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ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · JANUARY 2014

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Identification of Tobacco-Specific Nitrosamines as Disinfection Byproducts in Chloraminated Water

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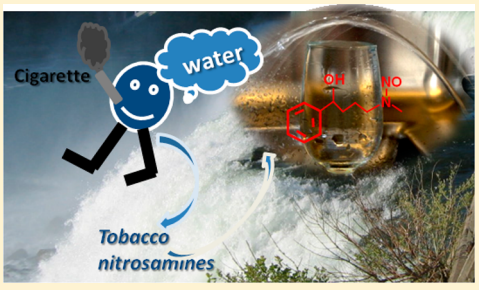
Supporting Information

ABSTRACT: Tobacco-specific nitrosamines (TSNAs) exist in environmental waters; however, it is unknown whether TSNAs can be produced during water disinfection. Here we report on the investigation and evidence of TSNAs as a new class of disinfection byproducts (DBPs). Using five common TSNAs, including (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) as the targets, we first developed a solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method capable of rapidly determining these TSNAs at levels as low as 0.02 ng/L in treated water. Using this highly sensitive method, we investigated the occurrence and formation potential (FP) (precursor test conducted in the presence of chloramines) of TSNAs in treated water from two wastewater treatment plants (WWTPs) and seven drinking water treatment plants (DWTPs). NNAL was detected in the FP samples, but not in the samples before the FP test, confirming NNAL as a DBP. NNK was detected in the treated wastewater before the FP test, but its concentration increased significantly after chloramination in two of three tests. Thus, NNK could be a DBP and/or a contaminant in wastewater. Moreover, these TSNAs were detected in FP tests of wastewater-impacted DWTP plant influents in 9 of 11 samples. However, TSNAs were not detected at full-scale DWTPs, except for at one DWTP with high ammonia where breakpoint chlorination was not achieved. The concentration of the sum of five TSNAs (0.3 ng/L) was 100-fold lower than NDMA, suggesting that TSNAs have a minor contribution to total nitrosamines in water. We examined several factors in the treatment process and found that chlorine or ozone may destroy TSNA precursors and granular activated carbon (GAC) treatment may remove the precursors. Further research is warranted into the efficiency of these processes at different DWTPs using sources of varying water quality.



INTRODUCTION

Water disinfection is a key measure in preventing water-borne diseases. Unfortunately, the disinfection process also generates unwanted byproducts.[1] Exposure to disinfection byproducts (DBPs) has been associated with bladder cancer in a number of epidemiological studies, but other cancer sites have been inconclusive.[2,3] The regulated DBPs (trihalomethanes, haloacetic acids) do not cause bladder cancer in animal bioassays, and the carcinogenic potencies and concentrations of these DBPs are not high enough to account for the cancer cases ascribed to high levels of DBP exposure in the epidemiological studies.[4] A possible group of putative DBPs that may be bladder carcinogens are nitrosamines derived from alkaloids.[4] Analysis of nitrosamines in drinking water has focused primarily on eight semivolatile nitrosamines (e.g., *N*-nitrosodimethylamine [NDMA]), some of which have been identified as DBPs.[5] However, based on a total *N*-nitrosamine assay, NDMA and the other volatile nitrosamines only contributed a small portion of the estimated total nitrosamines.[6] This suggests that the majority of nitrosamines are unknown.

Treated wastewater discharges are an important source of precursors for NDMA and other volatile nitrosamines in many drinking water supplies.[7] Nicotine and its metabolites are present in environmental waters (up to 100 ng/L in lake water), often as a result of wastewater impact (up to 10 μg/L in untreated wastewater).[8] Chlorination of nicotine has been shown to produce a number of different products, including cotinine and nornicotine.[9] Tobacco-specific nitrosamines (TSNAs) may be formed from nicotine and its metabolites by the addition of a nitroso group (Figure S1)[10,11] via similar mechanisms that produce the volatile nitrosamines from their secondary amines during water treatment.

