022, samples were collected from the Upstream Crow sampling site for six consecutive weeks to identify the precursors in the Crow river. Every week, the raw and lime softened samples were loaded onto mix-bed cartridge for non-target analysis to identify likely NDMA precursors. The same water samples before and after softening were also disinfected with chloramines and loaded onto mixed-bed cartridges. These post-disinfection extracts will allow us to examine changes in the chemical structures that occurred due to reaction with chloramines.

Update as of June 30, 2022

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022

In winter 2021, water samples were collected from the Upstream Crow River Site for six consecutive weeks to prepare four kinds of samples for non-targeted analysis to attempt to identify NDMA precursors. Raw water samples and lime-softened samples were prepared using the procedure described in the previous update. Half of the raw water samples (2 L) and lime-softened samples were then chloraminated under uniform formation conditions, during which NDMA precursors should have reacted with chloramines to form NDMA. The four kinds of water samples including raw samples, lime-softened samples, raw-chloraminated samples, and lime-softened-chloraminated samples were processed and analyzed using the SPE-LC-QTOF-MS method developed previously. The LC-QTOF-MS method enables us to identify unknown precursor chemicals that may be contributing to NDMA formation. After detecting all compound features in the four types of samples, potential NDMA precursor compounds in raw and lime-softened samples were identified by the sample comparison method. Specifically, chemical features in raw samples were compared to those in the corresponding raw post-chloraminated samples in which the NDMA precursors should have reacted with chloramines. Thus, features that are abundant in raw samples but decrease or disappear in the corresponding post-chloraminated sample

were selected as potential NDMA precursors. The same approach was used to identify possible precursor compounds in the lime-softened samples. Currently, analysis and interpretation of the data from the non-targeted analysis work is ongoing.

Seventh Update as of March 1, 2023

As described previously, water samples were collected from the main stem of Crow River for six consecutive weeks in the winter of 2022-23. Each water sample was lime softened and/or chloraminated, resulting in four sample types: untreated or raw samples (R), lime-softened samples (LS), raw-chloraminated samples (R-UFC), and lime-softened-chloraminated (LS-UFC) samples. Thus, there were 24 samples (6 weeks, four sample types each week) in total, analyzed by the LC-QTOF-MS protocol. A total of 5500 unique features were detected in the raw, lime-softened, raw-UFC, and lime-softened-UFC samples. Features that were detected in at least 8 samples among the 24 samples were regarded as High Detection Frequency (HDF) features. Among all features, there were approximately 900 HDF features in total, among which 108 HDF features were substantially removed (at least 50% decrease) during the UFC test, indicating these compounds could be NDMA precursors.

A list of 35 candidate compounds was compiled, and we have confirmed the identities of ten of these compounds, including the anti-depressant desvenlafaxine and opioid analgesic o-desmethyiltramadol (both are known NDMA precursors), and other pharmaceuticals, such as, gabapentin, metoprolol acid, alpha-hydroxy metoprolol, atenolol, mefenorex, 3-hydroxymorphinan, and the illicit substance cocaine. Ongoing work is focused on identifying the remaining candidate compounds. This will help us to assess if specific compounds and activities in the watershed are likely contributors to NDMA formation during water treatment.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023)

Water samples were collected from the main stem of the Crow River for six consecutive weeks, at a time and location where high levels of NDMA precursors were expected. A multi-sorbent solid phase extraction (SPE) cartridge, packed with five different sorbents, was used to extract the potential NDMA precursors from water samples before and after chloramination. The basic idea was that nitrogen-containing compounds that were degraded during disinfection with chloramines were potential precursors. We then used liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS) to analyze the chemicals extracted from the water samples. Features with a high detection frequency that showed significant degradation during chloramination were selected as candidates for identification via our a nontargeted analysis workflow that we developed.

NDMA precursors identified in the Crow River water samples included: tramadol, venlafaxine, metformin, 3-cyclohexyl-1,1-dimethylurea, amine oxide surfactants (myristamine oxide and lauramine oxide). In addition, precursors for other nitrosamines were also identified, including lidocaine (a commonly used local anesthetic), DEET (the most common active ingredient in insect repellents), tributylamine (industrial chemical), cocaine (narcotic), and trans-Nicotine-1-oxide (a derivative of nicotine). Some anthropogenic chemicals, that are not known N-nitrosamine precursors but were detected frequently in the Crow River were identified as well, including lamotrigine, carbamazepine, hydroxyatrazine, mefenorex, bupropion, ciclopirox, fexofenadine, and irbesartan. The majority of the identified NDMA precursors can be attributed to municipal wastewater effluents, which emphasizes the significance of municipal wastewater or other human waste discharges (e.g., septic systems) as an important source of NDMA precursors to the Crow River.

ACTIVITY 3: Investigate the effects of chlorine and ozone concentration and exposure time on destruction of precursors.

Description: There is some evidence that pre-oxidation of the precursors with free chlorine or ozone prior to addition of chloramines reduces the subsequent formation of NDMA. We will investigate the effects of chlorine and ozone concentration and exposure time on destruction of precursors. Mississippi River water samples will be lime softened as describe above and then treated with either free chlorine or ozone over a range of typical concentrations and contact times and then subjected to UFC testing to determine NDMA precursor levels. The

results will be compared to those without free chlorine or ozone treatment to assess overall treatment effectiveness.

ACTIVITY 3 ENRTF Budget: \$ 98,685

Outcome	Completion Date
1. Collect Mississippi River water samples and pretreat using chlorine or ozone.	April 30, 2022
2. Analyze the river water samples before and after treatment for NDMA precursor levels using the UFC test.	September 31, 2022

First Update March 1, 2020

No progress on this activity.

Second Update September 1, 2020

No progress on this activity.

Third Update March 1, 2021

Experiments to test the effects of pre-oxidation with ozone on NDMA precursor levels are currently being set up in the laboratory. Raw and lime softened water samples from the Minneapolis and/or Saint Paul water treatment facilities will be subjected to varying doses of ozone, incubated until the ozone dissipates, and then chloraminated (UFC conditions) and subsequently analyzed for NDMA. Ozonation of the water samples will occur in 1-liter amber glass bottles. A laboratory-scale ozone generator will be used to create an ozone stock solution by bubbling ozone gas into chilled ultrapure water. Initially, we will assess how quickly the ozone decays or reacts in the water samples. An aliquot of the ozone stock solution will be added to the water sample and incubated for 10-15 minutes with periodic sampling to measure the aqueous ozone concentration using the indigo method. After these initial ozone decay experiments, the water samples will be dosed with ozone and incubated until the ozone dissipates and then tested for NDMA precursor concentration. Results will be compared with those from the samples before ozone was applied.

Fourth Update September 1, 2021

Initial bench-scale (i.e., batch) experiments to quantify ozone decay rates in water samples are currently being conducted. Different ozone doses (1, 2, and 5 mg/L) and water temperatures (5 and 20 degrees Celsius) are being investigated. Ozone coupled with hydrogen peroxide to promote hydroxyl radical formation will also be explored. Treatment with ozone and hydrogen peroxide is considered an advanced oxidation process (AOP), with hydroxyl radicals serving as potent oxidizers that can potentially enhance destruction of NDMA precursors and limit subsequent NDMA formation upon disinfection with chloramines. Hydroxyl radicals will be measured indirectly in parallel with the dissolved ozone measurements by introducing a probe compound called para-chlorobenzoic acid (pCBA). The concentration of probe compound over time is quantified via high-pressure liquid chromatography (HPLC). The decrease of the probe compound in relation to the measured ozone at a given time is used to calculate the hydroxyl radical concentration. After these initial ozone decay (and hydroxyl radical formation) experiments, we will ozone water samples and test NDMA formation and compare the results with those from samples that had not been subjected to ozone treatment.

Fifth Update March 1, 2022

Chemical oxidation experiments were conducted on water samples collected on July 19, 2021 from the Saint Paul Regional Water Services (SPRWS) treatment facility. These water samples consisted of raw water (i.e., before any treatment), lime softened water, and lime softened and recarbonated water (i.e., re-carb water). The oxidation treatments included ozone (1, 2, and 5 mg/L), ozone (2 mg/L) coupled with hydrogen peroxide (1 mg/L) which is termed an advanced oxidation process (AOP), and free chlorine (4.24 mg/L as Cl₂). The free chlorine treatment mimics the free chlorine exposure used at the full-scale SPRWS treatment facility.

Experiments were performed at two temperatures (5 and 20 °C) to represent the range of water temperatures experienced at the facility. The AOP process is termed “advanced oxidation” because the process promotes the formation of highly reactive hydroxyl radicals that are able to rapidly degrade a wide variety of organic chemicals found in water. The formation of these hydroxyl radicals was tracked using a probe compound. Water samples before and after chemical oxidation were then tested for NDMA formation. Without oxidation, raw, lime softened, and re-carb water formed NDMA concentrations of 8.5, 20.7, and 26.3 ng/L, respectively. Chemical oxidation with ozone or AOP decreased NDMA concentrations to similar levels (ranging from 1.9 to 4 ng/L) regardless of treatment conditions. Free chlorine treatment of re-carb water at both 5 and 20°C was also effective, reducing NDMA concentrations from 26.3 ng/L to 2.1 and 1.4 ng/L, respectively. Analysis of hydroxyl radical formation indicated that more hydroxyl radicals were produced with increasing ozone doses for all water types. Finally, pre-oxidation experiments will be repeated on sample water collected from the Saint Paul water treatment facility on January 21, 2022 to investigate the impact of seasonal changes in water quality.

Update as of June 30, 2022

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022

Batch oxidation experiments were performed over a range of oxidant concentrations and temperatures of 5 and 20°C using winter samples collected from the Saint Paul Regional Water Services (SPRWS) water treatment facility. Specifically, water samples were collected from three stages along the treatment train: raw influent, directly after lime-softening, and re-carbonated water post lime softening. Pre-oxidation was explored with three common oxidants over a range of dosage: ozone (1, 2, and 5 mg/L), free chlorine (4.24 mg/L as Cl₂), and a combination of 2 mg/L ozone and 1 mg/L hydrogen peroxide. The treatment in which both ozone and hydrogen peroxide are used is termed an advanced oxidation process or AOP. NDMA precursor concentrations were determined before and after pre-oxidation using the uniform formation conditions test. NDMA precursor concentrations in the raw, lime softened, and re-carbonated samples collected in winter were 10.1 ng/L, 17 ng/L and 14 ng/L, respectively. All oxidation treatments applied to raw, lime-softened, and re-carbonated water effectively decreased the NDMA_{UFC} concentrations to 1.1 ng/L to 4.6 ng/L, which were all below the Minnesota Department of Health’s (MDH) guidance value of 5 ng/L and the State of California’s notification level of 10 ng/L. Furthermore, the effectiveness of pre-oxidation at destroying NDMA precursors was also investigated using a water sample collected from the Crow River. NDMA_{UFC} concentrations in raw and lime-softened Crow River samples were 33.7 ng/L and 36.5 ng/L, respectively. An ozone dose of 2 mg/L at 20°C was reasonably effective at destroying NDMA precursors in both raw and lime-softened Crow River water to 6.6 ng/L and 10.1 ng/L, respectively.

Seventh Update as of March 1, 2023

Ranitidine, a known NDMA precursor, was spiked into a sample of lime softened and recarbonated water (i.e., collected from the Saint Paul Regional Water Services water treatment facility in winter) to study the effectiveness of different pre-oxidation practices at destroying NDMA precursors when present at high concentrations. The water samples spiked with 1200 ng/L ranitidine and then pre-oxidized by dosing ozone (1 to 5 mg/L), 5 mg/L free chlorine, or 2 mg/L ozone followed by 5 mg/L free chlorine. Prior to oxidation, the ranitidine spike increased the average NDMA concentration in recarbonated water from 14 ng/L to 358 ± 3.1 ng/L. An ozone dose of 1 mg/L effectively reduced the mean NDMA formation to 21.3 ± 8.3 ng/L. Increasing ozone doses of 2 mg/L and 5 mg/L further decrease NDMA formation to 8.2 ± 1.5 ng/L and 4.2 ± 0.4 ng/L. Free chlorine alone decreased NDMA formation to 10.7 ± 3.8 ng/L. A combination of 2 mg/L ozone and 5 mg/L of free chlorine resulted in the lowest NDMA formation (2.4 ± 0.6 ng/L). All pre-oxidation treatments effectively destroyed ranitidine and any other NDMA precursors present in the water and decreased NDMA formation by 94% to 99%.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023)

Batch experiments were performed to investigate the effectiveness of pre-oxidation at destroying NDMA precursors. Samples included: 1) raw and lime softened Crow River water, and 2) raw, lime-softened, and re-carbonated water samples collected from the St. Paul Regional Water Services (SPRWS) treatment plant during the winter. SPRWS uses the Mississippi River as its main water supply. Selected water samples also were spiked with a model NDMA precursor, ranitidine, before further testing. The effect of ozone dose (1, 2, and 5 mg/L) and water temperature (5 and 20 degrees Celsius) were investigated. Free chlorine (4.24 mg/L as Cl₂) and ozone coupled with hydrogen peroxide to promote hydroxyl radical formation were also tested.

NDMA precursor concentrations in the raw, lime softened, and re-carbonated SPRWS water samples collected in winter were 10.1 ng/L, 17 ng/L and 14 ng/L, respectively. All oxidation treatments applied to raw, lime-softened, and re-carbonated water effectively decreased the NDMA_{UFC} concentrations to 1.1 ng/L to 4.6 ng/L, which were all below the Minnesota Department of Health's (MDH) guidance value of 5 ng/L and the State of California's notification level of 10 ng/L. Furthermore, the effectiveness of pre-oxidation at destroying NDMA precursors was also investigated using a water sample collected from the Crow River. NDMA_{UFC} concentrations in raw and lime-softened Crow River samples were 33.7 ng/L and 36.5 ng/L, respectively. An ozone dose of 2 mg/L at 20°C was reasonably effective at destroying NDMA precursors in both raw and lime-softened Crow River water to 6.6 ng/L and 10.1 ng/L, respectively.

The ranitidine spike increased the average NDMA concentration in recarbonated water from 14 ng/L to 358 ± 3.1 ng/L. An ozone dose of 1 mg/L effectively reduced the mean NDMA formation to 21.3 ± 8.3 ng/L. Increasing ozone doses of 2 mg/L and 5 mg/L further decrease NDMA formation to 8.2 ± 1.5 ng/L and 4.2 ± 0.4 ng/L. Free chlorine alone decreased NDMA formation to 10.7 ± 3.8 ng/L. A combination of 2 mg/L ozone and 5 mg/L of free chlorine resulted in the lowest NDMA formation (2.4 ± 0.6 ng/L). All pre-oxidation treatments effectively destroyed ranitidine and any other NDMA precursors present in the water and decreased NDMA formation by 94% to 99%. Overall, these results suggest that ozone at moderate doses of about 2 mg/L is very effective at destroying NDMA precursors. Furthermore, free chlorine at typical doses and contact times used for disinfection is also effective.

IV. DISSEMINATION:

Description: Findings will be disseminated directly to the two participating utilities as a written report and an in-person presentation. Findings will also be disseminated and archived via reports to LCCMR, peer-reviewed journal publications (open access), and presentations at conferences. We will also, if and when appropriate, disseminate results via press releases to the media and via the MDH website.

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the [ENRTF Acknowledgement Guidelines](#).

Dissemination Budget: \$ 37,050

First Update March 1, 2020

No progress on this activity.

Second Update September 1, 2021

No progress on this activity.

Third Update March 1, 2021

No progress on this activity.

Fourth Update September 1, 2022

An update on our research will be presented at the annual conference of the Minnesota Chapter of the American Water Works Association in Duluth in mid-September.

Fifth Update March 1, 2022

An update on our research was presented by graduate students Jiaqi Li and Eric Noe at the annual conference of the Minnesota Chapter of the American Water Works Association in Duluth in mid-September.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

An update on our research was presented by graduate students Jiaqi Li and Eric Noe at the annual conference of the American Chemical Society in San Diego in mid-March.

Seventh Update as of March 1, 2023:

A journal article on the testing of pre-oxidation for NDMA precursor destruction was submitted to the peer-reviewed scientific journal *Environmental Engineering Science* in February 2022.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

The findings of the project were presented at the annual conference of the Minnesota Chapter of the American Water Works Association in 2021, at the annual conference of the American Chemical Society in 2022, and at the annual conference of the AEESP in 2023.

A journal article concerning the pre-oxidation for NDMA precursor destruction was published in the journal *Environmental Engineering Science*. A journal article concerning the spatial and seasonal variations of NDMA precursors in the Crow River watershed and the effect of lime softening on NDMA formation was accepted by the journal *Environmental Science & Technology*.

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

Explanation of Capital Expenditures Greater Than \$5,000: N/A

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:

Enter Total Estimated Personnel Hours for entire duration of project: 5460	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 2.63
--	---

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:

Enter Total Estimated Contract Personnel Hours for entire duration of project: N/A	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: N/A
--	---

VI. PROJECT PARTNERS:

Name	Title	Affiliation	Role
William Arnold	Professor	University of Minnesota	Co-Investigator

A. Partners outside of project manager's organization receiving ENRTF funding: N/A

Name	Title	Affiliation	Role
-None -			

B. Partners outside of project manager's organization NOT receiving ENRTF funding

Name	Title	Affiliation	Role
George Kraynick	Water Quality Manager	MWTDS	Sampling, sharing data
Jim Bode	Water Production Manager	SPRWS	Sampling in Crow River

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

The main goals of the proposed research are to identify significant sources of NDMA precursors to the Upper Mississippi River and to develop pollution prevention strategies for reducing precursor inputs. For example, if it is found that a certain class of antibiotics given to farm animals is an important precursor, then one possible **strategy** would be to suggest a ban on that class of antibiotics for animal use. The **project impact** will be improved drinking water quality and protection of the health and safety of Minnesotans, particularly those in the Twin Cities and surrounding suburbs served by the Minneapolis and St. Paul water utilities.

C. Funding History: N/A

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted March 1 and September 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2023

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet

B. Visual Component or Map

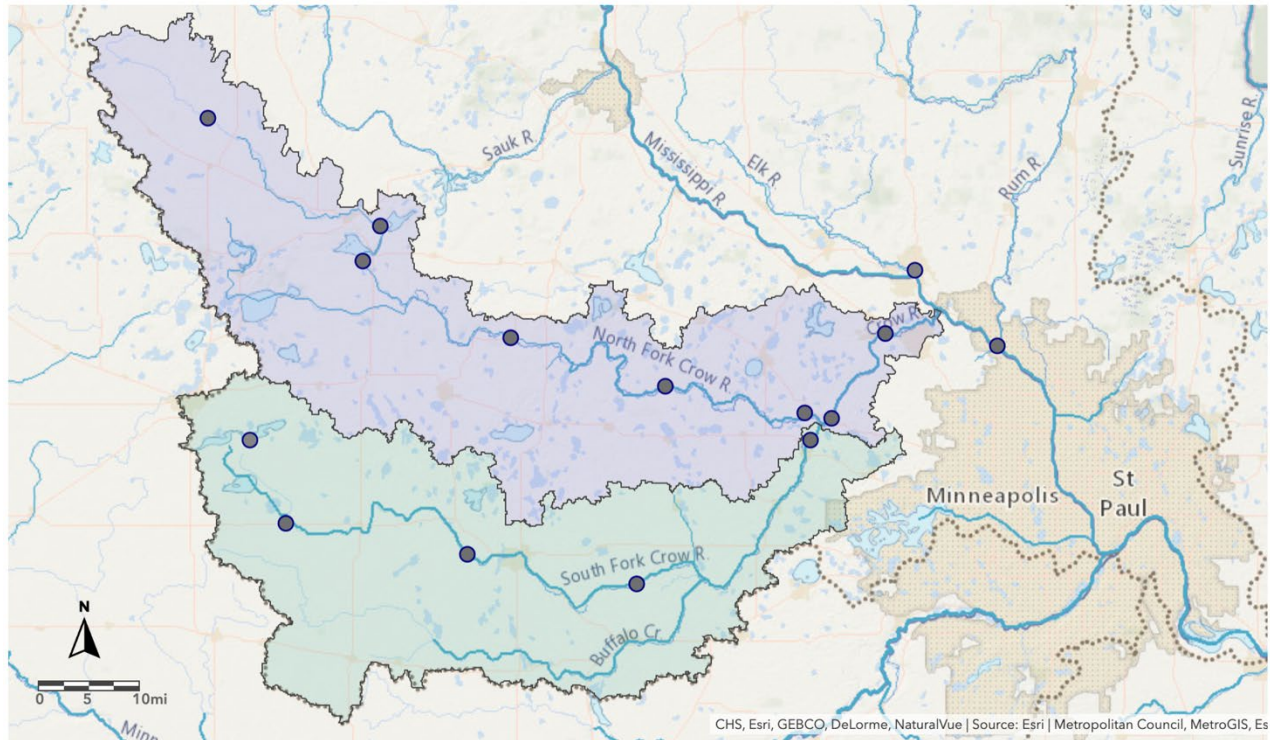


Figure 1. A map of sampling locations (black points) along the two tributaries (North Fork of the Crow River, South Fork of the Crow River), main stem of the Crow River, and upstream and downstream the confluent point of Crow river and Mississippi River. The watersheds for the North Fork Crow River and main stem of the Crow River are colored in purple while the watershed for South Fork of the Crow River is colored in green. The map is reproduced with permission from Esri. Map image is the intellectual property of Esri and is used herein under license. Copyright © Esri and its licensors.

