Analytical Methods for Predicted DBPs of Probable Toxicological Significance [Project #4089]

ORDER NUMBER: 4089

DATE AVAILABLE: April 2011

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OBJECTIVES:
The following were the main objectives of the study:

- Demonstrate the usefulness of a comprehensive rationale for selection of target compounds for analytical method development based on the likelihood of their occurrence in authentic drinking water systems and their toxicological significance at the concentrations that are likely to occur in drinking water
- Develop reliable, sensitive analytical methods to identify and quantify representative target compounds identified according to the priorities driven by the rationale specified above
- Investigate the occurrence and formation of these compounds in authentic source waters and finished water disinfected by chlorination and chloramination or under similar conditions in laboratory disinfection experiments

BACKGROUND:
A number of epidemiologic studies have found an association between the consumption of chlorinated drinking water and elevated risk of human bladder cancer more consistently than for any other cancer site. Some regulated water disinfection by-products (DBPs) induce cancer in experimental animals, but they do not account for the magnitude of increased risk of developing human cancer observed in epidemiological studies. One possible explanation is that other as yet unidentified carcinogens of higher potency are produced upon disinfection of drinking water. A prior Foundation project (2867) postulated the formation of several classes of compounds (DBPs) that are very likely to be formed in the chlorination or chloramination of drinking water. Some of these predicted DBPs also have a high probability of being potent carcinogens or developmental toxicants according to the quantitative structure toxicity studies (QSTR) and/or the literature available on related chemicals. This project examined whether four classes of predicted DBPs are actually formed under realistic conditions in waters representative of drinking water sources in North America and Australia.

APPROACH:
This project addressed the following four classes of chemicals as putative DBPs: halogenated quinones, halonitriles, *N*-chloramines, and nitrosamines. Halocyclopentenoic acids were not included due to lack of standards. For each class of the putative DBPs, two or three representative compounds were investigated in terms of development and validation of the analytical methods of appropriate sensitivity. Generally, the methods involve solid phase extraction (SPE) or alternatives for pre-concentration/clean up of the water samples, gas

chromatography (GC) or high performance liquid chromatography (HPLC) separation coupled with advanced tandem mass spectrometry detection. The developed methods have been validated and used to analyze representative source waters and finished water.

The haloquinone class of putative DBPs was of sufficient toxicological potency and likely to be carcinogenic that confirming how likely these contaminants are produced during drinking water disinfection is important. Toxicity of haloquinones is not known, however, a number of studies have reported toxicological effects of quinones through redox reactions that possibly generate radical species and reactive oxygen intermediates to cause DNA damage and DNA alkylation. Detection of haloquinones is challenging and consequently they have not been characterized as DBPs until the work of this project. For representative haloquinones, 2,6-dichloro-1,4-benzoquinone (DCBQ), 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ), 2,3,6-trichloro-1,4-benzoquinone (TCBQ), and 2,6-dibromobenzoquinone (DBBQ) were selected as the targets for the method development and to confirm their formation during chlorination and chloramination.

Organic *N*-chloramines represented a special case because insufficient toxicological data are available in the literature to allow prioritization of the class among other DBPs and there is no real basis for performing structure–activity relationships. To address this difficulty, a set of rapid bioassay systems have been developed with Australian funding at the Australian Water Quality Centre that allows evaluation of the ability of chemicals in this class to induce mutagenic and/or clastogenic effects, to affect apoptotic cell death, or to alter cell cycle kinetics. These data have been considered within the framework of the broad spectrum analysis of the members of organic *N*-chloramine class. Attempts made to develop analytical methods for likely *N*-chloramines were unsuccessful, largely because those of most interest appear unstable in the analytical instruments offering sufficient sensitivity.

A recent bioassay of dibromoacetonitrile by the National Toxicology Program (NTP 2010) indicates that this class may be of greater interest than the THMs. Analytical methods were developed to measure and detect longer chain halonitriles.

RESULTS/CONCLUSIONS:

An electrospray ionization tandem mass spectrometry technique based on the observation of unique electrochemistry that occurs during the electrospray ionization process was developed through this research. Selected chloro- and bromo-quinones were ionized through a one-electron reduction step to form $[M+H]^-$ under negative electrospray ionization. Addition of 0.25% formic acid to water samples was found to effectively stabilize the haloquinones in water and improve the ionization for analysis. These improvements were rationalized from the estimates of pKa values (5.8–6.3) of these haloquinones. The method of tandem mass spectrometry detection, combined with sample preservation, solid phase extraction, and liquid chromatography separation, enabled the detection of haloquinones in chlorinated water samples collected from a drinking water treatment plant. Selected haloquinones have been detected in drinking water after chlorination treatment, with concentrations ranging from 0.5 to 165 ng/L, and have not been detected in the untreated water. This SPE-LC-MS/MS method was used to investigate the occurrence of the four haloquinones in nine water treatment plants (WTPs). Preliminary results show that DCBQ and DBBQ are widely present as the predominant haloquinones in the treated

water but not in the source water. This method will enable further studies of occurrence and formation pathways of haloquinones in different treatment processes.