TSNAs are present in tobacco smoke and have been confirmed as carcinogens in animal tests.[12−14] For example, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is a more potent tumorigen than NDMA in F344 rats.[15] There has

Received: November 14, 2013
Revised: January 13, 2014
Accepted: January 14, 2014
Published: January 14, 2014

been speculation that TSNAs may occur in water.[16] One source of TSNAs could be from tobacco smokers. TSNAs are taken into the body during smoking, and some of the TSNAs are not fully metabolized in the body and are ultimately excreted through the urine to the wastewater. At the wastewater treatment plants (WWTPs), the removal efficiencies are unknown. In addition, some TSNA precursors may exist in the wastewater. TSNAs may be generated in the disinfection processes, at the WWTP and/or at drinking water treatment plants (DWTPs) where the source water was wastewater impacted.

Currently, no data are available on TSNA occurrence, either in source waters or in drinking water. Therefore, it is important to know the occurrence of TSNAs and their precursors in different types of water, including treated wastewater, raw drinking water, and treated drinking water. Moreover, because certain treatment/disinfection processes can destroy or remove NDMA precursors (e.g., chlorination, ozonation, granular activated carbon [GAC] adsorption),[17,18] it is important to determine the impact of these processes on TSNA precursors.

Based on NDMA and other volatile nitrosamine levels in drinking water, we anticipated that TSNAs are likely present at low ng/L levels. Therefore, we needed an analytical method that was sensitive, robust, and fast enough for monitoring purposes. To develop the analytical method, we focused on five TSNAs: *N*-nitrosonornicotine (NNN), *N*-nitrosoanabasine (NAB), *N*-nitrosoanatabine (NAT), NNK, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). We developed a solid-phase extraction (SPE) method to concentrate and preclean the samples, and a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to identify and quantify the TSNAs. We also used the method to determine whether TSNA precursors were present through formation potential (FP) tests. Furthermore, samples from two WWTPs and a number of DWTPs were tested to obtain preliminary occurrence data and to evaluate the impact of treatment processes on TSNA precursors.

EXPERIMENTAL SECTION

Chemicals and Solvents. The TSNA analytical standards for NNK, NNAL, NNN, NAB, and NAT were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Formic acid (FA, 49−51%, LC-MS grade) was purchased from Sigma-Aldrich (St. Louis, MO). The methanol and water used for bottle rinsing and sample preparation were Optima LC-MS grade purchased from Fisher Scientific, Inc. (Fair Lawn, NJ). All other chemicals were of analytical grade, unless otherwise indicated.

All of the TSNA stock solutions (at concentrations of 1 g/L, 1 mg/L, 100 μg/L, and 50 μg/L) were prepared in methanol and stored at −20 °C. All working solutions were freshly prepared prior to LC-MS/MS analysis.

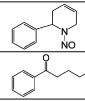
SPE of TSNAs. The analysis of TSNAs combined SPE with LC-MS/MS method.[19,20] The SPE was carried out using Oasis HLB cartridges (6 mL, 200 mg per cartridge; Waters, Milford, MA) mounted on a Visiprep SPE manifold (Supelco, Bellefonte, PA) connected to a vacuum. Prior to sample loading, each HLB cartridge was rinsed with 10 mL of methanol and equilibrated with 10 mL of water. Each sample (500 mL) was passed through the cartridges under vacuum at a flow rate of ∼3 mL/min. After sample loading, the cartridges were washed with 5 mL of 20% methanol and eluted with 5 mL of 80% methanol. The eluent fractions were collected and condensed to about 0.2 mL with a nitrogen stream and then reconstituted with water to 1 mL. The extracts were stored at 4 °C or were immediately analyzed. For each set of SPE experiments, controls using 500 mL of Optima LC-MS grade water were performed in parallel. When the samples were filtered prior to the SPE, another 500 mL of Optima water was also filtered as the filter control. A positive control of 500 mL of laboratory tap water containing 1 ng/L of each TSNA was also performed for each set of SPE.

To determine the SPE recovery, the standards were spiked into the laboratory tap water, which was used to mimic the real water samples. The standard mixture containing these five TSNAs was spiked into 500 mL of tap water at 1 ng/L and 10 ng/L each. The tap water and the Optima LC-MS grade water without spiking were used in SPE as the controls.

LC-MS/MS Analysis of TSNAs. The LC separation was performed on an Agilent 1290 series binary pump equipped with an autosampler. Separation was achieved using a Kinetex C18 column (100 × 3 mm × 2.6 μm; Phenomenex, Torrance, CA) and gradient elution.