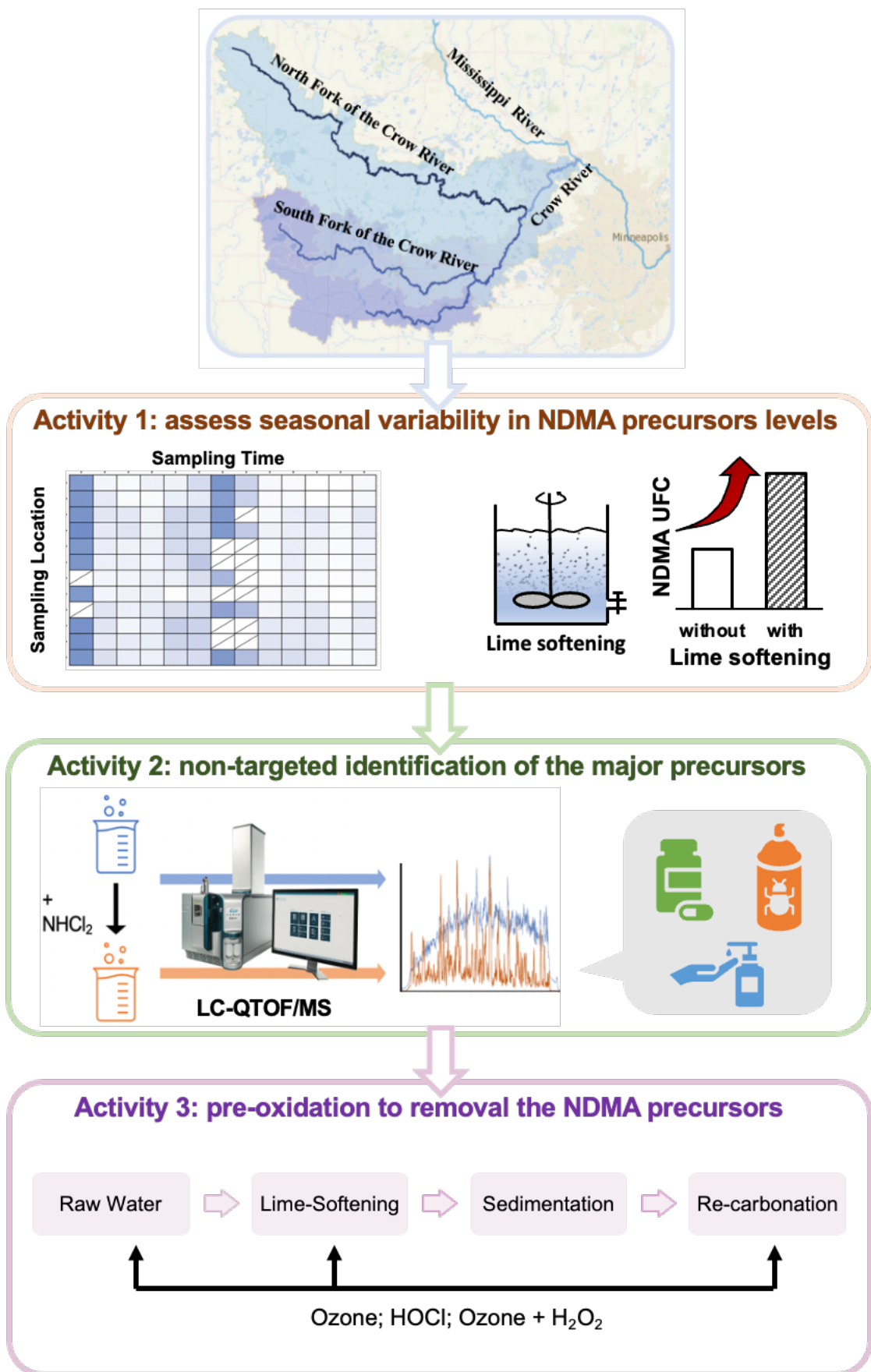


Figure 2. Visualization of this project: Improving Drinking Water for Minnesotans through Pollution Prevention

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2019 Budget Spreadsheet- FINAL

Legal Citation:

Project Manager: Raymond M. Hozalski

Project Title: Improving Drinking Water for Minnesotans through Pollution Prevention

Organization: University of Minnesota

Project Budget: \$345,000

Project Length and Completion Date: 3 years, June 30, 2023

Today's Date: 8/15/23

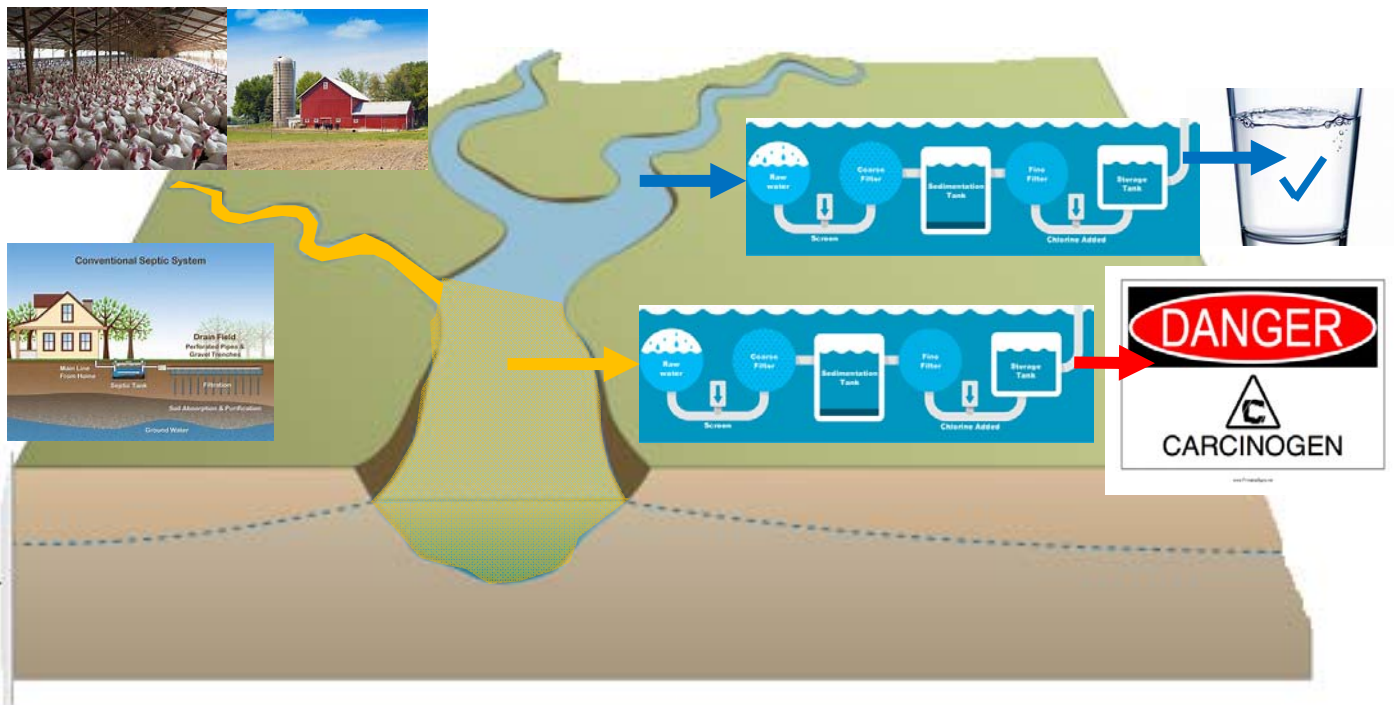


ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Budget 8/15/23	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	\$ 283,764	\$ 283,764	\$ -
Raymond Hozalski, Project Manager (75% salary, 25% fringe benefits). 8% FTE for years 1 - 3. Overall project coordination, lead Task 1 and Task 3 studies, co-lead Task 4. \$57,644			
William Arnold, Co-Project Manager (75% salary, 25% fringe benefits). 6% FTE for years 1 - 3. Lead Task 2 studies, co-lead Task 4. \$50,979			
Graduate student Research assistant 1, Perform environment fate studies for Task 1, perform computations in Task 3 (55% salary, 45% fringe benefits) 50% FTE for years 1-3. \$139,876			
Undergraduate researchers. Assist with laboratory experiments. 16 hrs per week during the calendar year for years 1 and 2 only. (100% salary). \$19,797			
Post-doctoral researcher, Train graduate student to perform river sampling and experiments (80% salary, 20% fringe benefits) 100% FTE for 3 weeks in year 1. \$3,704			
Equipment/Tools/Supplies			
Laboratory Supplies (chemical and isotopically labelled standards, chemical reagents, necessary glassware, solvents, consumable supplies, laboratory notebooks, software licenses, instrument	\$ 35,538	\$ 35,538	\$ -
Analytical time for quantification of NDMA in water samples.	\$ 10,000	\$ 7,300	\$ 2,700
Analytical time for identification of precursor chemicals using mass spectrometry.	\$ 10,000	\$ 5,455	\$ 4,545
Travel expenses in Minnesota			
University vehicle rental and hotel stays to collect water samples. Presentation of results at local conferences. Reimbursement will be according to University of Minnesota guidelines.	\$ 5,477	\$ 5,477	\$ -
Other (Open access fees for peer-reviewed journal papers)			
Publication charges to make to make published journal articles (2-3) immediately available via open access to maximize data availability and dissemination.	\$ 221	\$ -	\$ 221
COLUMN TOTAL	\$ 345,000	\$ 337,534	\$ 7,466

OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		\$ -	\$ -	
State: U of MN (In-kind; Indirect costs not charged to project)	Secured	\$ 167,067	\$ 114,247	\$ 52,820
In kind: Because the project is overhead free, laboratory space, electricity, and other facilities/administrative costs (54% of direct costs excluding permanent equipment and graduate student tuition benefits) are provided in-kind.		\$ -	\$ -	\$ -

PAST AND CURRENT ENRTF APPROPRIATIONS	Amount legally obligated but not yet spent	Budget	Spent	Balance
Current appropriation:		\$ -	\$ -	\$ -
Past appropriations:		\$ -	\$ -	\$ -

The carcinogen NDMA is formed when chlorine disinfectants react with precursors in human and/or animal waste discharges.



Outcomes:

1. Quantify carcinogen-forming precursor levels.
2. Identify times and places where maximum precursor inputs occur.
3. Optimize drinking water disinfection to minimize carcinogen formation.
4. Protect human health via pollution prevention.

Animal Feedlots and Domestic Wastewater Discharges are Likely Sources of *N*-Nitrosodimethylamine (NDMA) Precursors in Midwestern Watersheds

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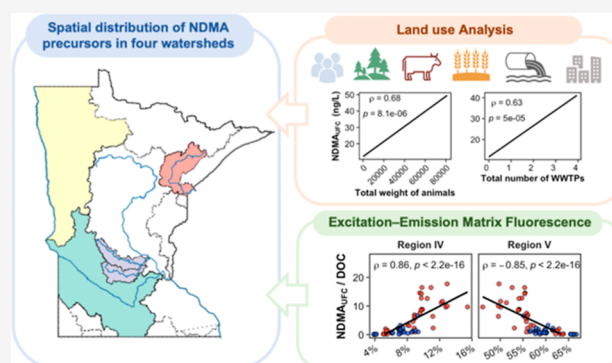
Article Recommendations



Supporting Information

ABSTRACT: *N*-nitrosodimethylamine (NDMA) precursor concentrations along four major rivers in Minnesota, USA were quantified and correlated with watershed land cover types, anthropogenic activity, and organic matter characteristics. River water samples (36 in total) were chloraminated under uniform formation conditions (UFC) before and after lime-softening treatment, and the resulting NDMA concentrations were quantified (NDMA_{UFC}). Regarding land cover, NDMA_{UFC} in raw river water exhibited weak positive correlations with urban land ($\rho = 0.33$, $p = 0.05$) and cropland coverage ($\rho = 0.35$, $p = 0.04$). For anthropogenic activity, NDMA_{UFC} in raw river water positively correlated with the number of feedlots ($\rho = 0.57$), total weight of animals ($\rho = 0.68$), and total number of domestic wastewater treatment plants (WWTPs; $\rho = 0.63$) with $p < 0.01$. NDMA_{UFC} positively correlated with region IV fluorescence intensity from fluorescence excitation–emission spectra ($\rho = 0.70$, $p < 0.01$). Lime softening of river water typically increased NDMA_{UFC} and preferentially removed organic matter that fluoresces in region V, suggesting that the organic matter in this region decreases NDMA_{UFC} by competing for available chloramines. Overall, animal feedlots, along with domestic WWTPs, are predominant sources of NDMA precursors in the studied watersheds, while croplands and urban runoff are of lesser importance.

KEYWORDS: chloramination, disinfection byproducts, land use, fluorescence excitation/emission matrices, natural organic matter (NOM), lime softening



INTRODUCTION

Disinfection is used to minimize the risk of exposure to pathogenic microorganisms via public water supplies.¹ Many drinking water treatment plants distribute water with chloramines as the residual disinfectant because it provides a stable residual while minimizing the formation of regulated and nonregulated halogenated disinfection byproducts (DBPs). Unfortunately, the use of chloramines may lead to the formation of nitrogenous DBPs such as *N*-nitrosamines and haloacetonitriles.^{2,3} *N*-nitrosodimethylamine (NDMA), the most frequently detected nitrosamine, is concerning to public health due to its toxicity, carcinogenicity, and mutagenicity.⁴ Currently, NDMA in drinking water is not regulated by any federal or state agencies in the United States (US). Many states, however, have established guidelines for NDMA in drinking water.⁵ The California Department of Public Health has set a public health goal of 3 ng/L and a notification level of 10 ng/L for NDMA, an advisory concentration based on health risks for unregulated chemicals.⁶ The Minnesota Department of Health has set a guidance value of 5 ng/L.⁵

NDMA precursors include a wide range of chemicals consisting of secondary and tertiary amines.⁷ Considering the

wide range of anthropogenic chemicals that contain secondary and tertiary amines, more research is needed to identify important NDMA precursor sources for potential mitigation measures to limit NDMA formation. Previous studies reported that nitrosamines and their precursors were negligible in pristine headwaters and that municipal wastewater effluents were the dominant source of nitrosamines in surface water.^{8,9} Agricultural sources of NDMA precursors include cropland runoff and wastewater from animal husbandry operations that could release herbicides (i.e., diuron and isoproturon) and veterinary antibiotics (i.e., tetracyclines and macrolides), respectively.^{8,10,11} Urban runoff also releases NDMA precursors into surface waters, especially during rainfall events.¹² Nitrosamines and their precursors may also be released from

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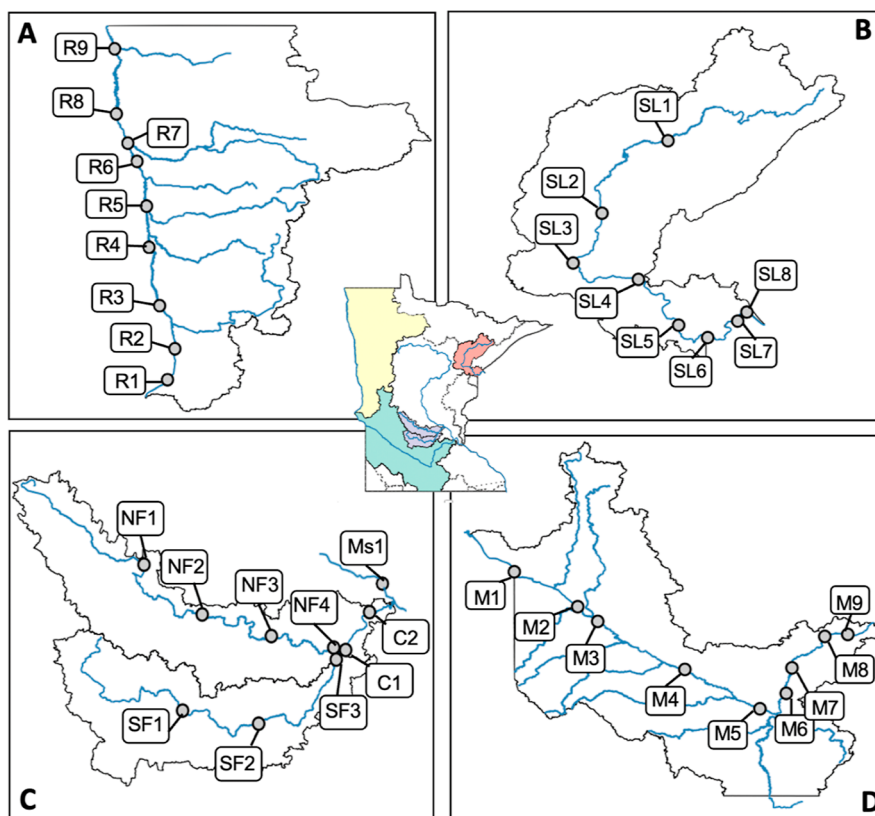


Figure 1. Sampling locations in the four watersheds (A) Red River (R); (B) St. Louis River (SL); (C) Crow River (NF = North Fork of the Crow River, SF = South Fork of the Crow River, C: main stem of the Crow River, and Ms = Mississippi River); and (D) Minnesota River (M) in Minnesota, USA.

scrap tires, which likely contribute to the NDMA precursor pool in urban runoff.¹³ Many prior investigations involved sampling directly from point sources, such as WWTP effluents, hospital wastewater, laundry water, agricultural runoff channels, stormwater drains, and manure or waste lagoons at animal operation facilities.^{8,14–17} The receiving waters, which serve as the source water for drinking water treatment plants, have received considerably less attention. Additionally, research assessing the relationships between the spatial distribution of NDMA precursors in surface water and surrounding land uses via geostatistical analysis is lacking.

Several studies have tried to use representative fluorescence peaks from fluorescence excitation–emission matrix (EEM) spectroscopy to predict NDMA formation.^{18–20} A positive correlation was reported between NDMA formation and protein-like components in raw and finished water samples from nine drinking water treatment plants in South Carolina, USA.²⁰ Similarly, a study of *N*-nitrosamines in ten drinking water treatment plants in China revealed that all *N*-nitrosamines, except *N*-nitrosodiphenylamine and *N*-nitrosomorpholine, demonstrated a strong correlation with a protein-like component in the drinking water.¹⁹ More recently, the removal of protein-like fluorescence correlated with a decrease in NDMA formation at a membrane-based wastewater treatment plant.¹⁸ Correlations between NDMA formation and the organic matter fluorescence of surface water samples collected from watersheds with varying land use characteristics could provide additional insight into NDMA formation. Additionally, NDMA formation in prior investigations was evaluated using formation potential (FP) testing, wherein an excess chlorine

dosage and prolonged reaction time were applied in an attempt to maximize NDMA formation.²¹ Thus, further examination of the association between representative fluorescence peaks and NDMA formation using surface water samples under more realistic disinfection conditions, such as using the uniform formation conditions (UFC) test, is needed.

This study examined spatial variations of NDMA precursors along rivers in four major watersheds with varying land use characteristics in the State of Minnesota, USA. A geostatistical analysis was performed to extract information about anthropogenic activities around each sampling location to assess relationships between land use and the NDMA formed using the UFC test (NDMA_{UFC}). Because previous work in our laboratory identified an increase in NDMA formation following lime softening,²² the effect of lime softening on NDMA formation was further explored in this study. In addition, correlations between selected fluorescence peak intensities and NDMA formation using the UFC test were examined.

MATERIALS AND METHODS

Chemicals and Reagents. Supplies and purities of all chemicals and reagents are listed in the [Supporting Information](#).

Sample Collection. River water samples were collected in spring/summer 2022 from four major watersheds in Minnesota, USA: the Minnesota River (8 sites), the Red River (9 sites), the St. Louis River (8 sites), and the Crow River (10 sites), with locations shown in [Figure 1](#). The collection dates and GPS coordinates of all sampling sites are

listed in Table S1. All river water samples were collected from the center of the river below the water surface using a high-density polyethylene (HDPE) bucket, which was lowered from a bridge using a rope. Water samples were stored in 20 L HDPE square jars and transported back to the laboratory in coolers on ice within 1 day of collection. To better understand the contribution of WWTP discharges to the NDMA precursor pool in surface waters, samples were also collected from the discharge point and from one upstream and three downstream locations of three WWTPs (WWTP A in the Cannon River, WWTP B in the Minnesota River, and WWTP C in the St. Croix River; Figure S1). The Cannon River and Minnesota River WWTP discharge samples were collected twice (i.e., in the summer and fall of 2022), while the St. Croix WWTP discharge samples were collected only in the fall of 2022. These samples were collected by submerging 5 L HDPE jars from the side of a canoe. All HDPE containers were cleaned by soaking them in an Alconox solution overnight and then rinsing them with deionized water and ultrapure water multiple times.

Sample Preparation and UFC Testing. Raw water samples were filtered through a GF/F glass fiber filter (Whatman, WHA1825047, 0.7 μm , 47 mm) before the chloramination test.²² To further investigate the effect of lime softening on NDMA formation, lime-softened samples were prepared by using river water samples collected from different watersheds following a protocol mimicking the lime softening process applied at the St. Paul Regional Water Services drinking water treatment plant.²² Details for the lime softening experiments are provided in the Supporting Information.

For UFC testing, filtered raw and lime-softened samples were buffered to pH 8 (4 mM borate buffer) and dosed with 5 mg Cl_2/L of freshly prepared monochloramine solution. The samples were then incubated in the dark at room temperature for 3 days.^{23,24} Following incubation, the residual chloramine concentration was quantified using the 2,2-azino-bis(3-ethyl-benzothiazoline)-6-sulfonic acid-diammonium salt (ABTS) method and then quenched with 31 mg/L of L-ascorbic acid.²⁵ Details of monochloramine solution preparation and residual chloramine determination are provided in Supporting Information.

