"Old" and new categories of DBPs in aqueous matrices: Analytical methods and toxicity testing

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Abstract The by-products formed during disinfection of water and wastewater (DBPs) have been a subject of priority research since 1974 when trihalomethanes (THMs) were detected for the first time in chlorinated water by Rook. Since then, many other categories of such toxic byproducts have been identified by utilizing of advanced analytical techniques. More than 600 compounds are known today and the list is increasing. Some of them, like haloacetic acids (HAAs), have been regulated. Others, such as N-nitrosodimethylamine (NDMA), are still being investigated. New categories such as halobenzoquinones and other aromatic DBPs have been recently detected in chlorinated water, wastewater and saline water. In the present review paper, the advances in analytical methods and the main new findings are highlighted. The toxicity testing for the various categories is also presented, as some new DBPs were found to be much more toxic than the "old" ones.

Keywords: New DBPs, analytical methods, toxicity, aqueous matrices

1. Introduction

Research on DBPs, compounds formed when disinfectants react with naturally occurring organic matter present in water, has been of particular interest for the water sector during the last decades. Since the discovery of THMs (Rook, 1974), with dominant species the carcinogen chloroform and their subsequent regulation in drinking water, the aim of water treatment plants is to comply with the regulatory limits. In an attempt to minimise their formation, water treatment parameters and disinfectant types/combinations were altered, often resulting in the formation of other DBP categories. Haloacetic acids (HAAs), haloketones (HKs), haloacetonitriles (HANs) have been added to the list of DBP categories, while researchers continued optimizing and developing new analytical methodologies for their determination in trace levels in aqueous matrices. Besides those "old" categories of DBPs, NDMA, MX and related compounds were among the newer categories that were revealed to be formed in disinfected waters. They were found to be much more toxic than THMs, but to be present in much lower concentrations, in the order of ng/L in treated water. Research on DBPs continued, regarding method optimization, new compounds identification and toxicity testing, resulting in more than 600 DBPs having been detected today, with chlorobenzoquinones and halogenated phenols being the latest new categories added to the big list of known DBPs so far, while more than half of the total quantity of chlorinated DBPs in water remains unidentified.

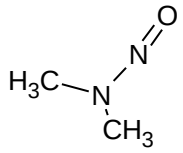
In order to effectively change the disinfection processes for the minimization of DBP formation, it is necessary to determine and identify which DBPs may be of health concern, as well as at which levels. This requires optimized analytical methodologies that can detect and identify as many DBPs as possible, in addition to techniques for toxicity testing for "old" and "new" DBP categories.

2. Optimization of DBP analytical techniques

Because DBPs occur at very low concentrations, from fractions of a ng/L to a few μg/L, analytical methods must have as high sensitivity and selectivity as possible, to accurately distinguish and identify target DBP from countless other ultra-trace substances in aqueous matrices. For the "old" categories of DBPs (THMS, HAAs, HKs, HANs), accurate methods are available and have been optimized (Nikolaou *et al.*, 2002a,b). The main analytical challenges nowadays regard the optimization of analytical techniques for the new DBP categories (NDMA, MX. chlorobenzoquinones, halogenated phenols).

Some DBPs may not be inert, which is more harmful for a consumer's health. N-nitrosodimethylamine (NDMA) belongs to the group of hazardous substances, however, earlier, the nitrosamine formation was only connected with the reaction of secondary amines with nitrite and its presence in drinking water was explained by its infiltration with raw water, but not by NDMA formation during the water treatment process (necessity to apply strong oxidants, both for the destruction of selected contaminants and for water disinfection). However, this situation leads to

the formation of disinfection/oxidation by-products including N-nitrosodimethylamie (NDMA), whose structural and molecular formula is presented in Fig1.



N-nitrosodimethylamine

Figure 1. NDMA

NDMA is a volatile, yellow, oily liquid marked by low viscosity. NDMA is highly soluble in water, alcohols, ethers and other organic solvents, as well as in fats. This compound is light sensitive, especially to the UV and it undergoes fast photolytic degradation. NDMA is flammable. NDMA molecular weight accounts to 78.08 g/mol and octanol/water coefficient (log Ko/w) to –0.57.