Headspace solid phase microextraction/gas chromatography–mass spectrometry (HS SPME/GC-MS) was developed and validated for the analysis of two primary halonitriles (HNs) of interest (2,2-dichloropropionitrile [2,2-DCPN] and 2,2-dibromobutyronitrile [2,2-DBBN]). Originally several halogenated propio- and butyronitriles were to be included, but lack of standard compounds resulted in confinement of the study to 2,2-DCPN and 2,2-DBBN. 2,2-DCPN was commercially available and attempts to synthesize the other halogenated nitriles were reasonably successful only for 2,2-DBBN. Although the synthesis yielded several other halogenated nitriles, those were produced in a low abundance and could not be separated into pure compounds. This project has demonstrated that the developed method may be used successfully for halonitrile compounds in general. The process of method development considered key variables, including choice of SPME fibre, SPME and GC-MS conditions, stability of standard compounds, and sample storage conditions to minimize degradation of the analytes. Matrix effects, linearity, precision, and limits of detection and determination were evaluated. The sensitivity of the developed method was excellent, with limits of detection for 2,2-DCPN and 2,2-DBBN at 0.5 and 0.9 ng/L, respectively, while recoveries of these compounds at the 20 ng/L level were >90% and reproducibility was <5% RSD.

A preliminary investigation to determine the likelihood of the presence of the longer chain halonitriles in drinking water was also conducted. Of the longer chain HNs, only 2,2-DCPN was formed in the laboratory-scale chlorination and chloramination experiments, suggesting that these compounds could form in disinfected drinking waters. The HNs of primary interest were not detected in any samples collected from drinking water distribution systems in Western Australia; however, 2,2-DCPN was measured in a treated water sample from western Canada. This suggests that the nature of the precursor material contained in the water sample plays a significant role in the formation of longer chain HNs. Therefore, future occurrence study of HNs should include diverse source waters and collection of water samples from diverse geographical locations.

Efficient utilization of HPLC and UPLC methods coupled with either triple quadrupole mass spectrometry or post-column reaction and UV detection has been shown to separate and quantify a diverse group of organic *N*-chloramines. With proper selection of mobile and stationary phases, excellent separation and retention of the *N*-chloramines was achieved. Solid phase extraction with a hydrophilic-lipophilic balance (HLB) phase was used to successfully pre-concentrate and enhance the detection of *N*-chloramines. These developed methods combined with solid phase extraction enabled the detection of *N*-chloramines at low concentration and could be used to facilitate further studies of formation and occurrence of *N*-chloramines in treated drinking water.

Cyto- and genotoxicity testing with the selected *N*-chloramines in a mammalian cell-based assay was also conducted. Overall, 17 organic *N*-chloramines were tested. The *N*-chloramine derivatives of glycine, histamine, lysine, and ethanolamine were found to be both cytotoxic and genotoxic at concentrations of the precursors that are expected to occur in raw waters and some finished waters. Glycine and ethanolamine monochloramines were shown to be stable for up to a week. This would allow ample time for transit through the distribution system and to reach a site

of action within a human consumer. While many other *N*-chloramines were not found to be genotoxic in the assays of single compounds, chlorine is known to transfer between amines, and so such apparently non-toxic species could potentially act as long-lived donors to more potent forms when they occur in mixtures. This preliminary evidence warrants further investigation of a wider range of amines, detailed studies of the mechanisms of action, and testing of selected candidates using *in vivo* carcinogenicity protocols.

Within the framework of the current project, the formation of *N*-nitrosodiphenylamine (NDPhA) from diphenylamine (DPhA) precursor using liquid chromatography tandem mass spectrometry was demonstrated. The effect of water pH and chloramination conditions on the formation of NDPhA was also thoroughly investigated. To identify precursors of NDPhA, raw water samples were collected from the same drinking water systems where NDPhA was previously detected. Analysis of the raw water samples showed the presence of 1.3 ng/L of DPhA and no detectable NDPhA. Seven hours after the treatment of the raw water with chloramines, the concentration of DPhA decreased to 0.4 ng/L with the corresponding formation of NDPhA (0.4 ng/L). Controlled experiments involving chloramination of DPhA in water showed that chloramines were essential to the formation of NDPhA, and that increasing pH from 4 to 10 resulted in 64-fold enhancement in NDPhA formation. Removal of DPhA and formation of NDPhA was found to be mass imbalanced, which led to the identification of two new DBPs, phenazine (MW 180 Da) and a chlorinated phenazine derivative (MW 216 Da). Both new DBPs were detected only in the treated water but not in the raw water. The results showed for the first time that nitrosamines and phenazines are co-produced during chloramination. Phenazine and *N*-chlorophenazine have never been reported as DBPs and their occurrence in drinking water and health effects are not known. A combined solid phase extraction with LC-MS/MS method with enhanced sensitivity and recovery for DPhA, NDPhA, and phenazine was developed, providing a tool for future studies on identification of precursors, formation, and occurrence of nitrosamines and phenazine-type of DBPs and toxicity of mixed nitrosamines and phenazines when they are co-present in water.

APPLICATIONS/RECOMMENDATIONS:
A comprehensive survey should be perform ed using the methods developed to determ ine the extent and magnitude of occurrence of the DBPs which can be monitored by these new methods. Further toxicology work to characterize the pote ntial health risk associated with the newly identified DBPs should be pursued.

RESEARCH PARTNER:
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