An API5500 QTrap mass spectrometer (AB Sciex, Concord, ON, Canada) was used for the determination of the TSNAs under positive multiple reaction monitoring (MRM) mode. Each TSNA was detected with two pairs of ion transitions, one for quantification with the other for confirmation. Table 1 describes the MRM transitions and the optimized MRM parameters, including collision energies (CE), declustering potential (DP), entrance potential (EP), and cell exit potential (CXP). The optimized instrumental conditions were as follows: curtain gas at 40 psi, ion source gas 1 at 50 psi, gas 2 at 30 psi, source temperature = 400 °C, ion spray voltage = 5000 V, and collision gas pressure at high.

Table 1. MRM Conditions of the 5 TSNAs[a]

Name	Structure	Precursor ion	Product ion	CE	EP	DP	CXP
NNN		178.0	148.0	15	10	60	15
		178.0	120.0	18	10	60	15
NAB		192.0	162.0	18	10	80	15
		192.0	133.0	20	10	80	15
NNK		208.1	121.9	27	10	80	15
		208.1	148.0	25	10	80	15
NAT		190.1	105.8	22	10	60	15
		190.1	160.1	22	10	60	15
NNAL		210.1	149.0	28	10	60	15
		210.1	180.1	25	10	60	15

[a]Note: NNN, N-nitrosonornicotine; NAB, N-nitrosoanabasine; NAT, N-nitrosoanatabine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

Quantification Method. Due to matrix effects from real water samples, a standard addition method was adopted for quantification. From the 1.0 mL of reconstituted SPE extract, an aliquot of the extract (100 μL) was analyzed with LC-MS/MS to estimate the concentration levels of the analytes, and then the standards were added to the extracts according to the estimated concentration. In detail, a set of solutions of 4 aliquots (100 μL each) were used to establish a calibration curve by adding 10 μL of mixed standards according to their estimated levels. A calibration curve was made by plotting the MRM chromatogram peak areas against the spiked concentration and was used for determining the original concentration of the analytes. In some real samples, the peak intensity was too low due to the existence of matrix effects, which would likely

suppress the ionization. If there was no TSNA peak or if a very low TSNA peak was detected, a 20% dilution with water (Optima LC-MS grade) was applied to the extracts, because dilution is an effective and simple way to reduce the matrix effect.[21,22] The diluted extracts were analyzed by LC-MS/MS again.

The method detection limit (MDL) of the SPE-LC-MS/MS was determined from triplicate tap water samples (500 mL) spiked with TSNA standards at a concentration of 0.1 ng/L each. The SPE and the LC-MS/MS were performed on these samples as described previously in the Experimental Section. The signal-to-noise ratios (S/N) were determined by dividing the target peak intensity with the noise near the peak. The MDL was determined as the concentration of each analyte at S/N of 3.

Sample Collection and Preparation. Samples were collected at a number of DWTPs and two WWTPs using amber glass bottles. DWTP samples were collected at the plant effluent and in the distribution system, and ascorbic acid (0.5 g/L) was added to quench the free chlorine residual. Some of the DWTPs were sampled for TSNA precursors in the plant influents and, in some cases, after chlorination, ozonation or GAC treatment. One WWTP was sampled at the secondary effluent and at the nitrifying biotower effluent, which was part of the tertiary treatment process. The other WWTP was sampled within the receiving water near the outfall pipe. Selected samples were analyzed in duplicate. All the FP and raw water samples were filtered to remove insoluble particles prior to SPE.