As a group of chemical compounds, nitrosamines have been known for over 100 years, but at the beginning they did not attract much attention. Only in 1954 John Barnes' and Peter Magee's, discovery contributed to the wide interest in this compound group. They showed that N-nitrosodimethylamine has carcinogenic impact on rats (Barnes and Magee, 1954). This discovery contributed to further research on nitrosamines, during which carcinogenic effect of 90% out of 300 investigated compounds was proved. At present, it is known that nitrosamines, particularly N-nitrosodimethylamine (NDMA), are compounds with high carcinogenic, mutagenic and teratogenic effects. The United States Environmental Protection Agency (US EPA) (USEPA, 2007) placed this compound in B2 group, i.e. among the compounds which are probably carcinogenic to humans. A noticeable attribute of secondary nitrosamines is the decrease of carcinogenic effect along with the increasing aliphatic chain. N-nitrosodiethylamine is only the exception from this rule and it is characterized by higher carcinogenic effect than N-nitrosodimethylamine. Nitrosamines were identified in many consumption products as well as in soil, wastewater and drinking water (Andrzejewski *et al.* 2005 and cited therein).

In 2002 Mitch and Sedlak (Mitch and Sedlak, 2002) and, independently, Choi and Valentine (Choi and Valentine, 2002) informed that N-nitrosodimethylamine (NDMA) was formed during the chloramination of drinking water containing dimethylamine (DMA), or water discharged from a wastewater treatment plant.

The mechanism of NDMA formation was explained as chain reactions of chloramines with dimethylamine. In further research it was shown that NDMA is also formed during chloramination of other organic precursors which contain a nitrogen molecule. In spite of the fact that the efficiency of chloramination reaction, which leads to NDMA formation, is not so high (under 1%), the hazardous nature of N-nitrosodimethylamine justifies the limitations to its amounts in drinking water, ranging from several to 100 nanograms per liter. These amounts are close to the concentrations recorded in municipal water so far: to 19 ng/L (Ontario, Kanada), to 100 ng/L (Alberta, Kanda) or 53 ng/L (USA) (Andrzejewski and Nawrocki, 2005 and cited therein).

However, it should be remembered that NDMA formation during the application of strong oxidants is not only connected with water treatment technologies but also with wastewater treatment. This problem was observed for the first time during the search for reasons of NDMA appearance in wells supplied with chloraminated discharge water from wastewater treatment plants (Mitch and Sedlak, 2002 and Choi and Valentine, 2002). The concentrations of NDMA precursors, including DMA, in wastewater are higher (e.g. DMA 50-120 μg/L) and oxidation technologies (or disinfection ones) applied assume the application of higher oxidant concentration or highly-efficient coupled technologies known as advanced oxidation processes (AOP). Therefore, in the discharge water from wastewater treatment plants, the concentrations of NDMA are higher, particularly when the discharged water is disinfected by chloramination (in the USA, the recorded NDMA concentrations amounted to 5000 ng/L (Nawrocki and Andrzejewski, 2012 and cited therein). On the other hand, the discharged waters are mainly drained to water reservoirs where impurities are diluted in considerable extend.

NDMA however can be also formed as the result of reaction of strong oxidants such as chlorine dioxide (Andrzejewski and Nawrocki, 2007), ozone (Andrzejewski and Nawrocki, 2007, and Andrzejewski *et al.*, 2008) or permanganate (Andrzejewski and Nawrocki, 2009) with dimethylamine presented in water. Moreover NDMA can also forms during ozone reaction with dimethylamine containing compounds (Oya *et al*, 2008).

Mechanism of NDMA formation during mono- or dichloramine reaction with DMA (or dimethylamine containing compounds) is still a matter of deliberates, however milestones of this reaction are out of discussion. These milestones are:

- reaction of monochloramine with DMA or dichloramine with DMA, which results with 1,1-dimethyl hydrazine (UDMH) or chloro-dimethyl hydrazine (Cl-UDMH) formation.

- 1,1-dimethyl hydrazine (UDMH) or chloro-dimethyl hydrazine (Cl-UDMH) are oxidized to form NDMA. (Mitch and Sedlak, 2002 and Choi and Valentine, 2002).

In these reactions DMA served as source of dimethyl amine- part of NDMA molecule and reaction seems to run according to molecular mechanism of reaction, however radical mechanism is also considered.

Several mechanisms of NDMA formation during ozonation of DMA or dimethylamine containing compounds were proposed, however currently formation of UDMH as an intermediate, is considered as the milestone of this mechanism. UDMH is subsequently oxidized to form NDMA, however detailed mechanism of UDMH formation is still discussed (Yang *et al.*, 2009). Despite of discussion on NDMA formation mechanism details (Yang *et al.*, 2009 and Andrzejewski et. al., 2012), DMA served both as source of dimethyl amine-part as well as nitrozo-part of NDMA molecule. That's way yield of NDMA/DMA conversion rate is lower than in case of chloramination of DMA containing water.