NDMA Determination. Duplicate 0.5 L samples were spiked with 10 ng/L NDMA-d6 and loaded into activated carbon solid phase extraction (SPE) cartridges (United Chemical Technologies, Enviro-Clean EPA Method 521²⁶) under vacuum at a flow rate ≤ 5 mL/min. Following 20 min of drying under vacuum, the cartridges were eluted with 15 mL of dichloromethane (DCM) at a flow rate of 2 to 3 mL/min. The DCM extracts were evaporated under nitrogen to remove all DCM, leaving only a small amount of water in the tubes, which was then reconstituted to a final volume of 0.5 mL using ultrapure water. The NDMA was quantified by capillary liquid chromatography (LC) mass spectrometry (MS) with positive electrospray ionization using a TSQ Vantage triple quadrupole MS (Thermo Scientific) connected to a high-performance liquid chromatography (HPLC) system (Dionex Ultimate 3000 rapid separation LC, Thermo Scientific). Quantification ions were $m/z = 58.17$ and 64.17 , and qualification was $m/z = 43.13$ and 46.13 for NDMA and NDMA-d6, respectively. Detailed parameter settings for the method are given in Table S2. The recovery of NDMA and method detection limit are given in Supporting Information. Results from additional

experiments to test the effects of SPE cartridge drying time on the potential formation of NDMA during extraction²⁷ suggest that this mechanism is not important for our water samples for drying times of up to 30 min (details in the Supporting Information).

NOM Size Distribution by High-Pressure Size Exclusion Chromatography. High-pressure size exclusion chromatography (HPSEC) was performed on an Agilent 1200 Infinity HPLC with an XBridge Protein BEH SEC column (125 $\text{\AA}$, 3.5 μm , 7.8 mm $\times$ 300 mm) purchased from Waters. The mobile phase consisted of 2 mM phosphate buffer at pH 7 with 0.1 M NaCl. The flow rate was 1.0 mL/min over an elution time of 15 min. Acetone and polystyrenesulfonate (PSS) standards with nominal molecular weights of 18,000, 8000, 4600, and 1000 Da were prepared in the mobile phase solution. Standards and all of the NOM samples were detected at 254 nm. Acetone (detected at 280 nm) was used to calibrate the void volume of the column.^{28,29}

NOM Characterization and UV-vis and Fluorescence Spectroscopy. Raw and lime-softened samples were filtered through 0.22 μm poly(ether sulfone) (PES) membrane filters before collection of EEM spectra. Absorbance at 254 nm (UV_{254}) was measured using a UV-vis spectrophotometer (Beckman Coulter DU 530). Dissolved organic carbon (DOC) was analyzed using a total organic carbon analyzer (TOC-L CPH, Shimadzu). Specific UV absorbance (SUVA) at 254 nm was calculated by normalizing the UV_{254} by the DOC concentration (mg C L^{-1}). The EEM and UV-visible light spectra were scanned using a Horiba Aqualog fluorescence spectrophotometer using a 1 cm quartz cell according to a previously developed method.³⁰ Samples were diluted with ultrapure water before measurement if $\text{UV}_{254} > 0.6 \text{ cm}^{-1}$. The excitation wavelength was varied from 220 to 600 nm with an increment of 3 nm, and the emission wavelength ranged from 240 to 800 nm with a 2.33 nm increment. The integration time was set to 1 s for raw samples with a UV_{254} generally close to 0.4 and 2 s for lime-softened samples with a UV_{254} typically lower than 0.1. Ultrapure water served as the blank. The blank spectra were subtracted from the EEMs of all samples to remove the Rayleigh effect, and inner filter effects were corrected and normalized using the Raman peak intensity in the drEEMs toolbox.³¹ For the Horiba Aqualog instrument used in this study, a highly significant linear relationship ($R^2 = 0.99995$) was observed between the excitation intensity at 254 nm and the absorbance at 254 nm after inner filter effect correction for a series of dilutions from a concentrated sample ($\text{UV}_{254} = 0.8 \text{ cm}^{-1}$), confirming the validity of the inner filter effect correction and its utility for concentrated CDOM analysis.³²

Data Analysis and Statistics. Land Use. The influence of land cover and anthropogenic activity on NDMA_{UFC} at each sampling site was assessed for circles with different radii (2.5, 5, 7.5, 8.5, 10, 12.5, and 15 km) and drawn around a point upstream of each sampling site at various distances (1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 km). Previous work evaluating anthropogenic chemical inputs has used a similar protocol.^{33–35} Within each circle, land cover and anthropogenic activity information were extracted, processed, and analyzed via R and ArcGIS online.^{36–38} All anthropogenic activities within each circle, including the number of WWTPs, the WWTP design flow, the number of feedlots, the total weight of livestock, the number of brownfields, and the number of septic tanks, were extracted from the Minnesota Geospatial

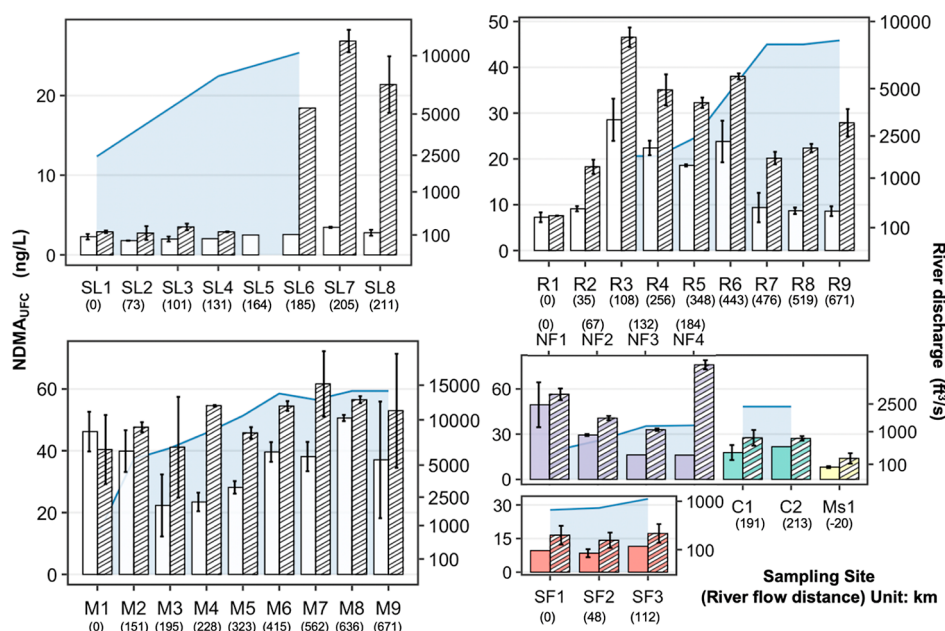


Figure 2. Spatial distribution of NDMA precursors along the (a) St. Louis River, (b) Red River, (c) Minnesota River, and (d) Crow River. All river samples were collected between May and July in 2022. Pattern-filled bars represent the lime-softened samples, while blank bars correspond to the raw samples. The sampling sites were named by the initials of each river and numbered from upstream to downstream (SL: St. Louis River; R: Red River, M: Minnesota River; NF: North Fork of the Crow River; SF: South Fork of the Crow River, C: Crow River main stem, and Ms: Mississippi River). The river discharge data were obtained from the CSG database of the Minnesota Department of Natural Resources. Detailed information for all gauges was shown in [Supporting Information](#). Error bars represent the difference between duplicate measurements.

Commons of the Minnesota Department of Health³⁹ using R.³⁷ The land cover within each circle, including forest, cropland, pasture, open water, and urban area, was obtained from the National Agricultural Statistics Service provided by the United States Department of Agriculture.⁴⁰ The extracted land cover and anthropogenic activity data were used to investigate potential correlations with the NDMA_{UFC} of water samples collected from each sampling site. Besides the circle upstream of each sampling site, information on land cover and anthropogenic activities for the entire catchment of each watershed was also extracted. The protocol and R script for data extraction, organization, processing, and analysis are provided in the [Supporting Information](#).

Excitation–Emission Matrices. EEM data sets were processed by fluorescence regional integration analysis.⁴¹ The regional fluorescence intensity ratio (P_i) was calculated from the volumetric fluorescence integration within each region (ϕ_i), normalized to the total volumetric fluorescence (eqs 1 and 2)

$$\phi_i = \sum_{\text{ex}} \sum_{\text{em}} I_{\lambda_{\text{ex}}\lambda_{\text{em}}} \Delta\lambda_{\text{ex}} \Delta\lambda_{\text{em}} \quad (1)$$

$$P_i = \phi_i / \phi_{\text{total}} \quad (2)$$

where ϕ_i is the volumetric fluorescence intensity within region i , ϕ_{total} is the sum of the volumetric fluorescence intensities within regions I to V, $I_{\lambda_{\text{ex}}\lambda_{\text{em}}}$ is the fluorescence intensity at a specific excitation and emission wavelength, $\Delta\lambda_{\text{ex}}$ is the excitation increment (3 nm), and $\Delta\lambda_{\text{em}}$ is the emission increment (2.33 nm). The five EEM regions are region I (ex 220–250, em 250–330), aromatic protein and tyrosine; region II (ex 220–250, em 330–380), aromatic proteins and tryptophan; region III (ex 220–250, em 380–550), mainly fulvic-like substances; region IV (ex 250–300, em 250–380),

soluble microbial-byproducts, including proteins and biopolymers; and region V (ex 250–400, em 380–550), humic-like substances.^{18,42,43}

Statistical Tests. Spearman's rank-order correlation, a bivariate method, was used to analyze the association between NDMA_{UFC} and land use, fluorescence peak intensity, and water quality indicators via R. The river miles between the sampling sites were obtained using the “riverdist” package in R. River discharge data were obtained from the Cooperative Stream Gauging (CSG) database of the Minnesota Department of Natural Resources.⁴⁴ The gauge information and river flow rate at the time of sampling are listed in [Table S4](#).

For any pair of upstream distances (i of 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 km) and radius (j of 2.5, 5, 7.5, 8.5, 10, 12.5, and 15 km), the sum of the absolute value of all of the Spearman correlations was calculated from eq 3, denoted as $\rho_{\text{sum}}(i,j)$. The radius and upstream distance that demonstrated the largest $\rho_{\text{sum}}(i,j)$ were chosen for detailed analysis of correlations between anthropogenic activity and NDMA_{UFC}.

$$\begin{aligned} \rho_{\text{sum}}(i,j) = & |\rho_{\text{N feedlots}}(i,j)| + |\rho_{\text{animal weight of feedlots}}(i,j)| \\ & + |\rho_{\text{N domestic WTPs}}(i,j)| + |\rho_{\text{capacity of domestic WTPs}}(i,j)| \\ & + |\rho_{\text{population}}(i,j)| + |\rho_{\text{N septic tanks}}| + |\rho_{\text{N brownfields}}| \\ & + |\rho_{\text{urban coverage}}| + |\rho_{\text{cropland coverage}}| \\ & + |\rho_{\text{forest coverage}}| + |\rho_{\text{wetland coverage}}| \\ & + |\rho_{\text{open space coverage}}| \end{aligned} \quad (3)$$

RESULTS

General Water Quality Characteristics. A summary of water quality parameters, including dissolved organic matter (DOC) concentration, SUVA, UV_{254} , total fluorescence intensity, and the ratio of volumetric fluorescence intensity to total fluorescence intensity for regions II to V are provided in Tables S5 and S6 (watershed averages) and Figure S3 (site-by-site values). For the raw water samples, the St. Louis River had the highest average SUVA, UV_{254} , and total fluorescence intensity, followed by the Red River, the Crow River, and the Minnesota River. DOC concentrations in the St. Louis River (24 ± 2 mg C/L, $n = 8$) were the highest, followed by the Minnesota River (15 ± 4 mg C/L, $n = 9$), Red River (14 ± 4 mg C/L, $n = 9$), and Crow River (8 ± 2 mg C/L, $n = 10$) samples. After lime-softening treatment, the average values of SUVA, UV_{254} , DOC concentration, and total fluorescence intensity across watersheds were ranked as follows: St. Louis River > Red River $\approx$ Crow River $\approx$ Minnesota River. In contrast to the obvious differences across watersheds, no clear spatial variation in these measured water quality parameters was observed along a single river (Figure S3).

Watershed-Scale Geospatial Distribution of NDMA_{UFC} along Rivers in Different Watersheds. NDMA formation under UFC test conditions (NDMA_{UFC}) demonstrated an apparent spatial variation along rivers in the four Minnesota watersheds differentially impacted by anthropogenic activities (Figure 2). Of the four watersheds investigated, the St. Louis River watershed is the least impacted, with wetlands and forests covering more than 80% of the land. Low concentrations of NDMA_{UFC} (2.1 ± 0.3 ng/L) were consistently observed across the five upstream sites (SL1–SL5) in the St. Louis River watershed. After lime-softening treatment, the NDMA_{UFC} in SL1–SL5 slightly increased to 3.0 ± 0.3 ng/L. The NDMA_{UFC} remained low (<4 ng/L) in the raw water samples collected from the downstream SL6, SL7, and SL8 sites even as land use became more developed. After lime-softening treatment, however, the NDMA_{UFC} values of those samples increased to 18.4, 26.8, and 24 ng/L, respectively.

The headwaters area sample from the Red River (R1) had NDMA_{UFC} values of 7.3 and 7.6 ng/L before and after lime-softening treatment. The NDMA_{UFC} gradually increased from R1 to R3 and remained elevated from R3 to R6 (mean: 21.6 ± 2.7 ng/L) over 335 km of river distance, followed by a decline to 9.3 ng/L at R7. An enhancement in NDMA formation was observed for all Red River samples after lime-softening treatment, with an average increase of $95.1 \pm 64.8\%$.

A high NDMA_{UFC} concentration was observed for the M1 site (46.2 ± 6.4 ng/L) on the Minnesota River located at the outlet of Big Stone Lake at the Minnesota/South Dakota border and for the M2 site (40 ± 6.4 ng/L) located 151 km downstream of M1. The river flow doubled from M3 to M8, and the NDMA_{UFC} in the raw water samples also increased steadily from 20 to 45 ng/L, indicating an increase in the total mass flux of NDMA precursors. The NDMA_{UFC} typically increased by an average of $49 \pm 43.1\%$ ($n = 9$) after lime-softening treatment.

Consistent with our previous findings,²² an elevated NDMA_{UFC} concentration was observed for the sample from the headwaters of the North Fork of the Crow River (NF1, 49.5 ± 14.9 ng/L), then gradually decreased downstream with increasing river discharge. Relatively low NDMA_{UFC} concen-

trations were observed in the South Fork of the Crow River (9.9 ± 1.6 ng/L) and the Mississippi River sample (8.1 ± 0.6 ng/L). After lime-softening treatment, an enhancement in NDMA formation was observed for all samples ($100 \pm 88.9\%$), with the NF4 sample exhibiting the greatest NDMA_{UFC} increase from 16.1 to 76.1 ng/L.

NDMA Precursors in Rivers Around WWTP Discharges. To investigate the contribution of treated municipal wastewater to the total NDMA precursor pool in the rivers, NDMA_{UFC} concentrations were measured for raw and lime-softened water samples collected in the rivers near WWTP effluent discharge points (Figure S4). For most sampling events, the raw and lime-softened NDMA_{UFC} concentrations were consistent for the upstream, effluent, and downstream locations, suggesting that municipal wastewater effluents had little impact on the NDMA precursor levels at these sites. Furthermore, there was little or no difference in the NDMA_{UFC} concentrations between the raw and lime-softened water samples. For the samples collected around a Minnesota River WWTP effluent in the fall, however, when the river flow was low, and the contribution of the WWTP effluent to the river flow was the highest (5.82%), an apparent effect of the WWTP effluent was observed. The raw water NDMA_{UFC} concentration increased from the upstream site (16.7 ± 0.03 ng/L; 1.2 km upstream) to the discharge site (27.5 ± 4.8 ng/L), followed by a decrease to 18.1 ± 1.1 ng/L for the three downstream sites (2.5, 5.5, and 8.2 km downstream of the discharge point). There was also a slightly elevated NDMA_{UFC} concentration for the water at the discharge point after lime softening (Figure S4). With the exception of the Minnesota River in the fall, when the wastewater discharge represented 5.82% of the river flow, the NDMA_{UFC} concentrations appeared to be driven by upstream/integrated precursor inputs and not the direct input of wastewater effluent at these three sampling locations.

Characterization of the Effect of Lime Softening Treatment via HPSEC and EEM Spectra. The effects of lime softening on NOM were determined by quantifying DOC, fluorescence intensity, and molecular weight distribution (via HPSEC) before and after lime-softening treatment (Figure S5). A significant decrease in DOC concentration was observed for all water samples after lime-softening treatment, with mean values for raw and lime-softened water of 14.3 ± 6.1 and 5.9 ± 3.9 mg C/L ($n = 55$), respectively. Total fluorescence intensity decreased by 20, 51, 37, and 52% for regions II, III, IV, and V, respectively. More effective removal of region III and V substances led to an increase in the fluorescence intensity ratio of regions II and IV and a decrease in the fluorescence intensity ratio of regions III and V. Lime-softening treatment did not alter the amount of organic matter with a molecular weight <500 Da, but substantial removal of NOM with >500 Da molecular weight was observed (Figure S6). After lime-softening treatment, the number-average molecular weight decreased by $28.5 \pm 8.8\%$, and the weight-average molecular weight decreased by $30.6 \pm 6.6\%$ (Figure S7), indicating that higher molecular weight organic matter was removed more effectively by lime-softening treatment.

NDMA Precursors and Land Use. The influence of circle radius and upstream distance on the Spearman correlation for each land cover, anthropogenic activity category, and NDMA_{UFC} concentration was evaluated, with results shown in Figures S8–S13. The cumulative Spearman correlations, $\rho_{\text{sum}}(i,j)$, were calculated for each pair of radius and upstream distance. Across different radii and upstream distances for the

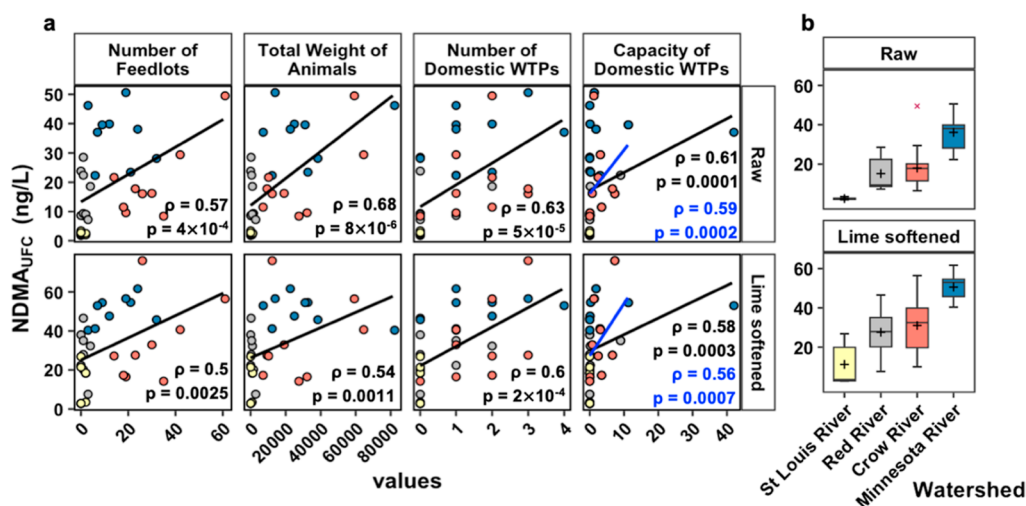


Figure 3. (a) Correlations between NDMA_{UFC} concentration in raw (top row; $n = 35$) and lime-softened (bottom row; $n = 35$) samples and the total number of feedlots, the total weight of animals (unit: lbs), the total number of domestic WWTPs, and the total capacity of domestic WWTPs (unit: cfs). Anthropogenic activity data were extracted for a circle with a 7.5 km radius centered 12 km upstream of each sampling site. The Spearman correlation coefficient and p -value are shown in each plot. For the capacity of WWTPs, correlation coefficients, and p -values for correlations with (black) and without (blue), the plant with the highest capacity are shown. (b) Box-and-whisker plots of NDMA_{UFC} concentrations in the raw (top) and lime-softened (bottom) samples from the four watersheds.