FP Experimental Conditions and Procedures. Because NDMA formation is favored by chloramines (and free chlorine can destroy NDMA precursors), the FP test for TSNAs was conducted with chloramines.[5] The total organic carbon (TOC) and ammonia concentration of the samples were determined prior to the FP setups. The dose of chlorine was based on the level of TOC, at 3 times the TOC (on a mass basis). The amount of ammonia was calculated based on the chlorine dose, at a Cl_2/N mass ratio of 3:1. The FP setup was performed by first adding ammonia and then chlorine under intensive stirring to minimize the contact of free chlorine with the precursors. The pH of the sample was initially adjusted to pH 8 with NaOH or H_2SO_4 if needed, and then a borate buffer was added to maintain the pH and the sample was kept at 25 °C for 3 days. When a sample contained raw-water−ammonia, an appropriate amount of ammonia was added to reach the Cl_2/N mass ratio of 3:1. For poorly nitrified WWTP samples, no ammonia was added and the Cl_2/N ratio usually ended up at ~2:1 (or less). An FP control experiment was also performed using the Milli-Q water. If the FP blank resulted in high NDMA formation, the FP reaction of the samples was rerun, as either the Milli-Q water was contaminated or there was a systemic problem. This procedure has been successfully used for determining precursor levels of NDMA and other volatile nitrosamines at WWTPs and at DWTPs.[23]

RESULTS AND DISCUSSION

Method Development. Figure 1 shows a typical LC-MS/MS determination of the five TSNA standards that were separated based on differences in both chromatography and *m*/*z* ratio. The separation was achieved in 3 min, in which NAT and NAB were separated primarily based on different ion transitions. Analysis of a real sample took 5 min, which included time for column cleaning and equilibration. Shorter analysis time is desirable to provide higher throughput when a large number of samples will be tested in a survey. The LC-MS/MS method provided a dynamic range from 50 ng/L to 5 μg/L with R^2 of 0.9968 to 0.9994. Figure S2 shows the calibration curves of the five TSNAs in ultrapure water, demonstrating excellent linear relationships and reproducibility.

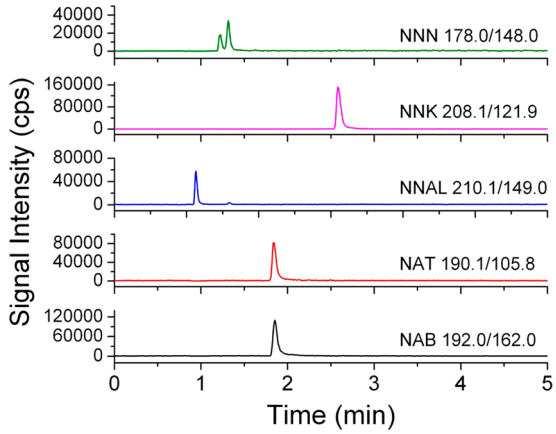


Figure 1. MRM chromatograms of 200 ng/L of the 5 TSNAs by LC-MS/MS. Mobile phase: (A) 0.1% FA in water; (B) 0.1% FA in methanol. Gradient: 0−3 min, 20−95% B; 3−4 min, 95% B; 4−4.1 min, 95−20% B; 4.1−5 min, 20%. Flow rate, 0.4 mL/min.

To examine the effect of sample matrix on the performance of the SPE-LC-MS/MS method, the five TSNAs spiked into tap water were analyzed. Figure S3 presents the calibration curves of the TSNAs when these samples were prepared in tap water at concentrations of 0.1 to 20 ng/L. The results demonstrate that the method can provide a linear dynamic range of 0.1 to 20 ng/L (R^2 > 0.99) and good reproducibility with a relative standard deviation (RSD) <6% (n = 3). The SPE recoveries of the TSNAs in tap water were evaluated at 1 and 10 ng/L. Table 2 summarizes the recoveries of these five TSNAs: in the range of 68−96% at 1 ng/L and 82−97% at 10 ng/L. The MDLs of the SPE-LC-MS/MS method were in the range of 0.02 to 0.05 ng/L (NNN, 0.05 ng/L; NAB, 0.02 ng/L; NAT, 0.03 ng/L; NNK, 0.03 ng/L; NNAL, 0.03 ng/L). Compared to the reported methods,[24−27] the new method provides faster separation (5 min instead of 13 min) and lower MDLs, enabling analysis of TSNAs in water.