NDMA and other nitrosamines are commonly determined in water by isotope dilution and liquid-liquid or solid phase extraction prior to GC-MS analysis, however other techniques were also proposed. Bellec *et al.* proposed a technique based on UV photohydrolysis of nitrosamines with the formation of nitrite ions, which is subsequently analyzed using the colorimetric method with Griess reagent. Cardenes *et al.* reported the application of dansylation in a microwave technique as the method preceding HPLC technique. Fluorimetric detection at 531 nm (irradiation 339 nm) allowed for the determination of the simplest N-nitrosodialkiloamines at the level of 70 picograms per injection, which is related to 27/ug L -1 in water. (Andrzejewski *et al*, 2005 and cited therein)

An emerging category of halogenated DBPs is Halobenzoquinones (HBQs) that identified recently. The HBQs determination in drinking water has shown that the concentrations are found at ng/L levels. They are suspected to be potential bladder carcinogens. Brominated and iodinated HBQs present higher cytotoxicity and genotoxicity than chlorinated HBQs. A halogen and/or alkyl and hydroxyl groups on a benzoquinone (BQ) ring constitute the basic structure of HBQs. For the determination of HBQs are applied the methods of GC-MS with electron ionization (EI) or chemical ionization (CI). For nonvolatile or thermally unstable HBQs prior to GC-MS analysis a derivatization step is required because in this form they are not amenable to direct GC-MS. In this case, HBQ analogues (2-tert-butyl-HQ and 2,6-di-tert-butyl-1,4-BQ) were converted to tertbutyldimethylsilyl derivatives by addition of N-methyl-N-(tertbutyldimethylsilyl)-trifluoroacetamide (MTBSTFA). As it concerns the (semi-)volatile, thermally-stable, non-ionic, and nonpolar HBQs, they are directly introduced to GC-MS. High performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) techniques have also been developed for trace analysis of HBQs. Liquid chromatography electrospray ionization (ESI) – mass spectrometry/mass spectrometry (LC-ECI-MS/MS) techniques present high sensitivity and accuracy for the analysis of HBQs. They analyze both HBQs and their transformation products (Wang *et al.*, 2016).

3. Identification of new DBPs

Research on analytical method development resulted in the identification of new DBPs. A new category is HBQs, that was discovered after being predicted to be found in chlorinated drinking water in Canada. HBQs are important because they appear to be many times (up to 1000 to 10,000 times) more toxic than the THMs. However, HBQs were found to occur in drinking water at low ng/L concentrations rather than the thousand fold higher concentrations of the THMs. The investigation of HBQs in nine treatment plants using chlorination, chlorination/chloramination, chloramination, and ozonation/chloramination treatments found that 2,6-dichlorobenzoquinone is present in all cases while 2,6-dibromorobenzoquinone in 72% of the cases. Ozonation, another disinfection process alternative to reduce THMs was found to increase DBBQ levels in the finished water (Zhao *et al.*, 2012).

Other new DBPs identified include two nitrosamines, NDPhA and nitrosopiperidine and two related nitrogenous DBPs, phenazine and N-chlorophenazine. These DBPs were investigated in 38 drinking water treatment plants in Canada and the USA. NDMA was the most frequently detected, followed by NDPhA which was detected as a DBP in both treated surface water and ground water. The main factor driving nitrosamine occurrence was chloramination, a disinfection process alternative commonly adopted to meet current regulatory limits for THMs.

Recently, a novel precursor ion scan method using electrospray ionization-triple quadrupole mass spectrometry (ESI-tqMS) has been developed for fast selective detection of polar halo-DBPs. When coupled with ultra performance liquid chromatography (UPLC), the ESI-tqMS can be applied for identification of unknown polar halo-DBPs (Zhai and Zhang, 2011; Xiao *et al.*, 2012; Zhai *et al.*, 2014). With the novel method, a number of polar halo-DBPs have been identified in chlorinated saline wastewater effluents, including 3,5-dichloro-4-hydroxybenzaldehyde, 3,5-dibromo-4-hydroxybenzaldehyde, 2,4,6-tribromophenol, 2,4,6-triiodophenol, 2,6-dichloro-4-nitrophenol, 2,6-dibromo-4-nitrophenol, 2,6-diiodo-4-nitrophenol, 2,4-dibromophenol, 4-bromophenol, 5-bromosalicylic acid and bromomaleic acid (Yang and Zhang, 2013). Most of these new DBPs are halophenolic compounds. Moreover, decreasing the bromide concentration in a wastewater effluent could shift the halophenolic DBPs from being more brominated to being less brominated during chlorination. Thus, for a bromo-DBP newly identified in a chlorinated saline wastewater effluent, its partially and fully chloro-analogues could generate during chlorination of other low bromide effluents. Halophenolic DBPs have also been found in chlorinated and chloraminated drinking waters (Zhai and Zhang, 2011; Zhai *et al.*, 2014),