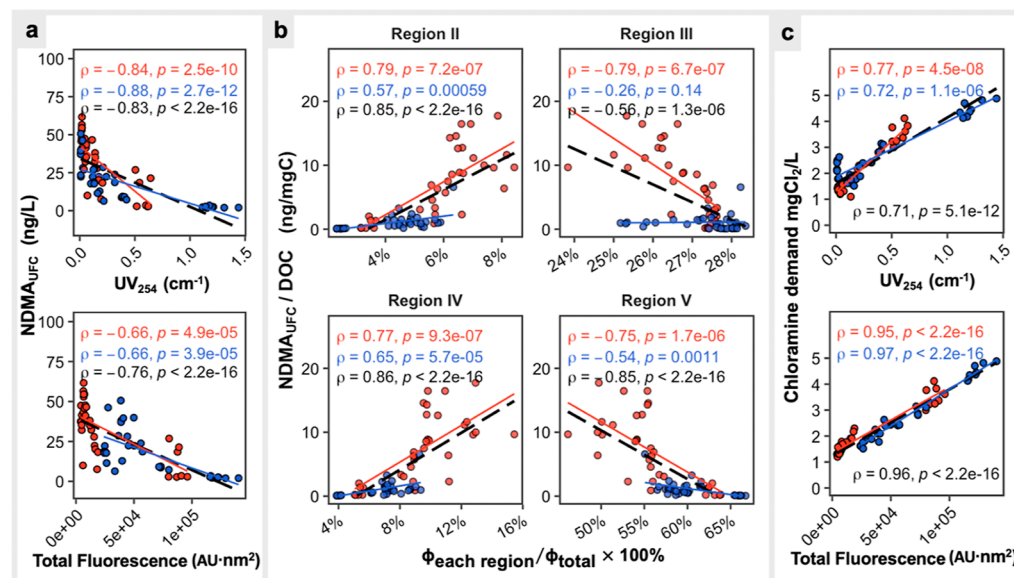


Figure 4. (a) Correlations between NDMA_{UFC} concentration and total fluorescence and UV₂₅₄. (b) Correlations between NDMA_{UFC} concentration and fluorescence intensity in each region normalized to total fluorescence. (c) Correlations between chloramine demand and total fluorescence and UV₂₅₄. The Spearman correlation coefficient and p -value are shown in each panel. Points and regression lines in blue represent raw samples, and those in red represent lime-softened samples. The overall regression is shown with a black dashed line.

extraction circle, robust positive correlations were observed between NDMA_{UFC} concentration and total weight of animals (Raw: $\rho = 0.57 \pm 0.11$, $p = 0.01 \pm 0.03$; LS: $\rho = 0.48 \pm 0.1$, $p = 0.02 \pm 0.05$) and number of feedlots (Raw: $\rho = 0.49 \pm 0.08$, $p = 0.01 \pm 0.03$; LS: $\rho = 0.45 \pm 0.08$, $p = 0.02 \pm 0.04$). In contrast, the correlations between the NDMA_{UFC} concentration and both the total number and the capacity of domestic WTPs were substantially weaker when the upstream distance was less than 6 km, and the radius was larger than 12.5 km. Overall, an upstream distance of 12 km and a radius of 7.5 km exhibited the highest $\rho_{\text{sum}}(i,j)$ (Figure S13).

Correlations of the NDMA_{UFC} concentration in raw and lime-softened water samples with various metrics of anthro-

pogenic activity within a circle of 7.5 km radius centered 12 km upstream of each sampling site are shown in Tables S7 and S8. A positive correlation between NDMA_{UFC} concentration and the total weight of livestock for raw samples ($\rho = 0.68$, $p < 0.01$) and lime-softened samples ($\rho = 0.54$, $p < 0.01$) was observed. The number of feedlots was also positively correlated with NDMA_{UFC} concentration in raw ($\rho = 0.57$, $p < 0.01$) and lime-softened samples ($\rho = 0.50$, $p < 0.01$). A detailed statistical analysis of feedlots across various livestock categories showed a significant correlation between NDMA_{UFC} concentration and the total weight of animals, as well as the total number of dairy, beef, and swine feedlots for both raw and lime-softened samples ($p < 0.01$; see Table S7).

Positive correlations were observed between NDMA_{UFC} concentration and the number of domestic WWTPs for both raw ($\rho = 0.63$, $p < 0.01$) and lime-softened water ($\rho = 0.60$, $p < 0.01$). NDMA_{UFC} concentration was also positively correlated with the total capacities of domestic WWTPs and NDMA_{UFC} concentration for the raw ($\rho = 0.61$, $p < 0.01$) and lime-softened water samples ($\rho = 0.58$, $p < 0.01$). As for industrial WWTPs, no correlation was observed between the NDMA_{UFC} concentration and the total number or capacity of these WWTPs. NDMA_{UFC} concentration positively correlated with the human population for raw ($\rho = 0.37$, $p = 0.03$) and lime-softened samples ($\rho = 0.48$, $p < 0.01$). There were weak positive associations between the number of septic tanks and the number of brownfields with NDMA_{UFC} concentration ($p > 0.1$, Table S7).

In terms of land cover within each circle of a 7.5 km radius, NDMA_{UFC} concentration positively correlated with urban land coverage for raw ($\rho = 0.33$, $p = 0.05$) and lime-softened samples ($\rho = 0.45$, $p < 0.01$). There were weak positive associations between cropland coverage and NDMA_{UFC} concentration (raw: $\rho = 0.35$, $p = 0.04$; lime-softened: $\rho = 0.21$, $p = 0.23$). NDMA_{UFC} concentration negatively correlated with forest ($\rho = -0.32$, $p = 0.06$) and wetland coverage ($\rho = -0.40$, $p = 0.02$) for raw samples but not for lime-softened samples.

The four watersheds in Minnesota with varying land use distributions demonstrated different levels of NDMA precursors (Figure 3b). A summary of feedlots and WWTPs for each watershed is shown in Table S9. The Minnesota River, with abundant feedlots ($n = 4290$) and WWTPs ($n = 193$) in the watershed, had the highest mean NDMA_{UFC} concentration for raw (36.1 ± 9.7 ng/L) and lime-softened water samples (50.6 ± 7.3 ng/L). The Crow River watershed, also with abundant feedlots ($n = 1045$) and WWTPs ($n = 46$), ranked second with 17.9 ± 9.85 and 33.4 ± 16 ng/L for raw and lime-softened water samples, respectively. The mean NDMA_{UFC} concentrations were 2.4 ± 0.5 ng/L in raw samples and 11.2 ± 10.6 ng/L in lime-softened St. Louis River samples, a watershed with a small number of feedlots ($n = 19$) and WWTPs ($n = 24$). The Red River (feedlots, $n = 655$; WWTPs, $n = 102$) had mean NDMA_{UFC} concentrations of 15.2 ± 8.2 and 27.6 ± 11.84 ng/L for raw and lime-softened water samples, respectively.

NDMA Formation, Chloramine Demand, and Organic Matter Properties. All parameters characterizing bulk NOM concentration or spectral properties were negatively correlated with NDMA_{UFC} concentration when raw and lime-softened samples were evaluated together or separately, as shown in Figures 4a and S14. Strong negative correlations were observed between NDMA_{UFC} and UV₂₅₄ ($\rho = -0.83$, $p < 0.01$), SUVA ($\rho = -0.69$, $p < 0.01$), and total fluorescence intensity ($\rho = -0.76$, $p < 0.01$). The correlation between NDMA_{UFC} and DOC concentration was weak ($\rho = -0.17$, $p = 0.05$). With lime-softening treatment, the mean DOC concentration decreased from 14.3 ± 6.1 to 5.9 ± 3.9 mg C/L, and total fluorescence intensity decreased by $54.2 \pm 14.9\%$, but the negative correlations of NDMA_{UFC} to UV₂₅₄, SUVA, DOC, and total fluorescence remained as robust as those for raw samples.

Correlations between NDMA_{UFC} normalized to the DOC concentration (NDMA_{UFC}/DOC) and regional fluorescence normalized to the total fluorescence for all samples or raw and lime-softened samples separately are shown in Figure 4b. The

NDMA_{UFC} in raw water samples positively correlated with the fluorescence intensity within region IV ($\rho = 0.65$, $p < 0.01$) and region II ($\rho = 0.57$, $p < 0.01$). For the lime-softened samples with less organic matter, the positive correlation between NDMA_{UFC}/DOC and region II ($\rho = 0.79$, $p < 0.01$) and region IV ($\rho = 0.77$, $p < 0.01$) remained robust. A negative correlation was observed between NDMA_{UFC}/DOC and fluorescence intensity within region V for raw ($\rho = -0.54$, $p < 0.01$) and lime-softened samples ($\rho = -0.75$, $p = 0.06$). The trends in region III are difficult to interpret given the weak negative relationships for lime-softened samples.

A positive correlation was observed between chloramine demand and UV₂₅₄, SUVA, total fluorescence, and DOC concentration for both raw and lime-softened samples when considered separately or when combined (Figures 4c and S15). For the combined data set, total fluorescence intensity exhibited the strongest correlation with chloramine demand ($\rho = 0.96$), followed by UV₂₅₄ ($\rho = 0.72$), TOC concentration ($\rho = 0.58$), and SUVA ($\rho = 0.36$) with p values all < 0.001 . For raw samples alone, total fluorescence exhibited the strongest correlation with chloramine demand ($\rho = 0.97$, $p < 0.001$), followed by UV₂₅₄ ($\rho = 0.77$, $p < 0.001$), and SUVA ($\rho = 0.35$, $p < 0.001$), and TOC concentration ($\rho = 0.27$, $p = 0.03$). For lime-softened water samples alone, where much of the NOM had been removed, the correlation between chloramine demand and total fluorescence intensity ($\rho = 0.95$, $p < 0.001$) was still the strongest. Among the fluorescence regions, region V exhibited the strongest positive correlation with chloramine demand for both raw ($\rho = 0.7$, $p < 0.001$) and lime-softened samples ($\rho = 0.85$, $p < 0.001$) (Figure S16), while region II and region IV were negatively correlated with chloramine demand ($p < 0.001$).

DISCUSSION

Sources of NDMA Precursors in Minnesota Waters.

Animal feedlots appear to be the most important source of NDMA precursors (as NDMA_{UFC}) in the studied watersheds based on the positive association of NDMA_{UFC} concentrations with the number of feedlots and the total weight of livestock. The watersheds that were abundant in feedlots, namely, the Crow and Minnesota Rivers, had a higher mean NDMA_{UFC} than the other two watersheds. Among all livestock categories, beef cattle, dairy cattle, and swine feedlots appear to be the main contributors to the NDMA precursor pool, considering their strong association with NDMA_{UFC} (Table S7). The high NDMA_{UFC} could be associated with amines in animal waste and antibiotics used in livestock operations. Animal operation facilities were found to release amines including triethylamine, tributylamine, triethylamines, dimethylamine, and trimethylamines, which can serve as *N*-nitrosamine precursors.^{8,45–48} Veterinary antibiotics used in animal agriculture, including tetracyclines and macrolides, were found to form NDMA during chloramination, with molar conversions ranging from 0.04 to 4.9%.¹¹ A previous study also found a notably high concentration of NDMA (1110 ± 110 ng/L) and other nitrosamines, including *N*-nitrosodiethylamine, *N*-nitrosopyrrolidine, and *N*-nitrosopiperidine, in swine wastewaters even without any chloramination.¹⁷

Domestic WWTPs also contribute to the total NDMA precursor pool in surface waters in Minnesota. Positive correlations were observed between NDMA_{UFC} concentrations and the number and capacities of domestic WWTPs (Table S7), but the correlations were affected by the radius and

upstream distance of the circle used for the data extraction approach. An evident increase in NDMA_{UFC} in raw and lime-softened samples was observed from R2 to R6 in the Red River, during which the river flows through major cities, including Fargo and Grand Forks. Similarly, the NDMA_{UFC} of lime-softened samples increased dramatically after the St. Louis River transitioned from forested and wetland areas to more urbanized areas. These results are consistent with previous research showing elevated NDMA formation in wastewater effluents and other urban waters.^{8,9,49,50} The weak positive correlation between cropland and NDMA_{UFC} concentrations (Table S8) suggests that crop production is a minor source of NDMA precursors for the studied watersheds. The consistently low levels of NDMA_{UFC} from SL1 to SL5 indicate forests and wetland areas with minimal anthropogenic impacts are not major contributors to the NDMA precursor pool in surface waters. The influence of an individual WWTP discharge on the total NDMA precursors in the receiving surface water was notable when river dilution was limited. However, the contribution from the WWTP effluent was no longer observable 2.5 km downstream of the discharge point.

The NDMA precursors present at a given point in a river appear to be an accumulated effect of all anthropogenic activities upstream. Furthermore, the precursor loading rate exceeds the rate of any natural attenuation process. This is supported by the gradual increase of NDMA_{UFC} in the Minnesota River and a consistently high NDMA_{UFC} from R3 to R7 in the Red River. There was no substantial change in NDMA_{UFC} around single WWTP discharge points, which is contrary to previous studies that reported an increase in NDMA precursors after WWTP plants.^{9,51} However, in this study, the contribution of each individual WWTP to total river flow was relatively small. Nevertheless, the accumulated upstream WWTP discharges likely represent an important fraction of the total NDMA precursor pool.

Using a geostatistical approach to identify likely sources of NDMA precursors in four major Minnesota rivers, we found that the NDMA precursor sources are predominantly animal operations and domestic wastewater discharge, with cropland and urban runoff being of minor importance. This finding challenges the currently accepted view that WWTPs are the primary contributors of NDMA precursors to drinking water treatment plants. The divergence from prior research may be attributed to differences in land use patterns within the study areas. Animal operations, especially concentrated animal feeding operations, are clustered in certain states, especially many states in the Midwest like Minnesota,⁵² where field studies on sources of NDMA precursors are scarce. Further study of discharges from animal operations is needed to confirm their role as potentially important sources of NDMA precursors and to identify the predominant precursor compounds as possible targets for source reduction strategies. Furthermore, this study only represented a snapshot of conditions throughout four watersheds, and NDMA precursor concentrations can be highly dynamic.^{22,53}

Natural Organic Matter Spectral Characteristics, NDMA_{UFC}, and Chloramine Demand. The characterization of NOM using fluorescence emission–excitation spectra revealed that various NOM components affect NDMA formation during chloramination. The negative correlation between Region V (humic acid-like) and NDMA_{UFC} indicates that humic acid-like substances are the primary competitors with NDMA precursors for chloramines. This is consistent

with increased NDMA_{UFC} after lime softening^{22,54} because this process removes the high MW fractions of NOM (i.e., humic acids). The weak association between NDMA_{UFC} concentrations and region III indicates that fulvic acid does not significantly affect NDMA formation. The strong positive correlation between NDMA_{UFC} and region IV fluorescence intensity ratio suggests that microbial byproducts or materials similar to this type of NOM can serve as NDMA precursors.⁴¹ Region II was also positively related to NDMA_{UFC} but more weakly than region IV, indicating that aromatic proteins also may contribute to NDMA formation but are less important than the materials that make up the region IV signal. The correlations between NDMA_{UFC} and fluorescence intensities within different regions remained robust after lime softening, during which much of the NOM (as measured by DOC and HPSEC) was removed. Because only NDMA, which is the most frequently detected and most abundant nitrosamine,⁸ was assessed, more research is needed to investigate the formation of total nitrosamines and other individual *N*-nitrosamines to elucidate potential correlations between NOM fluorescence and total *N*-nitrosamine formation.

Previous research on the relationships between NOM fluorescence and NDMA formation has been limited to water samples from water and wastewater treatment plants. A significant positive correlation between NDMA_{FP} and protein-like substances with a Spearman coefficient ($\rho = 0.628$) in a drinking water treatment plant and a Pearson correlation coefficient ($R^2 > 0.89$) in a wastewater treatment plant was reported.^{18,20} Herein, a strong positive correlation between protein-like fluorescence and NDMA formation was observed for samples collected along four rivers in watersheds with different land uses that exhibited distinct water quality characteristics. Taken together, these results suggest that fluorescence intensity within region IV is a potential indicator of NDMA precursors in surface waters, which could be useful for assessing when a system is susceptible to elevated levels of NDMA formation or to evaluate the success of treatments to reduce NDMA formation. For example, fluorescence excitation–emission matrices were used to characterize NOM in a drinking water treatment train, and the data showed that ozonation effectively oxidizes protein-like substances.⁵⁵ Ozonation has also proven to effectively attenuate NDMA precursors during drinking water treatment.^{56–58} Considering the link between protein fluorescence and NDMA_{UFC}, this further supports the potential for fluorescence intensity to serve as an indicator of NDMA precursor abundance, providing a fast and convenient alternative to the laborious batch-scale chloramination experiments that necessitate a lengthy incubation period and a complicated analytical procedure.

NDMA formation under UFC generally increased following lime-softening treatment, where a combination of lime, alum, and ferric chloride was used. This agrees with results from our previous study, in which NDMA_{UFC} typically increased (average 134.9%, maximum 1184%) after lime softening over a 15-month sampling campaign in the Crow River watershed.²² The negative correlations of DOC, SUVA, and UV₂₅₄ with the NDMA_{UFC} concentration indicated that aromatic NOM competes with NDMA precursors for available chloramines. Thus, the removal of NOM, and especially humic acid-like NOM, during the lime-softening process decreased competition for chloramines, which resulted in enhanced NDMA formation. More detailed kinetic studies that monitor chlor-

amine, NDMA formation, and NOM spectral properties as a function of time during incubation in UFC testing are needed to fully explore the dynamics of this chloramine competition.

Among all NOM-related parameters, total fluorescence intensity showed the strongest positive correlation with chloramine demand in samples varying in water quality characteristics before and after lime softening treatment. This opposing relationship between NDMA_{UFC} and total fluorescence and chlorine demand and total fluorescence (Figure 4a,c) further supports that the competition between NOM and NDMA precursors affects the extent of NDMA formation. TOC concentration is often believed to be a strong indicator of chlorine demand and, hence, is commonly incorporated into equations used to compute the required chlorine dosage. In the present study, TOC concentration demonstrated the weakest correlation with chloramine demand among the parameters measured for raw and lime-softened samples. Thus, total fluorescence intensity was a better predictor of chloramine demand. This is consistent with results from a one-year investigation in three different full-scale water distribution networks in China, where equations incorporating fluorescence moieties related to humic substances were most effective for predicting the required chlorine dose ($\rho = 0.95$, $p < 0.0001$).⁵⁹

Environmental Significance. A geostatistical analysis was performed to investigate potential sources of NDMA precursors along rivers in four watersheds with diverse land use characteristics. Our results suggest that animal operations were the most important source of NDMA precursors in the studied watersheds. Municipal WWTP discharges likely also were a significant contributor to the total NDMA precursor pool in the rivers, even though the contribution from a single WWTP effluent was difficult to discern as the individual WWTP discharges accounted for a small fraction of the river flows. Mitigating NDMA formation during water treatment is challenging as processes that remove NOM, and hence precursors for regulated and other halogenated DBPs, such as lime softening or conventional coagulation, do not appear to remove many, if any, NDMA precursors. In fact, the removal of NOM may decrease competition for chloramines, leading to increased nitrosamine formation.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.3c09251>.

Chemicals and reagents, procedures for lime softening treatment, monochloramine solution preparation and residual chlorine concentration determination and procedures for land use information extraction, and processing and analysis; information on sampling locations, river discharges, a summary of water quality characteristics, instrumental method setting for NDMA determination, correlations between NDMA_{UFC} and anthropogenic activities; and a summary of land use information across watersheds; a map of sampling locations around the WWTP discharge point, a map of buffer zones for land use information extraction, the spatial distribution of the water parameters across watersheds, the spatial distribution of NDMA precursors along rivers impacted by the wastewater treatment discharge point, comparisons on NOM component and size distribution between raw and lime-softened samples,

and correlations between NDMA_{UFC}, chlorine demand and water parameters (PDF)

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Notes

The authors declare no competing financial interest.

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Spatiotemporal Variability in *N*-Nitrosodimethylamine Precursor Levels in a Watershed Impacted by Agricultural Activities and Municipal Wastewater Discharges and Effects of Lime Softening

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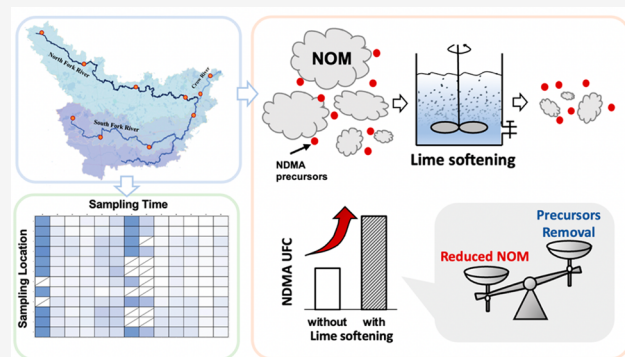
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ABSTRACT: The Crow River, a tributary of the Mississippi River in Minnesota, U.S.A., that is impacted by agricultural activities and municipal wastewater discharges, was sampled approximately monthly at 12 locations over 18 months to investigate temporal and spatial variations in *N*-nitrosodimethylamine (NDMA) precursor levels. NDMA precursors were quantified primarily by measuring NDMA formed under the low chloramine dose uniform formation conditions protocol (NDMA_{UFC}) and occasionally using the high dose formation potential protocol (NDMA_{FP}). Raw water NDMA_{UFC} concentrations (2.2 to 128 ng/L) exhibited substantial temporal variation but relatively little spatial variation. An increase in NDMA_{UFC} was observed for 126 of 169 water samples after lime-softening treatment. A kinetic model indicates that under chloramine-limited UFC test conditions, the increase in NDMA_{UFC} can be attributed to a decrease in competition between precursors and natural organic matter (NOM) for chloramines and reduced interactions of precursors with NOM. NDMA_{UFC} concentrations correlated positively with dissolved nitrogen concentration ($\rho = 0.44$, $p < 0.01$) when excluding the spring snowmelt period and negatively correlated with dissolved organic carbon concentration ($\rho = -0.47$, $p < 0.01$). Overall, NDMA precursor levels were highly dynamic and strongly affected by lime-softening treatment.