TSNAs and Their Precursors in Treated Wastewater. Having established the analytical method, we used it to preliminarily address the question of whether treated wastewater is a source of TSNAs and their precursors. NNAL was detected in the FP (chloraminated) samples of WWTP A and B samples (Table 3), supported by the MRM chromatograms of NNAL (Figure 2). In contrast, no NNAL peak was detected in the SPE control, filter control, and unchloraminated samples, supporting that the NNAL was a DBP. In addition, NNK was detected in the WWTP A and B samples before and after FP. The peak height of NNK increased significantly in the chloraminated samples (Figure 3) compared to the unchloraminated samples for WWTP A, supporting that a portion of the NNK was produced during chloramination. However, for WWTP B, there was more NNK in the sample before FP testing. Thus, NNK was a wastewater contaminant as well as a DBP. Moreover, NNN was detected in WWTP A and B samples after FP testing, but not before. These results confirm that NNAL, NNK, and NNN can be generated as DBPs during the chloramination process. The reason for the existence of

dx.doi.org/10.1021/es405075g | Environ. Sci. Technol. 2014, 48, 1828−1834

Table 2. SPE Recovery of TSNAs in Tap Water[a]

	%				
Conc. ng/L	NNN	NAB	NNK	NAT	NNAL
1	70.2 ± 10.4	77.1 ± 7.2	83.5 ± 2.4	67.7 ± 11.8	96.5 ± 3.7
10	81.7 ± 12.1	84.8 ± 12.4	82.8 ± 8.6	84.1 ± 13.0	97.2 ± 7.4

[a]±: Standard deviation calculated from 3 replicates.

Table 3. Concentrations of Nitrosamines in the WWTP Samples before and after FP Tests[a]

			concentration (ng/L)								
WWTP	sample location	test	NNAL	NNK	NNN	NAT	NAB	NDMA	NPYR	NMOR	NPIP
A	Secondary effluent	Before FP	ND	6.3	ND	ND	ND	---	---	---	---
		After FP	361 ± 95	58.4 ± 2.9	0.24 ± 0.10	ND	ND	1360	131	36	50
A	Biotower effluent	Before FP	ND	3.2	ND	ND	ND	---	---	---	---
		After FP	18.4 ± 6.7	7.4 ± 1.8	0.24 ± 0.24	ND	ND	280	25	2.8	ND
B	Outfall	Before FP	ND	17	ND	ND	ND	3.3	ND	ND	ND
		After FP	7.6	3.2	0.075	ND	ND	330	48	15	ND

[a]ND: Not detected. ---: Not measured. ±: Calculated from 2 SPE replicates.

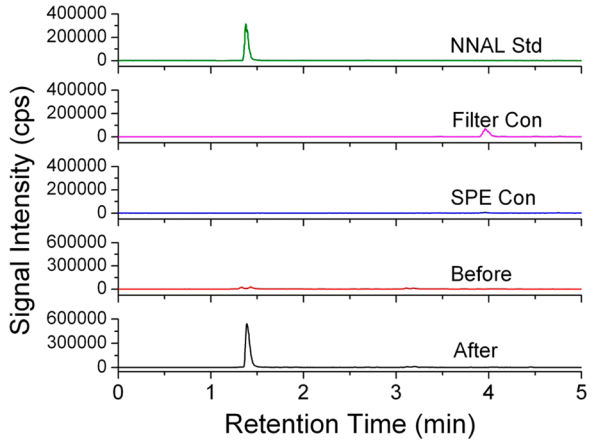


Figure 2. LC-MS/MS chromatogram of NNAL in a treated wastewater sample of WWTP A's secondary effluent (Std, standard; Con, control; before and after FP [chloramination] test).

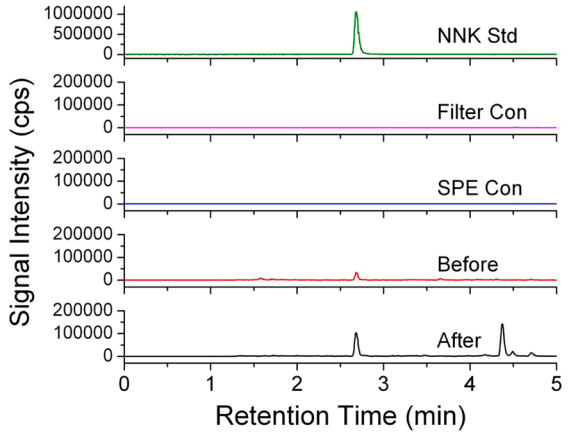


Figure 3. LC-MS/MS chromatogram of NNK in a treated wastewater sample of WWTP A's secondary effluent.

