Development and Application of a Total Nitrosamine Assay for Disinfected Waters [Project #4209]

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PRINCIPAL INVESTIGATORS: William A. Mitch and Ning Dai

OBJECTIVES:
The first objective of this research was to adapt an analytical method that had been developed for the analysis of total nitroso species in blood to the quantification of total N-nitrosamines in disinfected waters. The second objective was to apply this method to disinfected recreational waters, wastewaters, and drinking waters. When combined with the analysis of specific N-nitrosamines by EPA Method 521, the total N-nitrosamine assay would indicate the relative importance of the EPA Method 521 nitrosamines to the total nitrosamine pool.

BACKGROUND:
Following the discovery of N-nitrosodimethylamine (NDMA) as a chloramination disinfection byproduct in the 1990s, the EPA developed Method 521 to quantify the concentrations of seven specific, low molecular weight N-nitrosamines in drinking waters. Application of this method to a range of disinfected waters has identified several of these N-nitrosamines in disinfected waters, although NDMA has generally predominated. Due to the high cancer potencies exhibited by these nitrosamines, and likely many others, the EPA is considering the regulation of these compounds. A critical issue in assessing the nitrosamine-related cancer risk of disinfected waters is whether NDMA accounts for the majority of the exposure, or only a small piece of a larger nitrosamine pool. Were the correct N-nitrosamines targeted by EPA Method 521? The development of a total N-nitrosamine assay is critical to evaluate the fraction of the total pool accounted for by EPA Method 521 nitrosamines.

APPROACH:
An analytical method that had been applied to the analysis of N-nitroso species in blood was adapted for use in disinfected waters. The reaction chamber and detection conditions were optimized to increase the sensitivity of the method to N-nitrosamines. Using existing sample pre-treatment methods, the specificity of the method to N-nitrosamines was verified, provided existing sample pre-treatment techniques were employed to destroy interferences from nitrite and S-nitroso compounds.

Even after optimization of reactor chamber conditions, sample concentration was needed to observe the low concentrations of N-nitrosamines anticipated for disinfected waters. Continuous liquid-liquid extraction with ethyl acetate was pursued to achieve 1000-fold concentration factors, and extraction efficiencies were defined.
A sample preservation technique enabling destruction of nitrite prior to extraction without artifactual nitrosation of amines was developed. Artifactual nitrosation by nitrite was observed to be significant during extraction/concentration, even at the low nitrite levels commonly observed in drinking waters.

The method was applied to disinfected recreational waters, wastewaters, and drinking waters.

RESULTS:
Using NDMA as a model N-nitrosamine, an instrumental method reporting limit of 30 pmol was derived for detection of sample injections to the reaction chamber using the optimized detection method. This reporting limit is equivalent to 60 nM total N-nitrosamines for a 500 μL injection volume (4.4 μg/L as NDMA). Because total N-nitrosamine concentrations in drinking waters were anticipated to be as low as ~10 ng/L, the research team developed a continuous liquid-liquid extraction method employing 400 mL ethyl acetate to extract 1 L samples overnight. Extracts were concentrated to 0.4 mL for an overall concentration factor of 2,500 for analysis of drinking waters. Continuous liquid-liquid extraction using ethyl acetate was the only extraction procedure amongst those tested to achieve reasonable extraction efficiencies for highly polar N-nitrosamines, such as N-nitrosodiethanolamine. Regardless, this method achieved only ~20% extraction efficiencies for polar N-nitrosamines, including NDMA and N-nitrosodiethanolamine. Extraction efficiencies were higher for the more hydrophobic N-nitrosamines, including an overall extraction efficiency of 52% for the EPA Method 521 standard mix.

Nitrite may cause artifactual N-nitrosamine formation during sample storage (Mirvish, 1975; Casado et al., 1984), and during EPA Method 521 analysis (Padhye et al., 2010). For the total N-nitrosamine assay, nitrite concentrations higher than 30 nM were found to generate significant artifacts; partial extraction of nitrous acid and amines into the ethyl acetate may have resulted in amine nitrosation during reflux. As nitrosamine formation has been associated with chloramination (Schreiber and Mitch, 2006b), nitrite interference is likely in nitrifying sections of the chloraminated distribution systems of interest to this study. Preservation methods to destroy nitrite were desirable. The research team developed two preservation methods to reduce nitrite to nitrogen gas: addition to 1 L water samples of 2 g/L sulfamic acid at pH 2 or addition of 2.6 g/L sodium sulfite at pH 5.5. Both preservatives were held overnight, and then the sample pH was readjusted to pH 7–8 prior to continuous liquid-liquid extraction. Both methods involved approaching pH 3.5, the pH at which N-nitrosation of amines by nitrite is maximized (Mirvish, 1975), and so both techniques may promote artifacts. By using elevated preservative to nitrite molar ratios to promote nitrite destruction at the expense of nitrosation, both methods successfully destroyed nitrite while avoiding the promotion of N-nitrosamine formation. The final method employed sulfamic acid preservation.