KEYWORDS: *N*-nitrosodimethylamine (NDMA), lime softening, natural organic matter (NOM), chloramination, disinfection byproducts



INTRODUCTION

Many U.S. water utilities employ chloramines (NH_2Cl and NHCl_2) as their secondary or terminal disinfectant to minimize the formation of chlorinated disinfection byproducts (DBPs, i.e., trihalomethanes and haloacetic acids).^{1,2} Another benefit of chloramines is a long-lasting chlorine residual, which provides protection against opportunistic pathogens including *Legionella pneumophila*.³ Unfortunately, a potential side effect of the use of chloramines is *N*-nitrosamine formation. The occurrence of *N*-nitrosamines in drinking water has been receiving increasing attention due to their carcinogenic, mutagenic, and teratogenic properties.⁴ Among the nitrosamines, *N*-nitrosodimethylamine (NDMA) is the most frequently detected and is categorized as a B2 probable human carcinogen and priority contaminant according to the United States Environmental Protection Agency (USEPA).⁵ To date, no national standard has been developed for NDMA in drinking water, but many states have set guidance values ranging from 0.69 to 18 ng/L, including the frequently cited California Department of Public Health notification level of 10 ng/L.^{5,6}

Chloramine-induced NDMA formation occurs via a nucleophilic substitution reaction between dichloramine and

unprotonated secondary amines, leading to the formation of a chlorinated dimethylhydrazine intermediate, which is further oxidized to NDMA and/or other products.⁷ Organic contaminants containing tertiary and quaternary amine structures are also known to be NDMA precursors.⁸ NDMA precursors are mainly anthropogenic, contrary to conventional DBPs that are derived from natural organic matter (NOM). Considering the high abundance of secondary or tertiary amine structures in anthropogenic chemicals, the source of NDMA precursors is likely to be diverse. For example, industrial wastewaters from textile washing and dry cleaning are important sources of nitrosamine precursors.⁹ Municipal wastewater discharges contain tertiary amine-containing pharmaceuticals and quaternary amine-containing personal care products.^{10–12} Agricultural runoff also could contribute to

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the NDMA precursor pool in surface waters because of the presence of amine-containing pesticides (e.g., diuron and isoproturon) and veterinary antibiotics (e.g., tetracycline).^{13,14} Some amine-based polymers used as coagulants for water treatment or in sludge dewatering operations have been identified as NDMA precursors (e.g., polyamines, polyDAD-MAC, and Mannich polymers).^{15–17} There also are natural processes that can release NDMA precursors. The natural metabolism of amino acids has been found to release NDMA precursors, such as dimethylamine, *N*-methylalanine, and alanine methyl ester.^{18,19} Wildfires have been observed to cause increased NDMA precursor in affected surface water, which is likely associated with the higher abundance of fulvic-like, tryptophan-like, and tyrosine-like compounds present in the water samples.²⁰ In addition, the occurrence of seasonal cyanobacterial blooms in eutrophic waters releases algal organic matter, which has the potential to form NDMA during chloramination.^{21,22}

To better control *N*-nitrosamine formation and propose effective in-watershed control measures, spatial and temporal variation in the occurrence of NDMA precursors in source and surface waters must be better understood. Uzun et al.²³ investigated the NDMA formation potential of water samples collected from rivers and lakes located in the southeastern United States and reported that the NDMA precursor concentration was influenced by the ratio of an upstream wastewater effluent discharge to the river discharge and that large reservoirs and impoundments attenuated NDMA precursors effectively. Zeng et al.¹¹ measured the NDMA formation potential of water samples collected from pristine headwaters, eutrophic waters, agricultural runoff, stormwater runoff, and wastewater effluents. Wastewater effluents were the greatest contributors of NDMA precursors, followed by eutrophic waters, stormwater runoff, and lastly agricultural runoff. More recently, studies have emphasized the significance of natural attenuation mechanisms, including photolysis, biodegradation, and adsorption, for destroying or removing NDMA precursors in natural environments.^{24,25} Nevertheless, investigations of spatial and temporal variations in NDMA precursor concentrations in natural waters and potential precursor sources are very limited.

Many surface waters impacted by NDMA precursors serve as drinking water sources, which pose a potential risk to human health if chloramines are used for disinfection. Coagulation is one important step in drinking water treatment aimed at removing NOM before the disinfection process to avoid the formation of DBPs. Conventional drinking water treatment plants typically apply metal salts such as aluminum sulfate or ferric chloride as coagulants to facilitate the removal of particles and NOM.²⁶ Various studies have shown that coagulation with alum or ferric chloride has little or no influence on NDMA precursor concentrations based on NDMA formed following very high doses of chloramines using formation potential test conditions.^{27–29} We are unaware of any investigations of the effects of lime-softening with coagulation treatment on NDMA precursor levels. The extreme pH conditions together with substantial NOM removals associated with such a treatment scheme have been shown to potentially enhance NDMA formation upon subsequent chloramination in preliminary studies in our laboratory.³⁰ In this study, spatial and temporal variation in NDMA precursor levels was investigated for a tributary of the upper Mississippi River impacted by both agricultural activities

and municipal wastewater discharges and known to have relatively high NDMA precursor concentrations. The effect of lime softening on NDMA precursor concentrations was assessed by performing jar testing and measuring NDMA formed before and after treatment using the uniform formation conditions (UFC) test. Finally, potential correlations between watershed characteristics and NDMA precursor concentrations were investigated.

MATERIALS AND METHODS

Chemicals and Reagents. Chemicals and reagents are listed in the [Supporting Information](#). Ultrapure water (Millipore Milli-Q Advantage A10, 18.2 M Ω -cm resistivity) was used to prepare all solutions.

Sample Collection and Analysis. The sampling campaign was conducted throughout the Crow River watershed, which is northwest of the cities of Minneapolis and St. Paul (MN, U.S.A.), from July 2020 to December 2021. In total, 162 water samples were collected approximately monthly from 12 sites located along the two forks and main stem of the Crow River and in the Mississippi River upstream and downstream of the mouth of the Crow ([Figure 1](#)). There

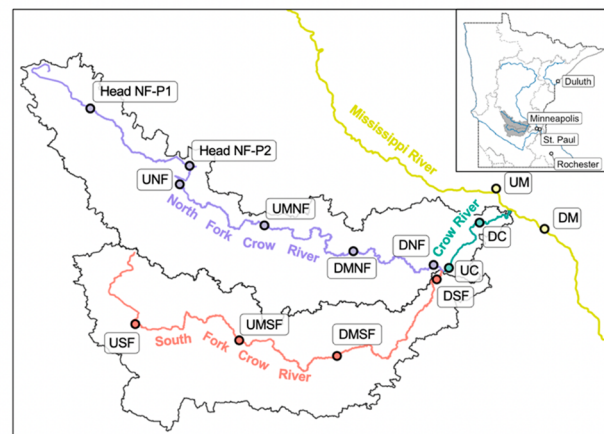


Figure 1. Sampling locations in the Crow River watershed. The GPS coordinates are listed in [Table S1](#). The inset shows the location of the Crow River watershed in Minnesota. The map was produced by the authors from data available in Minnesota Geospatial Commons.³¹

were four sampling sites in the North Fork of the Crow River (UNF, UMNF, DMNF, and DNF), four sites in the South Fork of the Crow River (USF, UMSF, DMSF, and DSF), two sites in the main stem of the Crow River (UC and DC), and two sites upstream and downstream of the confluence point of the Crow and Mississippi Rivers (UM and DM). The GPS coordinates for all of the sampling locations are provided in [Table S1](#). From April 2021 to December 2021, an additional 11 water samples were collected from two sites representing the headwaters area of the North Fork of the Crow River (Head NF-P1 and Head NF-P2). All river water samples were collected from the center of the river just below the surface by lowering a high-density polyethylene (HDPE) bucket from a bridge using a rope. The water was transferred to a 20 L high-density polyethylene jar. The HDPE bucket and jars were cleaned before use by soaking in Alconox solution overnight and rinsing with deionized water and ultrapure water multiple times. The water samples were transported to the laboratory in coolers on ice and stored in the dark in a refrigerator (<4 °C)

until subsequent use. For samples collected from August 2020 to June 2021, common physicochemical water quality indicators were analyzed, including total organic carbon (TOC), dissolved organic carbon (DOC), total nitrogen (TN), dissolved nitrogen (DN), nitrate, nitrite, ammonia, and UV absorbance at 254 nm (UV_{254}). Specific UV absorbance (SUVA) at 254 nm was calculated by normalizing UV_{254} (cm^{-1}) by the DOC concentration ($mg\ C\ L^{-1}$). The dissolved organic nitrogen (DON) concentration was calculated by subtracting the dissolved inorganic nitrogen (DIN) concentration (i.e., the sum of ammonia-N, nitrate-N, and nitrite-N concentrations) from the DN concentration. Details about the methods used to measure these water quality parameters are provided in the [Supporting Information](#).

Lime-Softening Treatment. Aliquots of the water samples were transferred into 2 L poly(methyl methacrylate) square flocculation jars and lime softened using a jar test apparatus. The softening protocol was designed to mimic the softening process applied at the full-scale drinking water treatment plant in St. Paul, MN, U.S.A.: (1) Rapid mix stage: Dose alum into each jar to achieve an initial concentration of 19 ppm as Al and stir at a speed of 300 rpm for 3 min. Add fresh lime slurry (10–15% w/v) to reach a pH of 11.1 as indicated by a pH probe, and then mix at a speed of 300 rpm for 3 min. (2) Flocculation stage: Stir for 27 min at 34 rpm, then dose 8 ppm (as Fe) ferric chloride solution, stir at 27 rpm for 24 min, and then stir at 20 rpm for 29 min. (3) Settling stage: Turn off the stirrer and allow 30 min for sedimentation of floc aggregates. The supernatant was withdrawn and adjusted to pH 8.5 using hydrochloric acid (32% w/v).

Chloramination Testing. All experiments were conducted in duplicate. Raw water samples were filtered through GF/F glass microfiber filters (Whatman, 0.7 μm) within 48 h of sample collection. No filtration of lime-softened samples was performed, because most particles were removed via settling during lime-softening treatment. Organic carbon concentrations before and after filtration of lime-softened samples were similar, indicating that a negligible amount of particulate organic matter remained in the lime-softened water. Filtered raw water samples and lime-softened samples were chloraminated under UFC conditions within one week of sample collection. UFC conditions simulate typical or average chloramination conditions used at water treatment plants in the United States.³² Chloramination was initiated by adding a preformed monochloramine solution into the sample (buffered at pH 8.0 with 4 mM borate) to achieve an initial concentration of 5 mg Cl_2/L . Preformed monochloramine (NH_2Cl) solutions were freshly prepared by dropwise addition of NaOCl solution into NH_4Cl solution to obtain a 1:1.15 molar ratio. The resulting concentration of NH_2Cl was determined spectrophotometrically. Chloraminated samples were held at room temperature in the dark for 3 days (typically $23 \pm 1\ ^\circ C$), and the residual chloramine concentration was measured by the ABTS method and then quenched with $\sim 31\ mg\ L^{-1}$ L-ascorbic acid.³³ Details about preparation and determination of the NH_2Cl concentration and residual chlorine are described in the [Supporting Information](#) (Texts S3 and S4).

A comparison study between NDMA formation under UFC test conditions and formation potential (FP) test conditions was conducted for selected samples. The FP test measures the maximum NDMA formation by dosing excess monochloramine to ensure that all precursors react with chloramines and

form NDMA. The FP test was initiated by adding 140 mg Cl_2/L of preformed monochloramine solution into the phosphate-buffered (10 mM, pH = 7) samples. The samples were incubated in the dark at room temperature for 7 days, and then 200 mg L^{-1} L-ascorbic acid was added to quench the residual chloramine.

NOM Dosing Experiments. River water samples were collected from the DNF site in the fall of 2022 and used to prepare the filtered raw and lime-softened samples, following the procedure described above. Mississippi River NOM (International Humic Substances Society, St. Paul, MN, U.S.A.) was added to raw and lime-softened samples to study the potential effect of NOM on NDMA formation. Stock solutions of NOM with a concentration of 500 mg of C/L were prepared in ultrapure water. The DOC concentration of the filtered raw and lime-softened samples was measured, and then 0.5 L of each raw and lime-softened sample was transferred to a separate clean amber glass bottle, followed by the addition of the NOM stock to increase the TOC concentration by 0.5, 1, 2, and 4 mg C/L. The NDMA formation was subsequently determined using the UFC test.

Photodegradation and Dark Incubation Experiments. Bench-scale photodegradation and dark incubation experiments were conducted using river water samples collected from the UNF site in fall 2022. Photodegradation experiments were conducted with an Atlas Suntest CPS+ solar simulator with a 1500 W xenon lamp. After filtering through Whatman GF/F and 0.22 μm hydrophilic PTFE filters, the river samples were transferred to a clean Pyrex dish and weighed. The samples were photolyzed at an intensity of $765\ W\ m^{-2}$ for 4 h. After photolysis, the beaker and sample were weighed again and reconstituted to the initial mass with ultrapure water to compensate for any evaporation. For the dark incubation experiments, raw, unfiltered river samples were placed into clean conical flasks topped with an autoclaved foam stopper to allow for air exchange and then incubated at $37\ ^\circ C$ in the dark for 7 days to allow for microbial transformations and nonphotochemical abiotic transformations of NDMA precursors and NOM to occur. As for the photolysis experiments, any water lost during the dark experiments was replaced with ultrapure water at the end of incubation.

NDMA Analysis. Duplicate 0.5 L samples were spiked with 10 ng/L NDMA- d_6 and passed through activated carbon cartridges (United Chemical Technologies, Enviro-Clean EPA Method 521 SPE Columns) under vacuum at a flow rate of 5 mL/min. After drying for 20 min under vacuum, the cartridges were eluted with 15 mL of dichloromethane (DCM) at a flow rate of 2 to 3 mL/min. The DCM extracts were collected in 15 mL conical test tubes and evaporated under nitrogen to remove DCM, leaving only a small amount of water remaining. The extracts were reconstituted to a final volume of 0.5 mL with ultrapure water. NDMA concentrations of the reconstituted samples were analyzed using a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific) interfaced to a Dionex Ultimate 3000 rapid separation LC (RSLC) high-performance liquid chromatography (HPLC) system (Thermo Scientific). Quantitation was based on $m/z = 58.17$ and 64.17 for NDMA and NDMA- d_6 , while $m/z = 43.13$ and 46.13 were used as qualifier ions. NDMA concentrations in filtered raw and lime-softened samples before chloramination were consistently below the method detection limit. Details about the analytical method for NDMA analysis are given in [Tables S3 and S4](#).

Data Analysis and Modeling. Data on anthropogenic activities in the Crow River watershed were obtained online. Cropland data were downloaded from the National Agricultural Statistics Service at the U.S. Department of Agriculture.³⁴ The distribution of feedlots, wastewater treatment plants, hospitals, ethanol plants, and cities/towns was obtained from Minnesota Geospatial Commons.³¹ Data extraction and processing were conducted using R and ArcGIS Pro. The approaches for analysis and visualization of land use data are described in the [Supporting Information](#) (Text S5). River discharge data for the main stem of the Crow River (gage ID: 18087001, GPS coordinates: 45.086711, -93.734457) were obtained from the Cooperative Stream Gaging (CSG) Web site of the Minnesota Department of Natural Resources.³⁵ Spearman correlation analysis, a bivariate method, was used to evaluate factors affecting NDMA formation.

Modeling was performed to predict the effect of a decrease in the NOM concentration (as TOC) on NDMA formation from the model precursor ranitidine under UFC conditions. A simple second-order model for the reaction of ranitidine with monochloramine (NH_2Cl) under Simulated Distribution System (SDS) conditions was developed by Zhang et al.³⁶ with the rate constant obtained by fitting experimental data³⁷ (i.e., model A; see the [Supporting Information](#)). In this model, the authors introduced a lag phase to address an observed delay in NDMA formation following precursor loss and effectively assumed that the molar yield of NDMA from ranitidine was 100%. Here, we modify this model slightly by removing the lag phase and adding a term to account for the observed molar yield (83.6%) in order to fit recently published data on NDMA_{SDS} formation from ranitidine as a function of NOM concentration (i.e., 1, 2.5, and 5 mg C/L; model B). For both models, the monochloramine concentration over time was computed using a chloramine decay model developed by the USEPA,³⁸ and the concentration of precursor (i.e., ranitidine) was determined by subtracting the product of the NDMA concentration and the assumed molar yield from the initial spiked precursor concentration. Details on the models and validation by fitting data from ref 37 are provided in the [Supporting Information](#).

RESULTS

General Water Quality Characteristics. Water quality parameter values are summarized in [Table S5](#). Plots showing seasonal and spatial variations of water quality parameter values are provided in [Figures S1–S3](#). The concentrations of DN, nitrate, ammonia, and nitrite were low in fall 2020 and increased in winter 2020, followed by an increase in March and April coupled with the increased river discharge due to spring snowmelt. TOC and DOC remained stable over time except for an increase from March to June 2021. In terms of spatial variation, both DOC and DN were higher in the South Fork of the Crow River than in the North Fork of the Crow River. Higher concentrations of DN were detected in the headwater samples of the North Fork (Head NF-P1 and Head NF-P2) compared to samples collected from other sites in the North Fork. Because 40 of 139 DON concentrations were negative, the method of obtaining DON concentrations indirectly by subtracting DIN concentrations from the corresponding DN concentrations was not appropriate for our data set.

Seasonal Variation of NDMA Precursors in Crow River Region. The concentration of NDMA formed under UFC conditions (NDMA_{UFC}), which we interpret as NDMA

precursor concentrations in the Crow River water, demonstrated a large variation during the 18-month sampling campaign ([Figure 2](#)). An abnormally high mean NDMA_{UFC}

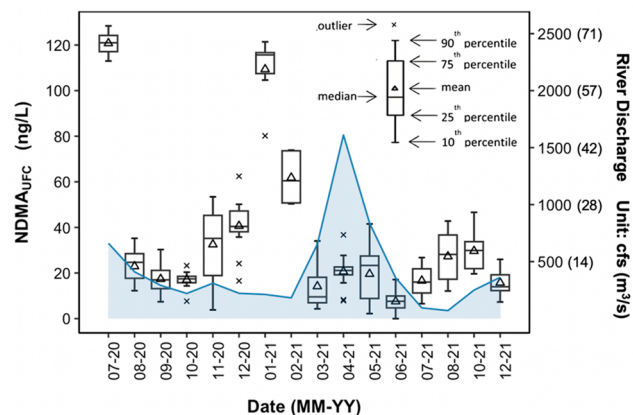


Figure 2. Seasonal variation of NDMA_{UFC} in raw water samples averaged across all sampling sites. River discharge is the monthly average discharge monitored at a gaging station located in the Crow River (gage ID: 18087001; closest to UC site) obtained from the Minnesota Department of Natural Resources Cooperative Stream Gaging Web site.

concentration of 121 ng/L was observed in July 2020, which was not repeated in July 2021. The NDMA_{UFC} was relatively low in fall 2020 (~ 20 ng/L). A gradually increasing trend in NDMA_{UFC} was observed from October to December 2020, reaching a peak in January. The mean NDMA_{UFC} concentrations were 17, 33, and 41 ng/L, respectively, in October, November, and December. In January and February 2021, mean NDMA_{UFC} concentrations in the Crow River samples were 109 and 62 ng/L, respectively. The NDMA_{UFC} decreased in March concomitantly with a dramatic increase in river discharge and then remained low for the following three months. Unlike in 2020, NDMA_{UFC} concentrations remained relatively low throughout fall 2021 (17 to 30 ng/L) and even decreased in December (16 ng/L). The monthly NDMA_{UFC} concentrations for samples collected at each location are shown in [Figure S4](#). Seasonal variations at each location were similar to those for the averaged whole watershed values. Residual chlorine concentrations at the end of each UFC test were consistently >1 mg of Cl_2 /L and typically >2 mg of Cl_2 /L ([Figure S5](#)).

Spatial Distribution of NDMA Precursors in Crow River Region. NDMA precursor concentrations were significantly greater in the North Fork of the Crow River than in the South Fork ([Figure 3a](#), $p = 0.03$, two-sample, one-tailed t test). NDMA precursor concentrations in the main stem of the Crow River were roughly equivalent to the concentration in the North Fork of the Crow River but were much greater than those in the Mississippi River ([Figure 3a](#)). The discharge of the North Fork (range: 50 to 830 cfs; mean: 256 ± 195 cfs) was typically greater than that of the South Fork (range: 27 to 638 cfs; mean: 167 ± 180 cfs) over the 18-month sampling campaign. Thus, during the sampling campaign, the North Fork of the Crow River was the main contributor of NDMA precursors to the main stem of the Crow River, which was, in turn, a contributor of NDMA precursors to the Mississippi River.

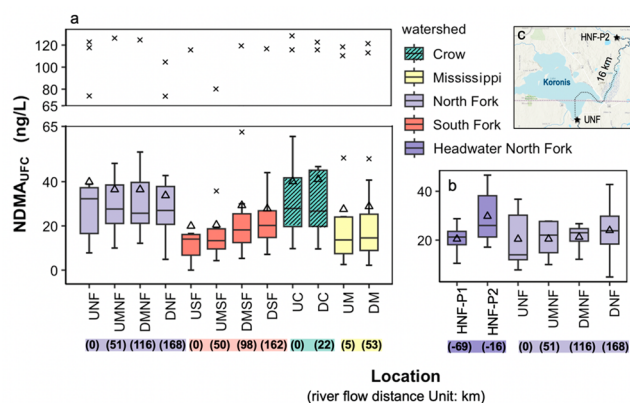


Figure 3. (a) Spatial distribution of NDMA_{UFC} in raw water samples collected during the 18-month sampling campaign with rivers differentiated by color. (b) NDMA_{UFC} concentration of raw water samples collected along the North Fork, including the headwaters area samples (HNF-P1 and HNF-P2) and the four downstream samples from April 2021 to December 2022. (c) Map for the river flow path between HNF-P2 and UNF. The map was reproduced with permission from Esri. Map image is the intellectual property of Esri and is used herein under license. Copyright Esri and its licensors. The symbols are defined in Figure 2.