NNK in the unchloraminated treated wastewater samples was likely due to the use of tobacco and certain tobacco products.[16] Furthermore, the WWTP samples were analyzed for volatile nitrosamines using the standard method with Ambersorb.[28] NDMA, *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR) were detected in all of the FP samples, and *N*-nitrosopiperidine (NPIP) was detected in the FP testing of WWTP A's secondary effluent.

Based on TSNA data in the literature,[29,30] the concentration of NNAL in urine ranges from 200 to 500 ng/L for smokers. The concentration of NAT was close to that of NNAL, whereas NNN and NAB were lower than NNAL.[31] The sum of TSNAs in urine was estimated at 0.5−1 μg/L levels. The TSNA concentration in the treated wastewaters studied was approximately 200-fold less than in smokers' urine. NNAL or NAT was not detected in the treated wastewater that we sampled, suggesting that TSNAs do not have significant contribution to total nitrosamines in treated wastewater. However, substantial amounts of TSNA precursors were present in the treated wastewater.

Analysis of TSNAs and Precursors at DWTPs. After preliminarily confirming that NNAL, NNK, and NNN were DBPs and their precursors were derived from wastewater, we investigated TSNA precursors at seven wastewater-impacted DWTPs in Canada and the U.S. We used two artificial sweeteners as indicators of wastewater impact, sucralose (SUC) and acesulfame (ACE), which are stable in the environment and are the most consumed artificial sweeteners.[32−34] Analytical methods for the artificial sweeteners are presented in the Supporting Information. In this study, sucralose was detected at concentrations of 0.3 to 10 μg/L in the plant influents (Table 4). In a U.S. survey, sucralose was reported in the source water for 15 out of 19 DWTPs at concentrations of 0.05 to 2.9 μg/L.[33]

Some TSNAs were detected in the FP samples of the plant influents from 9 of the 11 samples collected (Table 4). These included NNK and NNAL at concentrations, when detected, of 0.09−1.0 ng/L and 0.5−5.7 ng/L, respectively. NAT was also detected in some samples from 0.02 to 0.30 ng/L. All of the plant influents had NDMA precursors, as indicated by NDMA FP (56−220 ng/L). In DWTP 2, the amounts of TSNA precursors (or NDMA precursors) were similar in each of the two sampling events. In DWTP 1, TSNA precursors were detected in one sampling event but none in another sampling. NDMA FP was substantially higher in the sampling event in which TSNA precursors were detected at DWTP 1. In DWTP 3, TSNA precursors were detected in three sampling events, in

dx.doi.org/10.1021/es405075g | Environ. Sci. Technol. 2014, 48, 1828−1834

Table 4. Concentrations of TSNAs and NDMA in FP Samples and Artificial Sweeteners in DWTP Plant Influents[a]

		nitrosamine (ng/L)						sweetener (μg/L)	
DWTP	sample date	NNAL	NNK	NNN	NAT	NAB	NDMA[a]	SUC	ACE
1	1/11/13	ND	ND	ND	ND	ND	116	3.0	3.2
	4/17/13	5.4	0.8	ND	ND	ND	220	2.4	---
2	2/11/13	5.7 ± 0.4	0.6 ± 0.04	ND	ND	ND	89	0.3	0.38
	4/26/13	5.7	0.78	ND	0.30	ND	98	---	---
3	2/12/13	3.3 ± 0.2	0.2 ± 0.03	ND	ND	ND	131	1.6	3.2
	5/13/13	0.50	0.09	ND	0.06	ND	67	1.6	---
	6/3/13	5.2	1.0	ND	0.07	ND	95	0.8	0.98
4	5/27/13	1.5	0.2	ND	0.05	ND	110	0.4	0.69
5	1/7/13	ND	ND	ND	ND	ND	63	4.4	ND
6	4/8/13	1.9	0.31	ND	ND	ND	120	0.27	---
7	6/3/13	2.6	0.43	ND	0.02	ND	56	10	---

[a]±: Calculated from 2 SPE replicates. [b]Other volatile nitrosamines detected in some of the samples.