4. Advances in toxicity testing

A novel approach has been applied by Liu *et al.* (2015) allowing the evaluation of large numbers of samples and contaminant combinations with real time measurement of toxicity to a variety of human cell lines. Also, a novel method was developed by the same authors to assess the ability of various DBPs to bind to DNA, a potential marker of a chemical with the ability to damage DNA. Application of a cell culture technique was performed using real time – cell electronic screening. The ability of this technology to use human cell lines vs. single cell organisms like bacteria provides a very useful addition to the tools for rapidly assessing the toxic potential of individual DBPs. This technique was used to demonstrate the severe cytotoxicity of NDPhA by finding it to be 24 times more toxic than the more common NDMA, which is itself regarded as a strong carcinogen in rodents. The toxicity results provide important input into the assessment of potential health risks of DBPs. Evaluation of the reliability and robust behavior of this technique for environmental surveillance applications was performed. In addition a DNA-damage testing approach for DBPs was developed. The combination of measuring cell toxicity and DNA damage is very useful for characterizing the nature of toxic effects

and an indication of potential for causing cancer. According to the results, toxicity testing of four HBQs showed them to be highly toxic to the cells and HBQs also damaged DNA and proteins. Furthermore, a novel mass spectrometry technique was developed that was able to determine binding of certain DBPs to DNA. Public health risk issues associated with DBPs in drinking water are extremely complex, despite 40 years of investigation since THMs were first discovered. Initial regulations were precautionary based on evidence that chloroform was a rodent carcinogen. Drinking water providers have been responding with precautionary remedial actions to minimize DBP formation while recognizing that disinfection is essential to ensuring safe drinking water (Liu *et al.*, 2015).