Precursor concentrations increased gradually along the South Fork of the Crow River. In contrast, a consistently high concentration of NDMA precursors was observed in the North Fork of the Crow River from the upstream UNF point to the downstream DNF point (~117 river miles). NDMA_{UFC} precursor concentrations in the headwater HNF-P2 samples were even greater than those in the samples collected downstream (Figure 3b). In addition, an obvious decrease in NDMA_{UFC} was observed from HNF-P2 to UNF, even though the distance between the two points is only 6.5 river miles, but there is a lake between them.

The trends in spatial variation of NDMA_{UFC} for each month shown in Figure S4 were similar to the observed trends for the data averaged over the 18-month sampling period (Figure 3). One exception is that relatively high NDMA_{UFC} concentrations, comparable to those in the Crow River (i.e., UC and DC), were observed in water samples from the UM and DM sites in July 2020 (118 and 113 ng/L, respectively) and January 2021 (110 and 121 ng/L, respectively). A correlation analysis between land use and monthly averaged NDMA_{UFC} using a geostatistical approach was attempted, with a detailed methodology provided in the Supporting Information. No significant correlation was observed between NDMA_{UFC} and any anthropogenic activity factor (all *p* values > 0.1, Figure S6).

NDMA_{UFC} concentrations for raw water samples, photo-degraded samples, and samples after dark incubation from the UNF site are shown in Figure S7. The NDMA_{UFC} did not change during dark incubation but increased from 11.5 to 17.4 ng/L after photolysis. The TOC concentration did not change during either experiment (i.e., photolysis or dark incubation).

Effect of Lime Softening on NDMA Formation Potential. A comparison of NDMA_{UFC} for water samples before (i.e., raw) and after lime-softening treatment is shown in Figure 4a. The percent change after lime softening ranged from −68.9% to 1184% (Figure 4b). Among the 169 samples, NDMA_{UFC} increased after lime softening for 126 samples (70.4%), with a mean increase of 134.9% and a median increase of 53.7%. The NDMA_{UFC} decreased after lime

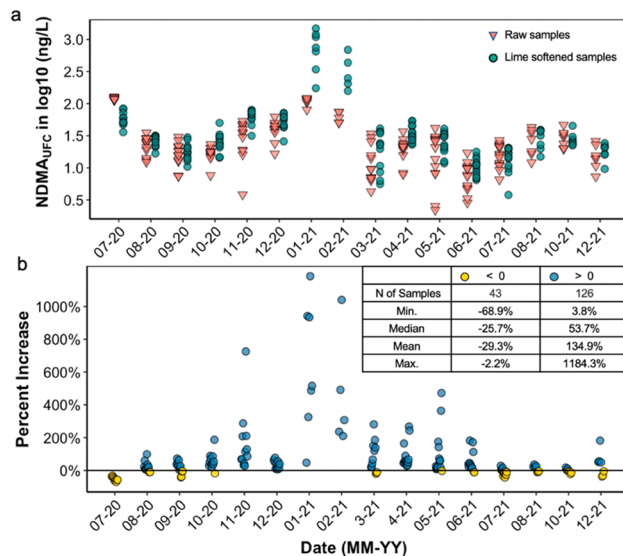


Figure 4. (a) Comparison of NDMA_{UFC} concentrations in raw and lime-softened water samples collected from the Crow River watershed from July 2020 to December 2021 shown on a log₁₀ scale. (b) The percent increase was calculated according to the formula: $\frac{\text{NDMA}_{\text{UFC,LS}} - \text{NDMA}_{\text{UFC,R}}}{\text{NDMA}_{\text{UFC,R}}} \times 100\%$, where NDMA_{UFC,LS} is the NDMA_{UFC} of samples after lime-softening treatment and NDMA_{UFC,R} is the NDMA_{UFC} of raw water samples. Data are classified into two groups to calculate the minimum, maximum, mean, and median value. Sample points with a percent increase less than 0 are “<0”, and sample points with a percent increase greater than 0 are “>0”.

softening for 43 samples, with a mean decrease of 29.3% and a median of 25.7%. Lime-softening treatment decreased the TOC concentration by an average of 77% (Table S6). Thus, regardless of the extent of TOC removal, lime-softening treatment typically increased the level of NDMA formation upon subsequent chloramination.

The percent increase in NDMA formation after lime softening was relatively minor from summer 2020 to fall 2020 and then gradually increased from fall to winter 2020, peaking in January 2021 and then declining slightly the next month. This lime-softening enhancement effect decreased from spring 2021 to summer 2021 and remained low, followed by a slight increase in December 2021. In general, the effect of lime softening on NDMA_{UFC} was greater in the late fall and winter as compared to the summer.

A positive average percent increase in NDMA_{UFC} after lime-softening treatment was observed for samples collected from each location (Figure S8). The average percent increase was the highest for the Mississippi River samples, followed by the South Fork of the Crow River, main stem of the Crow River, and North Fork of the Crow River. A high percentage increase was consistently observed in samples collected at the upstream sampling locations on the South Fork of the Crow River (USF and UMSF sites), where a constant low level of NDMA precursors was detected in the raw water prior to lime-softening treatment.

Effect of NOM and Chloramine Dosage on NDMA Formation. Bench-scale experiments were conducted to compare NDMA formation under the UFC and FP protocols for selected samples with and without the lime-softening treatment. The mean NDMA_{UFC} concentrations were 17.5 ± 7.2 and 33.8 ± 11.8 ng/L in raw and lime-softened samples,

respectively. The mean NDMA_{FP} concentrations were 73.3 ± 18.9 and 47.8 ± 8.3 ng/L in raw and lime-softened samples, respectively (Figure 5a). When comparing NDMA formation

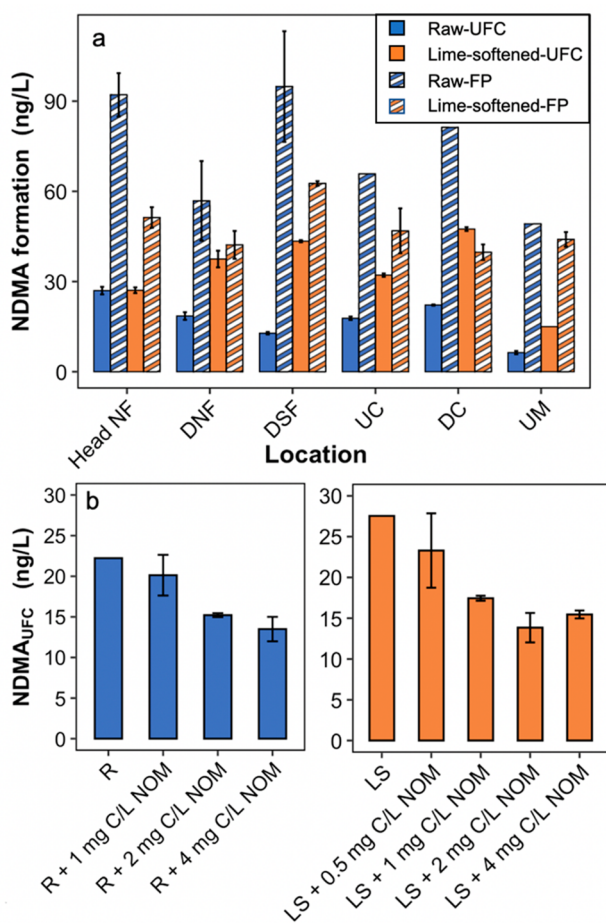


Figure 5. (a) NDMA formation potential under UFC and FP test conditions for samples before (raw, R) and after lime-softening treatment (LS). (b) NDMA_{UFC} of raw and lime-softened samples with increasing NOM addition. Error bars represent the range of duplicate measurements.

via the two protocols, the NDMA_{FP} concentrations were noticeably higher than the NDMA_{UFC} concentrations for raw samples, while for lime-softened samples the difference between NDMA_{UFC} and NDMA_{FP} concentrations was smaller. After lime-softening treatment, a clear increase was observed in the NDMA_{UFC} concentration, while a decrease was observed in NDMA_{FP}.

Assuming that NDMA_{FP} represents the total NDMA precursors in a sample, the percentage of NDMA precursors that reacted using the UFC test could be calculated as $\text{NDMA}_{\text{UFC}}/\text{NDMA}_{\text{FP}} \times 100\%$. The percentage of UFC-reactive precursors in each sample is shown in Table S7. The average percentage of UFC-reactive precursors is $23.8 \pm 8.4\%$ for raw water samples and $72 \pm 29.5\%$ for lime-softened samples, showing that a greater fraction of the total precursor pool reacted under UFC conditions for lime-softened samples than for raw water samples.

To study the effect of NOM concentration on NDMA formation, additional NOM was spiked into the raw and lime-softened samples collected from the DNF site in fall 2022, and then the NDMA formation was measured using the UFC test

(Figure 5b). The initial TOC concentrations were 19.1 and 3.9 mg C/L in the raw and lime-softened samples, respectively. With increasing dosage of NOM, the NDMA_{UFC} decreased gradually for raw and lime-softened samples. A roughly 50% reduction in NDMA formation was observed for both raw and lime-softened samples when 4 mg C/L of additional NOM was dosed into the samples.

Correlation between Physicochemical Water Quality Indicators and NDMA_{UFC}. A summary table of correlations between NDMA concentration and the measured water quality parameters is included in Table S8. The NDMA_{UFC} concentration in raw water samples was inversely related to the TOC concentration ($n = 138$, $\rho = -0.52$, $p = 1.2 \times 10^{-10}$) and the DOC concentration ($n = 138$, $\rho = -0.47$, $p = 1.2 \times 10^{-8}$) as shown in Table S8 and Figure S10. A negative correlation also was observed between NDMA_{UFC} in raw water samples and UV₂₅₄ ($n = 138$, $\rho = -0.47$, $p = 5 \times 10^{-6}$), but no correlation was observed between NDMA_{UFC} and SUVA ($n = 138$, $\rho = -0.13$, $p = 0.12$). The percent increase in NDMA formation after lime-softening treatment was positively correlated with the TOC concentration in raw samples ($n = 134$, $\rho = 0.23$, $p = 0.008$) but negatively correlated with NDMA_{UFC} in raw samples ($n = 169$, $\rho = -0.21$, $p = 0.0067$). The correlation between the percent increase in NDMA_{UFC} after lime-softening treatment and other water quality parameters was weak (Table S9).

No correlation was observed between NDMA_{UFC} and any nitrogen-related parameters. A high nitrogen load on the rivers was observed from March to June 2021. When this spring snowmelt period was excluded, a positive correlation between NDMA_{UFC} and DN was observed for the whole watershed ($n = 97$, $\rho = 0.44$, $p = 8 \times 10^{-6}$) and each river, including the Mississippi River ($n = 17$, $\rho = 0.5$, $p = 0.041$), Crow River main stem ($n = 17$, $\rho = 0.84$, $p = 1.6 \times 10^{-5}$), North Fork ($n = 34$, $\rho = 0.67$, $p = 6.5 \times 10^{-5}$), and South Fork ($n = 29$, $\rho = 0.42$, $p = 0.025$), as shown in Figure S11. The correlation between NDMA_{UFC} and DN is more robust in the samples collected from the North Fork of the Crow River and the Crow River main stem, where the NDMA precursors are more abundant.

Modeling the Effect of NOM Concentration on NDMA Formation from Ranitidine. In the absence of NOM, the second-order reaction rate constants used to predict NDMA formation for the two models were similar (1.3 and $1.37 \text{ M}^{-1} \text{ s}^{-1}$; Table S14). With increasing NOM concentration, the reaction rate constant decreased for both models but more dramatically for model B, where the rate constant decreased from 1.37 to $0.012 \text{ M}^{-1} \text{ s}^{-1}$ as NOM increased from 0 to 5 mg C/L. For an initial ranitidine concentration of 200 nM and the high NOM concentration condition (i.e., 6 mg C/L for model A and 5 mg C/L for model B), the predicted NDMA_{UFC} concentrations after 3 days of incubation were 161.8 and 21.2 nM for models A and B, respectively (Figure S14). With lower NOM concentrations (i.e., 2 mg C/L for model A and 1 mg C/L for model B), the predicted NDMA_{UFC} increased 23% for model A and 673% for model B. The differences in residual chloramine concentration between the high and low NOM concentration cases after 3 days were 0.67 mg of Cl₂/L for model A and 0.62 mg of Cl₂/L for model B (Figure S14), which is comparable to our experimental observations.

DISCUSSION

Lime Softening and Water Quality Affect NDMA Formation. Lime-softening treatment, where lime was added

together with alum and ferric chloride, typically resulted in an increase in NDMA formation using the UFC protocol. The enhancement was greatest when raw water samples had a relatively high level of NDMA precursors (>60 ng/L). For samples collected in January and February of 2020, NDMA formation increased more than 1000% to >1000 ng/L after lime softening (Figures 4 and S9). One possible explanation for the lime-softening enhancement effect is that the removal of NOM by softening and coagulation results in less competition for available chloramines between precursors and NOM during the UFC tests. The lime-softening process entailed the addition of iron, aluminum, and calcium, which subsequently undergo precipitation as solids as part of the treatment process. While the effect of these species on NDMA formation was not tested, it is expected that their effect would be minimal. Although different in terms of the pH during treatment, coagulation using polyvalent cations is similar to lime softening with coagulation in terms of the removal of NOM. Hence, the findings from the present study appear to contradict previous studies where coagulation with alum or FeCl_3 exerted little influence on NDMA formation potential despite effective removal of DOC and dissolved organic nitrogen.^{29,39–41} In these previous studies, however, the FP test was used (140 mg/L as Cl_2 of monochloramine and a 7 day-incubation period). In the present study, using the UFC protocol, which more closely mimics disinfection conditions in full-scale distribution systems, a consistent enhancement in NDMA formation following lime softening was observed.

The strong negative correlation between NDMA_{UFC} and the organic carbon concentration of raw water samples and the decrease in NDMA formation induced by spiking NOM implies that NOM is not a major source of NDMA precursors and that its inhibition of the reactivity of other precursors far outweighs its contribution to the precursor pool. NOM can inhibit NDMA formation, especially under UFC conditions, by serving either as a competitor with NDMA precursors for chloramines or by limiting the contact between precursors and chloramines by binding with precursors.^{37,42} At very high chloramine concentrations, a decrease in NDMA_{FP} was observed after lime-softening treatment, which is comparable to previous reports of a decrease in NDMA_{FP} after coagulation,^{29,40,41} suggesting that some NDMA precursors are removed along with NOM during these treatment processes. Nevertheless, while lime softening may remove NDMA precursors to a limited extent, the removal of NOM and the corresponding decreased inhibition of precursor reactivity with chloramines outweighs any precursor loss⁴³ and leads to increased NDMA formation under chloramine-limited conditions, as shown by the modeling results in Figure S14.

The enhancement effect of the lime softening treatment on NDMA formation was highly variable, which is likely influenced by the properties of NOM present in the water samples and the fractions removed by the lime softening treatment. Modeling results were consistent with our experimental observations in showing that NOM effectively inhibits the formation of NDMA under UFC test conditions even when residual chloramines are present throughout the reaction time. Specially, the NDMA_{UFC} predicted from model B was significantly lower than that from model A at comparably high NOM concentrations (5–6 mg C/L), which is likely related to differences in the composition of

the NOM in the water in the respective studies (e.g., SUVA_{254} of 2.3 L/mg·m in model A and 3.2 L/mg·m in model B).⁴²

Electrostatic interactions between contaminants and the NOM could also play a role. These electrostatic interactions can impede the contact of the NDMA precursors with chloramines.^{44–48} Secondary and tertiary amines (e.g., dimethylamine pK_a of 10.7)⁴⁹ are typically positively charged at circumneutral pH and thus possess the ability to engage in reversible electrostatic interactions with carboxyl and possibly quinone functional groups of NOM. The amine nitrogen in ranitidine ($\text{pK}_a = 8.2$),⁵⁰ is primarily positively charged over the pH range from 0 to 8.2. In addition, hydrogen bonding of tetracycline with high molecular weight and polar NOM fractions was found to effectively slow NDMA formation.⁵¹ By removal of NOM from the system to a greater extent than precursors, these effects would be lessened. A detailed characterization of NOM and its interactions with known or suspected NDMA precursor compounds would aid in understanding the role of lime softening and associated NOM removal in increasing NDMA formation.

There are confounding reports in the literature concerning correlations between NDMA_{FP} and nitrogen-related parameters. Some researchers observed a positive correlation between DON and NDMA formation,^{52–56} while others reported weak relationships between NDMA formation and nitrogen-related parameters.^{11,23,57} In the present study, a positive correlation between NDMA_{UFC} and DN concentration was observed, which suggests that the DN concentration is a potential surrogate for NDMA precursors released into the rivers sampled in this study. When high NDMA_{UFC} concentrations were detected in July 2020, January 2021, and February 2021, high DN concentrations were also observed. However, in January and February 2021, lower DOC concentrations were also observed, which would likely result in reduced inhibition of NDMA formation by NOM. This interpretation is supported by the relatively high residual chlorine concentrations observed at the end of the UFC test for these samples (Figure S5). Thus, the correlation between NDMA_{UFC} and DN may be coincidental. Nevertheless, if an elevated DN concentration is detected in the source water of a water treatment plant, then closer monitoring of NDMA levels in the drinking water distribution system may be warranted. Considering the high NDMA precursor concentration detected in the Crow River, a significant tributary of the Upper Mississippi River, ongoing nontarget analysis work is being performed in an attempt to identify dominant NDMA precursors and their sources in that river.

NDMA Formation Is More Variable Temporally than Spatially. Spatial and temporal variability in NDMA precursor levels were investigated by sampling at 12 locations along several rivers in one watershed monthly for a year and subsequently bimonthly for half a year. NDMA precursor concentrations in the Crow River determined via UFC testing varied seasonally from 7.5 ± 4.4 ng/L in June 2021 to 109.4 ± 14.0 ng/L in January 2021. The dilution effect of river flow appears to play a role in the seasonal variation of the NDMA precursor concentrations. For example, a sharp decrease in NDMA_{UFC} from February to March 2021 was coincident with the start of spring snowmelt and the resulting elevated river discharge. Similar studies concerning the seasonal dynamics of NDMA precursors in a watershed are scarce. A study on the occurrence of NDMA precursors in 12 locations (3 rivers and 9 lakes) in the Southeastern United States reported that the

monthly average NDMA_{FP} remained relatively constant in fall and winter (38–71 ng/L) but was more variable in spring and summer (34–93 ng/L), with a clear dilution effect as a result of rainfall.²³ No seasonal trend was observed in an investigation of NDMA_{FP} in the Buyukecekmece watershed in Turkey, in which a lake and its five tributaries were monitored.⁵⁸

A combined analysis of the spatial variations of NDMA_{UFC} and the geographical distribution of anthropogenic activities in the Crow River, including cropland, feedlots, wastewater treatment plants, hospitals, ethanol plants, and populated cities, provided clues as to potential sources of NDMA precursors in the watershed (Figure S12). Concentrated animal feeding operations (CAFOs) and municipal wastewater discharges are likely the major sources of NDMA precursors in the Crow River. There is no major city in the Crow River watershed (total population = 204,756; population of largest town = 15,825),⁵⁹ but many CAFOs are clustered in the headwaters region of the North Fork River, where high NDMA_{UFC} and DN concentrations (Figure S3) were measured. Several researchers reported that agricultural runoff from animal facilities, antibiotics used in animal operations, and swine wastewater were associated with elevated NDMA formation.^{11,13,60} In the downstream area of the Crow River watershed, there are fewer CAFOs and croplands but an increasing human population with corresponding municipal wastewater discharges, which appear to be associated with higher NDMA_{UFC} concentrations in the downstream portions of the North and South Forks as well as the main stem of the Crow River. Previous studies have shown that municipal wastewater effluents are an important point source of NDMA precursors, with urban runoff being an important nonpoint source of precursors.^{61–65} No significant correlation was found between NDMA_{UFC} and any land use factors, probably because the 12 sites are in one watershed, thus lacking the high spatial diversity in land use needed for a robust correlation analysis. The Crow River watershed, which is impacted by both agricultural activities and municipal wastewater discharges, was chosen because it was a suspected source of NDMA precursors to Upper Mississippi River drinking water supplies. To gain more insight into possible land-use effects on NDMA precursor levels, a follow-up study was performed involving extensive sampling along rivers in four different watersheds with widely varying land use characteristics.

Despite sampling at relatively high spatial and temporal resolution in the Crow River watershed, no hotspots were identified that would point to major point sources of NDMA precursors. NDMA precursor concentrations remained relatively consistent along the North Fork of the Crow River and gradually increased along the South Fork of the Crow River, with no step increases indicative of a substantial point source. Additional insight could be gained by nontargeted analysis to identify known or potential NDMA precursors (e.g., pharmaceuticals, agrochemicals) that could then be linked to specific sources or operations, but that was beyond the scope of the present study.

Environmental Significance and Limitations. A unique aspect of this study involved comparisons of NDMA formation before and after lime softening. The observed increase in NDMA_{UFC} after lime softening is likely related to the competition for limited chloramines by NOM. The differences in the percent increase in NDMA formation after lime softening may be related to differing NOM properties or

composition in water samples, as suggested by the modeling results. More work is needed to investigate the role of lime softening on NDMA formation including testing of waters from other watersheds to determine if the effects observed in the Crow River are representative of other hard surface waters.