The method was applied successfully to recreational waters, but the samples were concentrated only 1,000-fold to a 1 mL of ethyl acetate extract. For these waters, the preservation technique was not applied, as the free chlorine residual in these waters
generally held nitrite concentrations <30 nM. Combination of the total N-nitrosamine method with the analysis of specific N-nitrosamines by EPA Method 521 revealed that NDMA, the predominant specific N-nitrosamine, accounted for only ~10% of the total N-nitrosamine pool.

For wastewater effluents and drinking waters, the sulfamic acid preservation method was applied. For wastewater effluents, the 1,000-fold concentration factor was employed, similar to recreational waters. The increase in total N-nitrosamine signals due to the ~1 hour chloramine contact time in a municipal wastewater treatment plant effluent was insignificant. However, when secondary municipal wastewater effluents collected upstream of disinfectant application were treated with a formation potential analysis, involving the addition of 2 nM preformed monochloramine for 10 days, the levels of total N-nitrosamines formed were significant. Combination of the total N-nitrosamine method with the analysis of specific N-nitrosamines by EPA Method 521 revealed that specific N-nitrosamines generally accounted for <30% of the total N-nitrosamine signal prior to the formation potential analysis. However, after the formation potential analysis, NDMA accounted for nearly all of the total N-nitrosamine signal in four nitrified/denitrified wastewater samples collected from Connecticut, but only about half of the total N-nitrosamine signal in a California municipal wastewater effluent.

The total N-nitrosamine signals for drinking waters were significantly lower than those observed in recreational waters or wastewater effluents. Accordingly, application of the total N-nitrosamine procedure to drinking water samples employed the 2,500-fold concentration factor, with 100 μL injections. However, the greater concentration factor was associated with a reduction in the overall extraction efficiencies of model nitrosamines from a range of 37−75% to 24−52%. A method detection limit calculated using the 24% extraction efficiency relevant to NDMA was calculated at 0.5 nM as NDMA for 1 L samples concentrated to 0.4 mL with 100 μL injections. Although no instrumental response was observed for duplicate analyses of 1 L MilliQ water blanks preserved with sulfamic acid, this method detection limit was based upon the 30 pmol instrumental detection limit, assuming a 2,500-fold concentration factor and 24% extraction efficiency relevant to NDMA. The method detection limit calculated for seven analyses of 1 L MilliQ water spiked with 1.2 nM NDMA was 0.43 nM as NDMA. The 0.5 nM as NDMA method detection limit was adopted as the more conservative detection limit. For drinking water samples, NDMA, the only specific nitrosamine detected, accounted for 12−27% of the total nitrosamine signal. The wide range relates to the range in extraction efficiencies.

It is important to note that the method as applied to drinking waters was prone to contamination at these low method reporting limits. In particular, the continuous liquid-liquid extractors were a frequent source of contamination. The contamination was observed after analysis of samples with high TONO signals, as well as when the extractors had remained unused within the fume hood for several days between uses. To avoid contamination associated with these situations, the extractors were run with MilliQ water for 15 h to clean the extractors prior to extracting drinking water samples.
CONCLUSIONS:
Despite the issues regarding the application of the current method to drinking water samples, the method can be employed to draw important conclusions, even in its current form. The key research question driving the research was to evaluate what fraction of the total N-nitrosamine pool is captured by the specific N-nitrosamines targeted by EPA Method 521. As indicated in the introduction, N-nitrosamine formation pathways are general to a wide array of secondary, tertiary, and quaternary amines. Hence, it was anticipated that the array of N-nitrosamines would be limited only by the array of these amine precursors present in the waters. Generally, NDMA has been the dominant specific N-nitrosamine detected by EPA Method 521 in disinfected waters. As the EPA considers N-nitrosamine regulatory approaches, a significant question is whether NDMA is the major N-nitrosamine of concern in disinfected waters or whether a wider array of N-nitrosamines occurs that is not captured by EPA Method 521. In the latter case, it may be important to consider the occurrence of these other N-nitrosamines in evaluating the N-nitrosamine-related toxicity of a disinfected water, as many N-nitrosamines exhibit comparable cancer potencies.