Table 5. Concentrations of Nitrosamines at DWTP 6 (4/8/13)[a]

		concentration (ng/L)					
sample location	test	NNAL	NNK	NNN	NAT	NAB	NDMA
Plant inf	FP	1.9	0.31	ND	ND	ND	120
Recarb eff	FP	3.3	0.61	ND	HD	ND	69
Recarb eff	SDS/FP	0.09	0.06	ND	0.60	ND	4.5
Plant eff	Instant.	ND	ND	ND	ND	ND	15
DS/ave	Instant.	0.25 ± 0	0.08 ± 0.02	ND	ND	ND	57
DS/max	Instant.	0.22 ± 0.02	0.06 ± 0.01	ND	ND	ND	44

[a]FP: Formation potential. SDS: Simulated distribution system. Instant: Instantaneous (full-scale) result. DS/ave: Average detention time in distribution system. DS/max: Maximum detention time in distribution system. ±: Calculated from 2 replicates.

Table 6. Concentrations of TSNAs and NDMA in FP Samples of Plant Influents and Unit Process Effluents, Indicating the Destruction/Removal of Nitrosamine Precursors at Selected DWTPs[a]

			ng/L					
DWTP	sample location	sample date	NNAL	NNK	NNN	NAT	NAB	NDMA
1	Plant Inf	4/17/13	5.4	0.8	ND	ND	ND	220
	Filter Inf	4/17/13	6.9	1.1	ND	ND	ND	165
	GAC Eff	4/17/13	1.0	0.2	ND	0.03	ND	44
1	Filter Inf	7/15/13	1.68	0.27	ND	ND	ND	108
	Filter Eff	7/15/13	1.56	0.24	ND	ND	ND	171
	Filter Eff Cl_2	7/15/13	ND	ND	ND	ND	ND	8.4
	GAC Eff	7/15/13	0.14	ND	ND	ND	ND	42
3	Plant Inf	6/3/13	5.2	1.0	ND	0.07	ND	95
	Ozone Eff	6/3/13	0.66	0.17	ND	0.05	ND	44
3	Plant Inf	8/13/13	4.24	0.8	ND	ND	ND	80
	Ozone Inf	8/13/13	3.67	0.61	ND	ND	ND	89
	Ozone Inf Cl_2	8/13/13	ND	ND	ND	ND	ND	6.9
	Ozone Eff	8/13/13	ND	ND	ND	ND	ND	12
7	Plant Inf	6/3/13	2.6	0.43	ND	0.02	ND	56
	Ozone Eff	6/3/13	0.1	0.04	ND	0.03	ND	96

[a]Cl_2: Chlorine contact for 1 h before adding the ammonia.

which TSNA FPs were lower when the NDMA FP was lower. However, this database is too small to determine if there is a relationship between TSNA FP and NDMA FP. Nonetheless, these preliminary data show that wastewater-impacted DWTPs may have TSNA precursors in addition to NDMA precursors, as indicated by the FP data.

We analyzed the treated waters and distribution systems of a number of DWTPs for TSNAs, both wastewater-impacted and pristine (including ones not in Table 4). TSNAs were not detected at any DWTPs, except for DWTP 6. Notably, we detected NNK and NNAL in the distribution system of DWTP 6 (Table 5) (see chromatograms in Supporting Information). TSNAs were not detected in the plant effluent, but they were detected in the distribution system. Similarly, the concentration of NDMA was low in the finished water at DWTP 6 and increased in the distribution system (Table 5). The main difference between DWTP 6 and the other DWTPs studied was that the raw water in DWTP 6 had a much higher ammonia concentration (0.4−0.5 mg/L as N) and breakpoint was not achieved with a short free chlorine contact time (16 min) in very cold water (2 °C) and at quite high pH (pH 10). Thus, there was no free chlorine available to destroy NDMA (and

dx.doi.org/10.1021/es405075g | Environ. Sci. Technol. 2014, 48, 1828−1834

potentially TSNA) precursors. Moreover, the addition of chlorine to ammonia-containing water can result in a localized region (until the chlorine is fully dispersed) with a high Cl_2/N ratio, which can form dichloramine leading to the generation of more NDMA than monochloramine.[35] This may have factored into the formation of TSNAs at this DWTP.