One limitation of this study is that NDMA formation was measured by adding preformed chloramines via the UFC and FP tests. Many drinking water treatment plants in the United States use free chlorine as the primary disinfectant followed by the addition of NH₃ to convert the residual free chlorine to chloramines.^{66,67} Short contact with free chlorine potentially destroys some NDMA precursors, resulting in less NDMA formation after converting the residual chlorine to chloramines.^{68,69} Thus, the UFC and FP tests represent moderate to worst-case scenarios, respectively, for NDMA formation, while free chlorine exposure followed by NH₃ addition would result in lower and likely more realistic NDMA concentrations.³² The obvious trade-off with the free chlorine exposure strategy for preoxidizing NDMA precursors is the formation of halogenated DBPs including regulated compounds. Furthermore, investigation of the formation of other nitrosamines in addition to NDMA would provide a complete picture of the role of lime softening, NOM, and land use on total nitrosamines.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.3c01767>.

Supporting text, including chemicals and reagents, information on water quality analyses, methods to prepare monochloramine solution and determine residual chlorine concentration, and procedures for land use data analysis; supporting tables, including information on sampling locations, river discharges, summary of water quality characteristics, instrumental method setting for NDMA determination, NOM removal efficiencies of lime-softening treatment, percentages of UFC-reactive precursors in the selected Crow River samples, and Spearman correlation analysis results between all the measured water parameters and NDMA_{UFC} concentrations and percent increase in NDMA_{UFC} after softening; supporting figures, including the temporal and spatial variations in the water parameters, temporospatial concentrations of NDMA_{UFC} in the raw samples in the Crow River watershed, NDMA_{UFC} concentrations after photolysis and dark incubation, correlations between NDMA_{UFC} and water parameters, including DN, TOC, and DOC, and distribution of anthropogenic activities in the Crow River watershed (PDF)

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Notes

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Preoxidation Effectively Destroys *N*-Nitrosodimethylamine Precursors in Raw, Lime-Softened, and Recarbonated Surface Waters

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Abstract

Nitrosamines, including *N*-nitrosodimethylamine (NDMA), are disinfection by-products of concern for drinking water utilities that use chloramines as a disinfectant. In this study, batch experiments were performed to investigate the effectiveness of preoxidation with ozone, free chlorine, or both on removal of NDMA precursors in raw, lime-softened, and recarbonated water from a full-scale water treatment plant treating primarily Mississippi River water and in a tributary water sample before and after bench-scale lime softening. NDMA “precursor” concentrations were assessed indirectly by quantifying NDMA formed after addition of preformed chloramines using the uniform formation conditions (UFC) protocol. NDMA concentrations (NDMA_{UFC}) in the raw plant water were 8 ng/L in summer and 10 ng/L in winter and increased to 13–26 ng/L in lime-softened and recarbonated waters. NDMA_{UFC} in the raw tributary water was 33.7 ng/L. Preoxidation by ozone at doses of up to 5 mg/L effectively decreased NDMA_{UFC} concentrations in all water samples with and without hydrogen peroxide addition (hydrogen peroxide:ozone mass ratio of 0.5:1). The effectiveness of ozone at destroying ambient NDMA precursors was independent of water temperature and ozone dose. NDMA precursor destruction, however, increased with increasing ozone dose for a recarbonated water sample spiked with ranitidine (initial NDMA_{UFC} = 358 ng/L). Free chlorine at a dose of 5 mg/L as Cl₂ for 20 min of contact time (i.e., Ct ~ 70 mg-min/L) was most effective at destroying ambient NDMA precursors in recarbonated water, and similarly, free chlorine treatment following ozone (2 mg/L) was more effective at destroying NDMA precursors in the ranitidine-spiked water than ozone alone. Preoxidation with ozone, free chlorine, or both proved to be an effective strategy for limiting NDMA formation upon subsequent chloramination for surface waters impacted by agricultural activities and municipal wastewater discharges.

Keywords: chloramines; disinfection byproducts; drinking water; ozone

Introduction

DISINFECTION OF DRINKING water, primarily with chemical oxidants, has significantly improved public health by mitigating the risks affiliated with pathogen exposure. A chemical oxidant, however, can react with precursor compounds such as natural organic matter (NOM) or anthropogenic contaminants in source waters to form toxic and

potentially carcinogenic disinfection by-products (DBPs). DBPs were first discovered in the 1970s when free chlorine, the predominant disinfectant in the United States, was shown to react with NOM to form toxic and potentially carcinogenic trihalomethanes (THMs) (Rook, 1974). The concentrations of THMs and another class of DBPs discovered in the early 1980s, haloacetic acids (Miller and Uden, 1983), in drinking water have subsequently been regulated by the USEPA (2022a).

Although a national survey of water utilities conducted in 2017 indicated that ~70% of respondents still used free chlorine for disinfection, many utilities have transitioned to alternative disinfectants, such as ozone, chlorine dioxide, and chloramines, to reduce formation of regulated and other halogenated DBPs. Nonetheless, harmful DBPs are also associated with these alternative disinfectants: bromate and aldehydes with ozone; chlorite with chlorine dioxide; and

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nitrosamines with chloramines (Gilca et al., 2020; Shah et al., 2012). Chloramines are frequently used as a residual or secondary disinfectant, allowing time for nitrosamines to form in water distribution systems (Krasner et al., 2013).

With chloramination occurring in numerous utilities in the United States, nitrosamines are a DBP class of particular concern. Nitrosamines are potentially potent carcinogens and are associated with a 10^{-6} lifetime cancer risk when present in drinking water at nanogram per liter concentrations (USEPA, 2022b). Of the nitrosamines, *N*-nitrosodimethylamine (NDMA) is the most prevalent compound, and it has been detected in 34% of chloraminated drinking water samples according to a nationwide survey conducted as part of the Unregulated Contaminant Monitoring Rule 2 (Woods and Dickenson, 2015).

The main formation pathway for NDMA is the reaction of dichloramine, which co-occurs with monochloramine, with organic precursor compounds containing secondary, tertiary, or quaternary amine functional groups. Pesticides, fungicides, pharmaceutical and personal care products, and amine-based polymers used in water treatment have all been identified as NDMA precursors (Krasner et al., 2013; Sgroi et al., 2018). Municipal wastewater discharges often contain a variety of these anthropogenic precursor compounds and often contribute the highest load of NDMA precursors to a watershed, although agricultural activity and stormwater runoff are important sources of precursors as well (Aydin et al., 2012; Zeng et al., 2016).

Although there is no federal regulation for NDMA, NDMA was added to a draft of the USEPA drinking water contaminant candidate list 5 in 2021 (USEPA, 2021). Some states have established guidance or notification levels for NDMA, including a notification level of 10 ng/L in California and a guidance value of 5 ng/L in Minnesota (California Department of Public Health, 2013; Minnesota Department of Health, 2023). The World Health Organization guidance value for NDMA in drinking water is 100 ng/L (WHO, 2008).

Oxidizing precursors before chloramination may be a viable strategy for limiting NDMA formation upon disinfection. Several studies have demonstrated that oxidants such as ozone, free chlorine, permanganate, ferrate, hydrogen peroxide, or a combination of these oxidants can deactivate NDMA precursors (Chen and Valentine, 2008; Krasner et al., 2013; Lee et al., 2008; Lee et al., 2007a; Pisarenko et al., 2012; Selbes et al., 2014; Shah et al., 2012; Wang et al., 2015). Generally, ozone and free chlorine tend to be the most effective at decreasing NDMA formation, as both oxidants readily react with amine functional groups (Deborde and von Gunten, 2007; Lim et al., 2022).

There are examples, however, of direct NDMA formation upon ozonation of specific precursors (e.g., *N*-*N*-dimethylsulfamide, daminozide, metformin, and to a lesser extent dimethylamine) and wastewater samples (Liao et al., 2021; Padhye et al., 2011; Song et al., 2022; von Gunten et al., 2010; Yang et al., 2009). In addition, there are several reports suggesting that preozonation can increase NDMA formation upon subsequent chloramination (Andrzejewski et al., 2008; Gerrity et al., 2014; Liao et al., 2021; Marti et al., 2014; Oya et al., 2008; Schmidt and Brauch, 2008; Trogolo et al., 2015; von Gunten et al., 2010). Thus, the effect of preoxidation on limiting NDMA formation may be specific to a given water matrix.

The main objective of this study was to assess the effects of preoxidation on NDMA formation upon chloramination for water samples from the upper Mississippi River watershed. The upper Mississippi River was of interest because it serves as the drinking water supply for ~1 million residents of the State of Minnesota, USA, including those in the Twin Cities of Minneapolis and St. Paul, and has the potential to form more than 50 ng/L of NDMA upon addition of preformed chloramines (Hozalski et al., 2021). Raw water samples were collected from a water treatment plant supplied primarily with Mississippi River water, as well as a tributary to the Mississippi River impacted by agricultural activities and municipal wastewater discharges.

An important aspect of this study involved testing of water samples from a lime-softening plant, where the relatively high pH conditions (10.6–10.8 for lime-softened water and 8.6–8.9 for recarbonated water) should facilitate not only NOM removal but also hydroxyl radical formation, when ozonating without the addition of hydrogen peroxide. In addition, testing a wide range of oxidant doses and combinations of oxidants provides information to optimize treatment.

Selected water samples also were spiked with a model NDMA precursor, ranitidine, before further testing. Pre-oxidation was performed in batch reactors where three common oxidants were compared under a wide range of conditions: free chlorine, ozone, and a combination of ozone and hydrogen peroxide (i.e., an advanced oxidation process [AOP]). The effect of preoxidation on NDMA precursor levels was assessed by applying preformed chloramines to all water samples following the uniform formation conditions (UFC) protocol and measuring the resulting NDMA concentrations.

Materials and Methods

Site selection, sample collection, and chemical reagents

A drinking water treatment plant (Saint Paul Regional Water Services [SPRWS]) with the Mississippi River as its main source water was chosen for this study. Water is withdrawn from the Mississippi River and passed through a chain of lakes before entering the treatment plant. The water is treated by the addition of lime and coagulant (alum and ferric chloride), flocculation, sedimentation, recarbonation, and filtration before disinfection with free chlorine and then addition of ammonia to form chloramines before distribution. Raw water, lime-softened water, and recarbonated water were collected from SPRWS on two occasions in July 19, 2021 (summer), and January 21, 2022 (winter), to investigate potential seasonal water quality variations.

Water samples were collected in clean high-density polyethylene carboys, filtered (0.7 μ m glass microfiber), and stored in the dark at 4°C until used. An additional water sample was collected from the Crow River, a suspected contributor of NDMA precursors to the Mississippi River, on February 19, 2022 (45.088345, -93.733763). The Crow River water sample was then split, and half the sample was filtered (0.7 μ m glass microfiber) and the other half was lime softened using a jar test procedure that mimics lime softening at SPRWS. Both the raw and lime-softened Crow River water samples were stored in the dark at 4°C until used.

All chemical sources and purities are provided in the Supplementary Information. The jar test procedure used for lime softening Crow River water is also provided in the Supplementary Information.

Batch oxidation experiments

For batch experiments with ozone, ozone gas (up to 8% by weight) was produced from ultra-high purity oxygen gas (99.99%) using a Pacific Ozone O₃ generator and then sparged into ultrapure water cooled to 4°C in an ice bath (Supplementary Information). Ozone stock solutions ranged from 30 to 50 mg/L. In addition, hydroxyl radical (•OH) concentrations were measured indirectly for ozone and AOP experiments by monitoring the decrease of an added probe compound, para-chlorobenzoic acid (pCBA; ≥98.5%).

Batch ozonation and AOP experiments were performed in 1,000-mL amber glass bottles fitted with bottle top dispensers. A sample of 600 mL water with 10 μM pCBA was added to the bottle. Ozone was injected into the reactor by glass pipette to the desired dose, while continuously stirring the reactor contents with a magnetic stir bar. For the AOP experiments, hydrogen peroxide was added to the batch reactors just before the addition of ozone, at a mass ratio of 0.5:1 of hydrogen peroxide:ozone. All materials in the reactors were comprised of glass or polytetrafluoroethylene so as not to be reactive with chemical oxidants.

The raw, lime-softened, and recarbonated water samples were ozonated at room temperature (20.7°C ± 0.5°C) at doses of 1, 2, and 5 mg/L. Additional ozonation experiments were performed in cold water (5.2°C ± 0.2°C), but at only one dose (2 mg/L). AOP experiments were performed on raw and recarbonated water at both temperatures, but only one ozone dose (2 mg/L). For ranitidine spiking experiments, an aliquot from a 24 mg/L stock solution prepared in ultrapure water was added to a 600 mL sample of winter recarbonated water to obtain an initial concentration of 1,200 ng/L (3.8 nM). The raw and softened Crow River water samples were ozonated at room temperature at a dose of 2 mg/L only.

Water samples were withdrawn from the batch reactors using the dispenser for the measurement of residual ozone concentration after 30 s and then every minute thereafter until the ozone was depleted (up to 10 min). Concurrent subsamples for indirect measurement of •OH were also dispensed from the reactor using a bottle top dispenser into separate glass vials containing a final concentration of 10 mM of tert-butyl alcohol (TBA, an •OH quencher to halt reaction of pCBA) for every minute of ozone residual measurement. After this 10-min period, 475 mL of remaining sample water was then transferred to a 500 mL amber bottle to conduct the UFC test.

The UFC test mimics practical chloramination conditions, and the procedure was adopted from Zeng and Mitch (2015). Preformed monochloramine was prepared each day of UFC testing by mixing a cold 25 mM ammonium chloride solution with sodium hypochlorite at a 1:1 molar ratio (Soltermann et al., 2013). The resulting monochloramine concentration was then quantified using molar absorptivities from the methods of Schreiber and Mitch (2005) and Schurter et al. (1995). An aliquot of monochloramine was added to water samples that were buffered at a pH of 8 with a 4 mM borate solution to obtain an initial concentration of 5 mg/L as Cl₂.

The water samples were then incubated in the dark at room temperature for 3 days. After 3 days, the residual chloramine concentrations were measured. Residual chloramines were then quenched with excess L-ascorbic acid.

Free chlorine preoxidation testing was performed on recarbonated water and ozonated (2 mg/L) recarbonated water. A simulated distribution system (SDS) protocol developed by SPRWS was followed for the chlorination and chloramination phases. Briefly, the water sample was first buffered to a pH of 8 with borate buffer. The total organic carbon (TOC) of the water was then measured, and sodium hypochlorite prepared in pH 6.7 borate buffer was added to the water at a chlorine to TOC mass ratio of 1.2 Cl₂:1 TOC. The reactor contents were then mixed for 20 min with a PTFE-coated magnetic stir bar before adding ammonium sulfate at an initial chlorine (as Cl₂) to ammonia mass ratio of 4.6:0.94.

The initial chlorine dose ranged from 4.24 to 5 mg/L as Cl₂ to achieve a target residual concentration after 20 min of 3.5 mg/L as Cl₂ and a Ct of ~70 mg-min/L. The pH of the water was adjusted to 9 with sodium hydroxide and then the batch bottles were incubated for 3 days at room temperature in the dark. After incubation, the residual chloramine concentration was measured using the ABTS method and then quenched with L-ascorbic acid. Brief free chlorine exposure before addition of ammonia to form chloramines mimics common water treatment practice where free chlorine is used for primary disinfection and then converted to chloramines for secondary or residual disinfection in the water distribution system. A summary of the experimental matrix is shown in Table 1. Most experiments were conducted in triplicate, with exceptions noted in the presentation of results.

Analytical methods

Routine water quality parameters, including turbidity, TOC, dissolved organic carbon (DOC), alkalinity, and bromide concentrations, were measured following Standard Methods (American Public Health Association et al., 2023). Ozone concentrations were quantified by the indigo method (Bader and Hoigné, 1981). Residual chloramine concentrations were measured by the ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] method (Pinkernell et al., 2000). The decrease of pCBA concentration was measured by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 240 nm based on a method developed by Lee et al. (2007b). Details of all three methods and calculation of ozone exposures are provided in the Supplementary Information.

NDMA extraction and quantification followed a modified USEPA Method 521 (Munch and Bassett, 2004). Briefly, all samples were first spiked with an internal standard (d₆-NDMA) to a concentration of 10 ng/L before solid phase extraction (SPE) using Enviro-Clean 521 Activated Carbon cartridges (United Chemical Technologies). The cartridges were first conditioned with dichloromethane, methanol, and then Milli-Q water.

After SPE, cartridges were eluted with dichloromethane and the SPE extract was evaporated under a gentle N₂ stream to a volume of ~0.1 mL. Samples were then reconstituted to a proportional volume in microliters as the original UFC sample volume in milliliters (i.e., 485 μL to an original volume of 485 mL to account for the concentrating effect of

TABLE 1. SUMMARY OF BATCH OXIDATION EXPERIMENTS PERFORMED ON THE COLLECTED WATER SAMPLES

Sample source	Collection time	Sample type	Preoxidation						
			Control	1 mg/L O ₃	2 mg/L O ₃	5 mg/L O ₃	2 mg/L O ₃ + 1 mg/L H ₂ O ₂	Free chlorine	2 mg/L O ₃ + free chlorine
Drinking water treatment plant	Summer, Winter 2021	Raw	✓	✓	✓✓	✓	✓✓		
		Lime softened	✓	✓	✓✓	✓			
		Recarbonated	✓	✓	✓✓	✓	✓✓	✓✓	✓ ^a
	Winter 2021	Recarbonated ranitidine spike	✓	✓	✓	✓	✓	✓	✓
Crow River	Winter 2021	Raw	✓	✓					
		Lime softened	✓	✓					

Black marks indicate experiments conducted at room temperature (20.7°C ± 0.5°C), while gray checks indicate experiments conducted at low temperature (5.2°C ± 0.2°C).

^aIndicates this experiment was only conducted using samples collected in winter 2021.

nitrogen blow down) with liquid chromatography-mass spectrometry (LC-MS) grade water and transferred to HPLC vials. A full SPE procedure is in the Supplementary Information. NDMA was analyzed using a Thermo Scientific Vantage Triple-Stage Quadrupole Mass Spectrometer, and details are in the Supplementary Information. Results for samples in which the internal standard was not detected (15.8% of samples) were discarded.

The measured NDMA concentration was corrected for dilution from the addition of the ozone stock solution, hydrogen peroxide solution (if used), •OH probe compound solution, and UFC components (borate buffer and chlorine/chloramine solution). Total dilutions ranged from 2% to 18% of the original sample volume. Likewise, the measured pCBA concentration was corrected for the dilution from the addition of the ozone stock solution and TBA. A derivation of dilution corrections and sample calculations for NDMA and pCBA is in the Supplementary Information. Batch experiments with free chlorine resulted in minimal dilution (<1%), so no correction was needed.

Data analysis

Data analysis and visualization were performed with R software (R Core Team, 2021). Student's *t*-tests were performed to compare •OH formation in different water types and at different temperatures. Likewise, *t*-tests were performed to compare NDMA formation between similar pre-oxidation treatments and at different temperatures.

Results

Water quality

A summary of water quality indicators for both summer and winter samples collected from SPRWS is provided in Supplementary Table S1. The SPRWS summer raw water had a pH of 8.4 and a TOC of 6.9 mg/L, while for the winter raw water, the values were 7.9 and 8.4 mg/L, respectively. The lime-softened SPRWS water for summer and winter had an elevated pH (10.6–10.8) and the TOC concentration was reduced to 3.9 mg/L.

The removal of NOM by lime softening is also reflected in a decrease in UV absorbance (UV₂₅₄). Alkalinity, a source of •OH scavengers, also decreased ~3-fold by lime softening. The pH of recarbonated SPRWS water was 8.6 for the summer water and 8.9 for the winter water and the TOC was similar to the lime-softened water. For the Crow River water, the pH and TOC concentration were 7.9 and 34 mg/L, respectively, and the values after lime softening in the laboratory using a jar-test apparatus were 10.5 and 2.6 mg/L, respectively.

Ozone degradation kinetics and •OH formation

Ozone was completely consumed within 30 s under most experimental conditions, particularly at ozone doses of 1 and 2 mg/L and in the high pH lime-softened waters before recarbonation. In these cases, ozone exposure was estimated from the area under a linear decay curve from the known ozone dose at time zero to a concentration of 0 mg/L ozone at 30 s. The estimated ozone exposures were 0.25, 0.5, and 1.25 mg-min/L for ozone doses of 1, 2, and 5 mg/L, respectively.

Because ozone may have been completely depleted before the first residual measurement at 30 s, these exposures may be overestimates. At an ozone dose of 5 mg/L, ozone typically was detected for a minute or longer at room temperature, with calculated exposures ranging from 1.36 to 2.83 mg-min/L for raw and recarbonated water samples from SPRWS (Supplementary Table S2). Example plots of ozone decay for different water types are provided in Supplementary Fig. S1.

The amounts of •OH formed during batch experiments at 20°C for summer and winter water are compared in Supplementary Fig. S2. For experiments with ozone consumption within 30 s, the ratio of •OH to O₃ exposures (*R_{ct}*) (Elovitz and von Gunten, 1999; Elovitz et al., 2000) could not be calculated. Thus, the loss of pCBA at 1 min is presented to show changes in the extent of •OH production.