The combination of the specific N-nitrosamine analysis by EPA Method 521 with the total N-nitrosamine assay indicated that NDMA was the only specific nitrosamine detected, but NDMA was a minor component of the total N-nitrosamine pool in recreational waters and drinking waters. The identity of the other components of the N-nitrosamine pool remains unclear. However, the total N-nitrosamine assay provides a boundary on the size of this pool, and helps place the importance of NDMA in recreational waters (Walse and Mitch, 2008) or drinking waters in perspective.

As indicated in the introduction, the organic precursors for NDMA formation in drinking waters have been associated with two sources: cationic coagulants (e.g., polyDADMAC) or anion exchange resin polymers employed during treatment, or the use of wastewater-impacted waters. Regarding treatment polymers, one can predict the most likely N-nitrosamine structures to form based upon the known polymer structure. For example, the quaternary amine functional group in polyDADMAC has a dimethylamine moiety. Accordingly, NDMA is a likely product. The responsible reactions would involve cleavage of the two nitrogen-carbon bonds linking this dimethylamine moiety to the polymer backbone. However, cleavage of other combinations of the four nitrogen-carbon bonds in this quaternary amine group could result in the formation of other N-nitrosamines still bound to the polymer. This possibility requires further research.

Regarding wastewater-impacted water supplies, the results from the application of the formation potential analysis to municipal wastewater effluents are relevant. In these impaired water supplies, the total N-nitrosamine formation in disinfected drinking waters would be lower due to dilution of the wastewater effluents by background water in the source waters, and lower chloramine contact times. However, it is anticipated that the relative formation of NDMA and other EPA Method 521 specific nitrosamines to the total N-nitrosamine pool would be similar. Accordingly, this type of evaluation may be an indirect method of evaluating the relative importance of N-nitrosamine precursors. In a limited survey of five municipal wastewater effluents, NDMA accounted for nearly all...
of the total N-nitrosamine precursor pool in four Connecticut wastewaters and nearly half of the total N-nitrosamine precursor pool in one California wastewater. These results indicate that NDMA may be far more important in wastewater-impacted waters than was observed in the limited survey of recreational or drinking waters. Accordingly, although the preponderance of the evidence suggests that NDMA may be a minor component of the total nitrosamine pool in disinfected drinking waters, a wider survey of disinfected waters is needed to draw firm conclusions.

APPLICATIONS/RECOMMENDATIONS:
While progress was made in developing the method, further work is needed before it is possible to draw firm conclusions regarding the central question of the relative importance of NDMA to the total N-nitrosamine pool. With the current method, two items should be targeted. First, the formation potential assay that was applied to wastewater effluents should be applied either to a drinking water employing polyDADMAC, or even to polyDADMAC itself. In conjunction with EPA Method 521 analysis, the total N-nitrosamine assay would indicate the potential for polyDADMAC to form other N-nitrosamines. Second, this same approach should be applied to a wider array of municipal wastewater effluents to determine whether the high relative importance of NDMA precursors that was found is consistent across the United States.

Further application of the analytical technique to a wider array of drinking waters is warranted. There are two reasons to pursue further method development. First, it is important to note that while NDMA has been associated with wastewater-impacted waters (Mitch and Schreiber, 2006a), it is unclear whether this association holds for other N-nitrosamines. Although organic nitrogen tends to be higher in wastewater-impaired waters than in pristine waters (Westerhoff and Mash, 2002), the same is true for algal-impacted waters. Different types of amine precursors may occur in algal-impaired or pristine waters than in wastewater-impacted waters, leading to the formation of different types of N-nitrosamines. Second, once developed, this method may be less expensive, and easier to conduct than EPA Method 521, while providing a definitive boundary to the total N-nitrosamine pool. Thus, this method could serve as a more convenient monitoring technique, particularly if the EPA is interested in regulating N-nitrosamines as a group.

Note that the current method has been optimized to achieve a method detection limit within the range targeted for disinfected drinking waters (i.e., 0.5 nM or 37 ng/L as NDMA). However, to finalize the development of the method, improvements in the extraction technique enabling recovery of both hydrophilic and hydrophobic N-nitrosamines at high levels should be pursued. Because the identities of the N-nitrosamines contributing to the total N-nitrosamine signal are unclear, achieving comparable, and high recoveries for a wide range of N-nitrosamines is desirable. This goal is likely to be challenging, due to the difficulty of extracting highly hydrophilic N-nitrosamines, such as N-nitrosodiethanolamine, from water.