To further evaluate DWTP 6, a sample of the treated water (from the recarbonation effluent) was chlorinated under simulated distribution system (SDS) conditions for 1 h at 25 °C and at pH 8 in order to ensure that breakpoint was achieved. The concentrations of NNAL and NNK in an FP sample before chlorine treatment were 3.3 and 0.6 ng/L, respectively. (Note the recarbonation effluent represented water that had been softened with lime and treated with powdered activated carbon [PAC]. The use of PAC removed NDMA precursors, but not those of the TSNAs.) After 1 h of chlorination, the water was subjected to FP testing with chloramines. The concentrations of NNAL and NNK decreased to 0.09 and 0.06 ng/L, respectively, representing removal efficiencies of 97% for NNAL and 90% for NNK. These results are consistent with the impact of free chlorine on the NDMA precursors at this DWTP (93% destruction of the FP). This test and the lack of TSNA formation at other DWTPs with various amounts of free chlorine contact time before the addition of ammonia suggest that prechlorination may be a key factor in destroying the TSNA precursors. Thus, we did another batch of FP test of samples from DWTPs 1 and 3 with and without 1 h of free chlorine contact and found that the TSNA precursors were fully destroyed (below the MDL, Table 6).

In addition to chlorination, other treatment/disinfection processes may also destroy/remove the TSNA precursors. We collected samples from selected DWTPs at different treatment steps and performed FP tests (examples are shown in Table 6). Conventional treatment had little impact on nitrosamine precursors at DWTP 1 in April 2013. However, at DWTP 1 in April 2013, we found that GAC adsorption was most efficient in removing TSNA precursors (about 90% for both NNK and NNAL) and NDMA FP (73%). Similar results were observed for DWTP 1 in July 2013. For DWTP 3, ozone destroyed nitrosamine precursors in June 2013 (83% for NNK, 87% for NNAL, and 54% for NDMA), and in August the efficiency of destruction was essentially 100% (TSNAs were not detected in the ozone effluent FP sample). To further confirm the effect of the ozone, we collected samples form DWTP 7 and found the efficiency of ozone was high for the TSNAs (91% and 96% for NNK and NNAL, respectively), but resulted in an increase in NDMA FP (perhaps ozone made some of the NDMA precursors more amenable to nitrosation).

Some of the other data were not consistent with the effects discussed above. Clearly, more data are needed to better understand the impact of different treatment/disinfection processes on TSNA precursors. Nonetheless, these preliminary results possibly explain why TSNAs were not detected at most of the DWTPs. Our initial study efforts demonstrate that chlorination and some advanced treatment processes (i.e., ozonation, GAC treatment) may destroy/remove TSNA precursors, similarly to what has been previously observed for NDMA.

To provide a perspective on TSNAs as DBPs relative to NDMA, the sum of NNK and NNAL detected in the DWTP 6 distribution system was about 0.3 ng/L, which was 200-fold less than NDMA in the same DWTP 6 distribution system. The concentrations corresponding to a 10^{-6} cancer risk for TSNAs (NNN, NNK) and NDMA were 0.8 and 0.7 ng/L, respectively.[16] The toxicity of NNAL and NNK are similar based on the rat lung tumor induction test.[12] The health risk due to TSNAs in this instance was much less than that of NDMA. Considering the frequency of detection of TSNAs in drinking water to date, the risk of TSNAs is even lower than NDMA. Further investigations to date have shown that several important factors influence the removal/destruction efficiencies of TSNA precursors: contact with free chlorine; presence of GAC adsorption; preoxidation with ozone, where there was temporal variability in its removal efficiency. Additional testing needs to be conducted to better elucidate these possible control measures. Nonetheless, these preliminary findings are consistent with proven control measures for NDMA.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

LC-MS/MS methods for sucralose (SUC) and acesulfame (ACE). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Water Research Foundation (Project 4461) and managed by Djanette Khiari. The authors greatly appreciate the efforts by participating utilities to collect full-scale samples. Nitrosamine testing and FP setups at the Metropolitan Water District were also conducted by Raha Shirkhani and Eduardo Garcia. The SUC analysis was performed by Matthew Prescott.

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