The formation of •OH (as measured indirectly by pCBA decrease) increased with increasing ozone dose for all water types for both summer and winter samples. In addition, regardless of ozone dose, more •OH reacted with the probe compound in recarbonated SPRWS water, which had both

lower DOC and alkalinity, than the raw and lime-softened water for both seasons. The formation of $\bullet\text{OH}$ during AOP treatment resulted in a similar loss of the pCBA compared to the raw and recarbonated water at the same ozone dose (2 mg/L), indicating similar extent of $\bullet\text{OH}$ formation with and without the addition of H_2O_2 . The effect of temperature on $\bullet\text{OH}$ formation is shown in Supplementary Fig. S3. In recarbonated water, *t*-tests showed significantly greater $\bullet\text{OH}$ formation at 5°C than at room temperature.

NDMA precursor removal by preoxidation

The mean NDMA_{UFC} concentrations were 8.5 ng/L for SPRWS raw summer water (Fig. 1) and 10.1 ng/L for SPRWS raw winter water (Supplementary Fig. S4). All oxidation treatments for these two raw waters resulted in NDMA_{UFC} concentrations below 5 ng/L, with concentrations ranging from 3.1 to 3.8 ng/L for the summer water and 2.5 to 3.6 ng/L for winter water. In addition, the NDMA_{UFC} concentration following AOP treatment at 2 mg/L of ozone was similar to that for ozone alone at the same dose. Ozonation was also effective at limiting NDMA formation in a water sample from the Crow River (Fig. 2), which has substantially higher raw water NDMA formation (33.7 ng/L). An ozone dose of 2 mg/L at 20°C reduced the average NDMA concentration to 6.6 ng/L.

The two watersheds that the water samples originated from differ substantially in land use (Supplementary Table S3). The Crow River watershed is dominated by agricultural activity (73%), while the Mississippi River watershed in the Twin Cities area is more urbanized (47%), with agricultural activity accounting for only 22% of the land use in the watershed. These differences may introduce different pools of NDMA precursors.

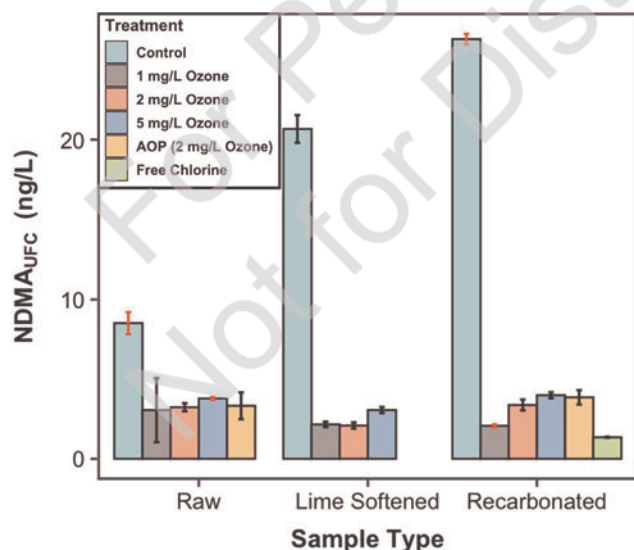


FIG. 1. Effect of preoxidation at 20°C on mean NDMA_{UFC} concentration for raw, lime-softened, and recarbonated SPRWS water collected in the summer. Error bars in red represent the difference between duplicate results, while error bars in black represent the standard deviation of triplicate results. NDMA, *N*-nitrosodimethylamine; SPRWS, Saint Paul Regional Water Services; UFC, uniform formation conditions.

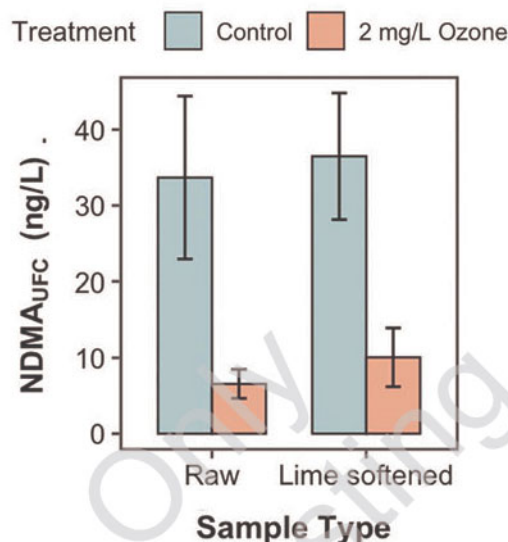


FIG. 2. The effect of preoxidation on mean NDMA_{UFC} concentration for raw and lime-softened Crow River water. Error bars represent the standard deviation of triplicate results. The pH and TOC of raw water were 7.9 and 34 mg/L, respectively, while the pH and TOC of lime-softened water were 10.5 and 2.6 mg/L, respectively. TOC, total organic carbon.

Lime softening of SPRWS raw water increased the formation of NDMA during subsequent UFC testing, with values of 20.7 ng/L for summer water (Fig. 1) and 17 ng/L for winter water (Supplementary Fig. S4). After recarbonation, the NDMA_{UFC} concentration increased further to an average concentration of 26.3 ng/L for summer water, but decreased slightly

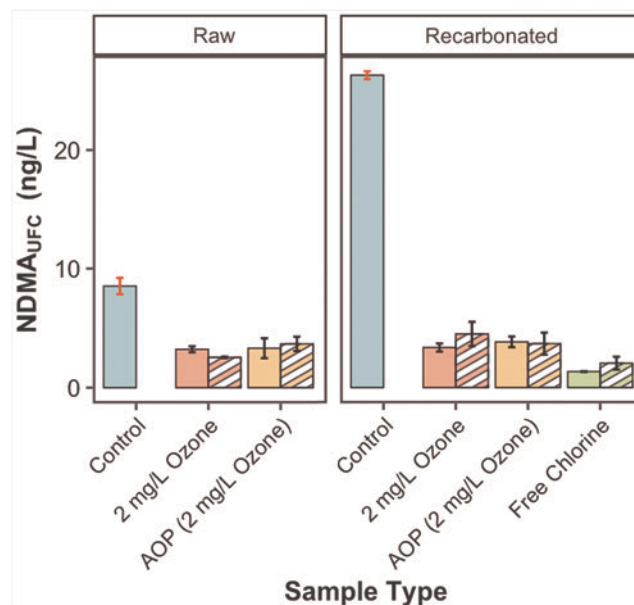


FIG. 3. Effect of preoxidation temperature on mean NDMA_{UFC} concentration for raw and recarbonated water collected in the summer. Error bars in red represent the difference between duplicate results, while error bars in black represent the standard deviation of triplicate results. Solid bars and diagonal hatched bars represent results for temperatures of 20°C and 5°C, respectively.

for winter water to 14 ng/L. No increase in NDMA_{UFC} was observed after lime softening of the Crow River water (Fig. 2).

Ozonation of lime-softened SPRWS water at ozone doses ranging from 1 to 5 mg/L decreased NDMA_{UFC} levels to 2.2–3.1 ng/L for the summer water (Fig. 1) and 1.5–2.7 ng/L for the winter water (Supplementary Fig. S4). For Crow River water, an ozone dose of 2 mg/L at 20°C decreased NDMA_{UFC} to 10.1 ng/L (Fig. 2). Ozonation of recarbonated SPRWS summer water decreased average NDMA_{UFC} to 2.1–4.0 ng/L (4.3–4.6 for winter).

Applying free chlorine using an SDS protocol to recarbonated SPRWS water at a dose of 4.24 mg/L as Cl₂ for 20 min (Ct ~ 70 mg-min/L) resulted in the lowest NDMA_{UFC} concentrations of all treatments (1.4 ng/L summer and 1.1 ng/L winter). AOP treatment at an ozone dose of 2 mg/L resulted in similar NDMA_{UFC} concentrations as ozone alone at the same dose. An additional experiment was performed in which recarbonated SPRWS winter water was ozonated at 2 mg/L followed by free chlorine exposure (dose of 5 mg/L for 20 min, Ct ~ 70 mg-min/L) and then ammonia addition to form chloramines. The mean (± standard deviation) NDMA_{UFC} after applying the two oxidants in series before chloramine formation (3.6 ± 2.5 ng/L) was similar to the values for either oxidant alone at the same doses.

Increasing the ozone dose did not correlate with NDMA_{UFC} concentration (Supplementary Fig. S5, $p=0.51$) in summer raw water. Unlike raw water, NDMA_{UFC} concentration slightly increased with increasing ozone dose at 20°C for the lime-softened and recarbonated water (see $p=0.00083$ and 0.0087 , respectively). No trend was observed in winter water (Supplementary Fig. S5). The increase observed in summer could be due to precursors that react directly with ozone to form NDMA. An ozone dose of 2 mg/L

was applied to the winter water samples and then directly analyzed for NDMA without the addition of chloramines as in a UFC test. For all three water types, ~3 ng/L of NDMA was formed by the addition of ozone without a UFC test (Supplementary Fig. S6).

The effect of preoxidation temperature on NDMA_{UFC} is shown in Fig. 3 (summer) and Supplementary Fig. S7 (winter). In only one case, raw water with an ozone dose of 2 mg/L, was the NDMA_{UFC} statistically different (2.6 ng/L at 5°C ozone dose, 3.2 ng/L at 20°C, $p=0.009$) between the two temperatures. There was no significant difference for the other water samples or preoxidation treatments.

Ranitidine spiking experiments

The effect of preoxidation on limiting NDMA formation in winter recarbonated water spiked with 1,200 ng/L of ranitidine is shown in Fig. 4. Before oxidation, the ranitidine spike increased the average NDMA_{UFC} concentration in recarbonated water from 14 to 358 ng/L (121% molar yield of NDMA from the spiked ranitidine). An ozone dose of 1 mg/L reduced the average NDMA_{UFC} concentration by 94% to 21 ng/L. Increasing ozone doses of 2 and 5 mg/L further diminished NDMA_{UFC} concentrations to 8.2 and 4.2 ng/L, respectively. A sequence of 2 mg/L ozone followed by 5 mg/L of free chlorine produced similar average NDMA_{UFC} concentrations (2.4 ng/L) as 5 mg/L of ozone alone. Free chlorine alone was also effective at diminishing NDMA_{UFC} concentrations by ~97%.

Discussion

Roles of ozone and hydroxyl radical

The longevity of the ozone residual is dependent on the pH, alkalinity, TOC, and temperature of the water matrix (von Gunten, 2003). A combination of these factors most likely played roles in producing low ozone exposures in the sample waters tested. For example, ozone decay rates have been observed to increase by an order of magnitude from 5°C to 25°C (Elovitz et al., 2000), and this is consistent with the rapid loss of ozone at 20°C observed herein.

In addition to temperature, differences in water quality in the different sample types affected ozone stability. Raw water had a lower pH than lime-softened and recarbonated water, which would lead to greater ozone stability because hydroxide initiates ozone decomposition. Raw water exhibited the highest total alkalinity, which would help promote ozone stability due to •OH scavenging by bicarbonate (von Gunten, 2003), because •OH also leads to ozone decomposition. The high TOC in raw water (6–9 mg/L), however, likely leads to the rapid loss of applied ozone, even at 5°C, outweighing any stabilization due to the pH or alkalinity. Rapid ozone residual consumption in lime-softened water is attributed to the high pH. The recarbonated water has the most persistent ozone residual due to the combination of lower pH, lower alkalinity, and reduced TOC.

Depending on the ozonation conditions, including pH, temperature, and TOC concentration, the ozone decomposition and subsequent •OH formation could mimic a traditional AOP using ozone alone (von Gunten, 2003). The similar amounts of •OH produced (as measured by pCBA loss) coupled with the similar extents of NDMA_{UFC} reduction for

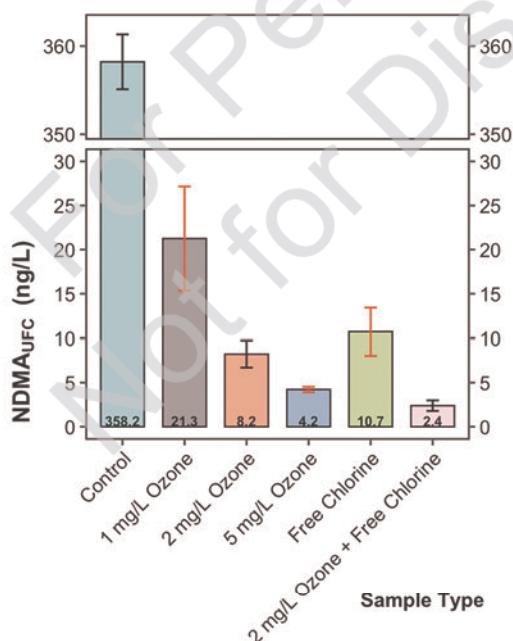


FIG. 4. Effect of preoxidation on mean NDMA_{UFC} concentration for recarbonated water collected in the winter and spiked with 1,200 ng/L ranitidine. Error bars in red represent the difference between duplicate results, while error bars in black represent the standard deviation of triplicate results.

the ozone and AOPs in this study suggest that $\bullet\text{OH}$ is the oxidant responsible for destruction of NDMA precursors. Furthermore, these results suggest that there was no benefit to adding H_2O_2 compared to ozone alone at the elevated pH (8.6–8.9) of the recarbonated water.

Regardless of ozone dose, there was more $\bullet\text{OH}$ exposure for recarbonated water compared to raw or lime-softened water. The recarbonated water had the lowest TOC concentration and alkalinity, both of which serve as $\bullet\text{OH}$ scavengers. Although the TOC concentration and alkalinity of lime-softened and recarbonated water were similar, the inorganic carbon in lime-softened water is mostly in the form of CO_3^{2-} , an $\bullet\text{OH}$ scavenger with a rate constant of $3.9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (Buxton et al., 1988).

At the pH of recarbonated water, the dominant form of inorganic carbon is HCO_3^- , with a rate constant for reaction with $\bullet\text{OH}$ of $8.5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (Buxton et al., 1988). Because many organic compounds have second-order rate constants with $\bullet\text{OH}$ at near-diffusion controlled reaction rate constants ($\sim 10^9 \text{ M}^{-1} \text{ s}^{-1}$), applying ozone to recarbonated water minimized $\bullet\text{OH}$ scavenging by NOM or inorganic carbon, leading to more oxidation of NDMA precursors (von Gunten, 2003).

NDMA precursor removal

Without preoxidation treatment, chloramination of precursors in the raw, softened, and recarbonated SPRWS water samples resulted in NDMA_{UFC} concentrations ranging from 8 to 26 ng/L, above the Minnesota Department of Health's guidance value of 5 ng/L. Ozonation at doses ranging from 1 to 5 mg/L, with or without peroxide addition, as well as preoxidation with free chlorine (dose of 4.24 mg/L as Cl_2 , Ct $\sim 70 \text{ mg-min/L}$) consistently resulted in NDMA_{UFC} concentrations of 5 ng/L or less.

A decrease in NDMA formation in drinking water following preoxidation has been reported previously (Pisarenko et al., 2012; Shah et al., 2012). Shah et al. (2012) investigated the preoxidation of raw water and water that had undergone lime softening and recarbonation. An ozone exposure of 0.4 mg/L-min decreased NDMA formation by 50% in the Shah et al. (2012) study, while ozone exposures as low as 0.25 mg-min/L (estimated) in this study decreased NDMA formation by 65–70% for raw water and 70–90% for recarbonated water. Although ozone performed better than free chlorine in terms of decreasing NDMA formation in Shah et al.'s (2012) study, free chlorine performed similar to or better than ozone in this work.

In addition, Shah et al. (2012) observed a greater decrease in chloramine-induced NDMA formation when preoxidation with free chlorine (dose of 2.5 mg/L Cl_2 for 30 min) was performed at 21°C than at 4°C, but observed little to no difference in NDMA formation when applying ozone at different temperatures. The experiments with SPRWS water produced similar results to Shah et al. (2012) in regard to ozonation at different temperatures, but differed with respect to free chlorine at different temperatures. Nevertheless, results in this study and in Shah et al.'s (2012) suggest that preoxidation with ozone or chlorine would be effective in decreasing NDMA formation over a wide range of water temperatures.

Ozone was also effective at reducing NDMA formation in the Crow River water sample, even though the raw water TOC concentration was relatively high at 34 mg/L. Thus, it

appears that competition between NOM and NDMA precursors for ozone was not significant as the decrease in NDMA_{UFC} was still substantial ($\sim 80\%$ for raw water). Lee et al. (2007a) observed decreases in NDMA formation potential range from 32% to 94% after applying ozone doses of 1 or 2 mg/L to natural waters with ambient precursor pools and much lower NOM content than the Crow River water.

The pool of NDMA precursors in the Crow River sample was likely different than that in SPRWS raw water due to greater agricultural activity in the Crow River watershed. The effective removal of NDMA precursors from raw and lime-softened water samples by ozone from two different surface waters over a wide range of conditions is promising. Nevertheless, more testing of the effects of preoxidation on NDMA precursor removal should be performed on a wider variety of surface waters to investigate potential benefits, as well as drawbacks in terms of formation of regulated or other DBPs.

For most of the conditions tested, there was no effect of increasing ozone dose on NDMA_{UFC}, suggesting that an ozone dose of 1 mg/L was sufficient for the removal of precursors at NDMA_{UFC} concentrations ranging from 8.5 to 26.3 ng/L before preoxidation treatment. However, when the precursor level was increased by spiking in a known precursor compound to achieve an extremely high NDMA_{UFC} of 358 ng/L, there was a clear effect of ozone dose on precursor removal. At that precursor level, an ozone dose of 5 mg/L or a combination of ozonation followed by free chlorine exposure was needed to decrease the NDMA_{UFC} to below 5 ng/L. Thus, these results suggest that low to moderate ozone doses (1–2 mg/L) should be effective for NDMA control, except in extreme circumstances.

It is important to note that NDMA concentrations produced from UFC tests may overestimate the amount of NDMA formed at a utility, because the water is dosed with preformed chloramines and then incubated for 3 days at room temperature (20–22°C) during UFC testing. It is common for water utilities to have brief free chlorine exposure for disinfection credit before converting the residual free chlorine to chloramines with the addition of ammonia. This process was investigated by performing an SDS test and consistently resulted in NDMA concentrations below 5 ng/L. Furthermore, in the winter, in temperate climates, rates of NDMA formation in chloraminated water should be less than those during UFC testing because of the lower water temperatures.

The reason for increased NDMA_{UFC} after lime softening could arise from more efficient removal of organic matter overall compared to NDMA precursors (either microcontaminants or the precursors in NOM). This would lead to a greater fraction of the chloramines available to react with NDMA precursors after softening. The elevated pH also could potentially liberate precursor compounds bound to NOM or alter the structure of NDMA precursors by base-catalyzed hydrolysis and making them more reactive. Based on our ongoing work, we suspect that the decrease in competition for chloramines from NOM is likely the most important factor.

Formation of NDMA and effectiveness of preoxidation with a known precursor

Reported molar conversions for ranitidine to NDMA range from 40% to 100% in distilled deionized water, ultrapure water, or finished tap water for a variety of formation potential tests (Selbes et al., 2018; Selbes et al., 2014; Shen and

Andrews, 2010; Wang et al., 2015; Zou et al., 2018). The observed yield was 121% for ranitidine spiked into lime-softened and recarbonated surface water in this study. Several other studies in the literature also have observed that the presence of NOM can enhance the formation of NDMA from ranitidine (Selbes et al., 2012; Zou et al., 2018).

Nonetheless, the data demonstrate that both free chlorine and ozone were effective at decreasing NDMA formation in ranitidine-spiked water. Both of these oxidants have been reported to diminish the NDMA formation potential of ranitidine, with ozone generally more effective than free chlorine (Selbes et al., 2014; Wang et al., 2015; Zou et al., 2018). In this study, a free chlorine dose of 4.24 mg/L as Cl_2 was slightly more effective than an ozone dose of 1 mg/L, but ozone performed better with increasing dose.

The combination of preozonation at a dose of 2 mg/L followed by free chlorine at a dose of 5 mg/L resulted in the largest reduction in NDMA_{UFC} among all preoxidation treatments tested. Although this study tested the effects of preoxidation on waters spiked with only one model NDMA precursor, the results suggest that moderate doses of ozone and free chlorine, especially when used in series, could effectively decrease NDMA formation for waters with extremely high levels of precursors.

Conclusions

The effects of preoxidation with ozone (1–5 mg/L), free chlorine (4.24–5 mg/L as Cl_2 , Ct ~70 mg-min/L), or both in series, on NDMA precursor levels (i.e., measured by UFC testing), were investigated. Ozone effectively removed NDMA precursors in raw and lime-softened water samples from two different watersheds, suggesting that preoxidation could be effective for a variety of drinking water supplies with different precursor sources.

In addition, NDMA_{UFC} concentrations after preoxidation were similar, regardless of ozone dose, suggesting that utilities using chloramination may only need low ozone doses (e.g., 1 mg/L) to effectively destroy NDMA precursors. When treating water with extremely high precursor concentrations, an ozone dose of 5 mg/L or a combination of ozonation followed by free chlorine exposure was needed to decrease the NDMA_{UFC} to below 5 ng/L. Overall, these results suggest that low to moderate ozone doses and free chlorine Ct values should be effective for NDMA control, except in extreme circumstances.

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Authors' Contributions

E.N.: methodology, investigation, validation, data curation, visualization, and writing—original draft; J.L.: methodology, investigation, visualization, and writing—review

and editing; W.A.: conceptualization, methodology, writing—review and editing, supervision, project administration, and funding acquisition; R.H.: conceptualization, methodology, writing—review and editing, supervision, project administration, and funding acquisition.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Information

Supplementary Table S1

Supplementary Table S2

Supplementary Table S3

Supplementary Figure S1

Supplementary Figure S2

Supplementary Figure S3

Supplementary Figure S4

Supplementary Figure S5

Supplementary Figure S6

Supplementary Figure S7

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