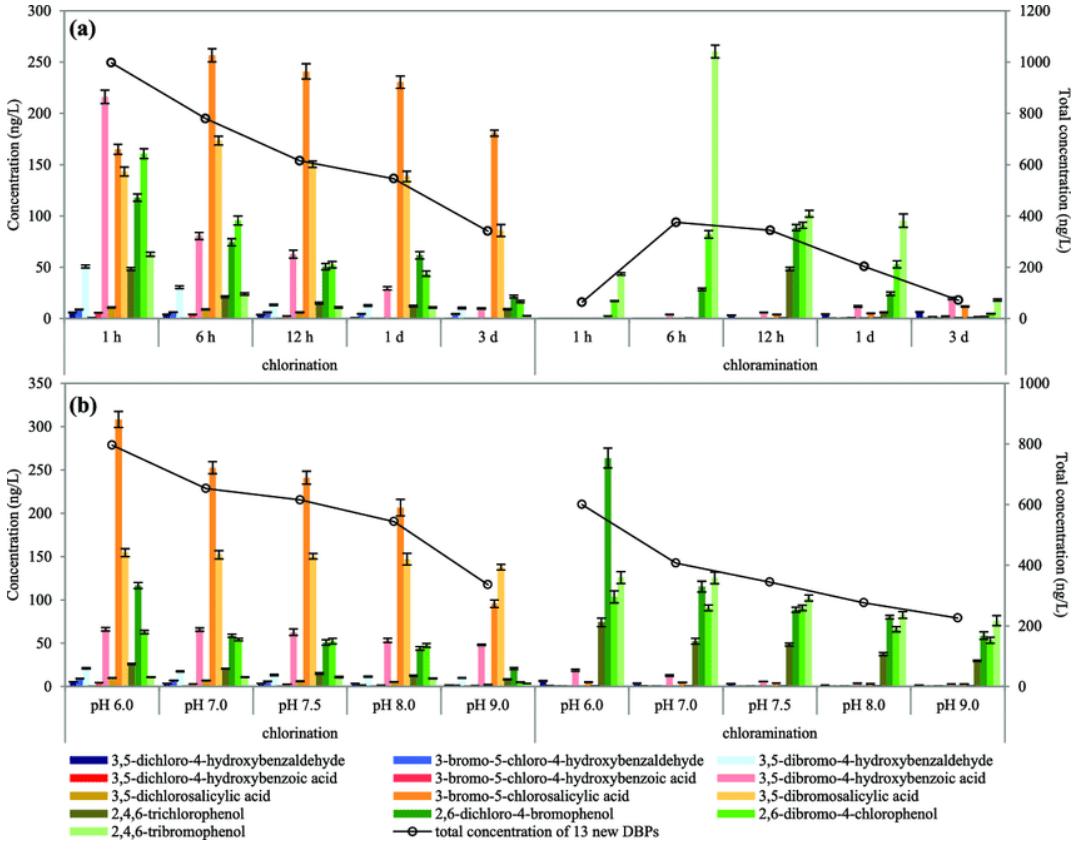


Figure 2. Formation of halophenolic DBPs in water (source: Pan *et al.*, 2017)

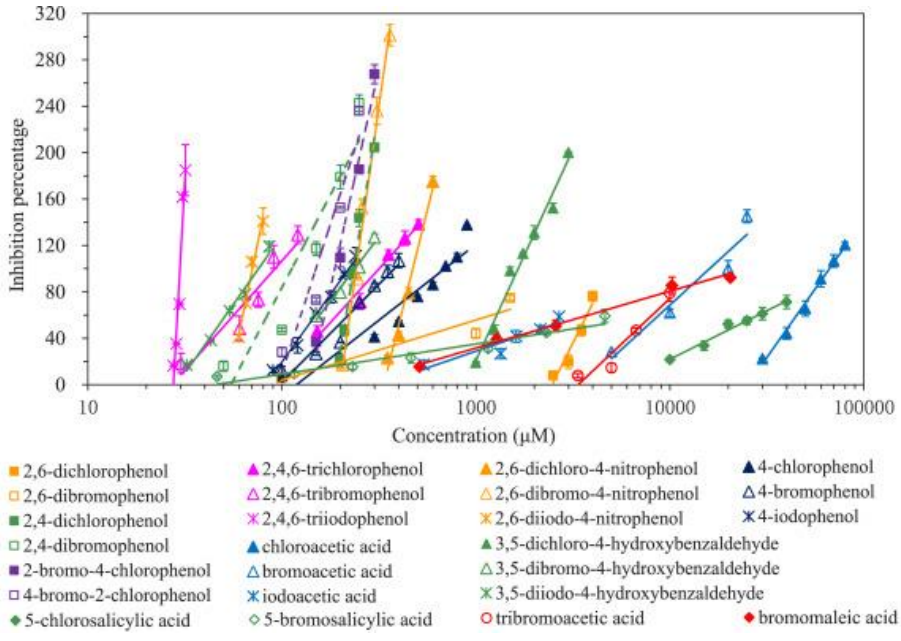


Figure 3. Toxicity of halophenolic and haloaliphatic DBPs (concentration-response curves of growth inhibition for T. marina) (source: Liu *et al.*, 2015)

The above research has demonstrated the risk of pursuing alternative disinfection technologies for DBP reduction without full evaluation of what possible new DBPs may be formed or increased by the alternative processes to achieve THM reduction. The challenge of balancing the certain health risk from microbial pathogens against highly complex and uncertain health risks from DBPs creates a difficult tension for drinking water providers. However, with an improved understanding of accurate knowledge about DBP health risks, drinking water providers can more confidently manage DBPs in a sensible precautionary manner. This research provides clear evidence for continuing with a suitably precautionary approach to minimize DBP formation with reasonable measures. Likewise, there is risk in blindly pursuing any alternate technology to reduce regulated DBPs without clearly understanding the potential of such options for creating alternative DBPs. These alternative DBPs may pose a greater health risk or alternate disinfection processes may compromise disinfection efficiency. The knowledge arising from this research provides reassurance that current levels of DBP regulation offer a reasonable, precautionary balance among the complex, competing risks involved (e.g., DBPs vs. microbial pathogens, regulated DBPs vs. newly discovered DBPs

The toxicity of new DBPs, halophenolic and haloaliphatic has been assessed also in chlorinated wastewater samples, in particular saline samples, where also an increase of bromo-analogues was observed. Using seawater for toilet flushing effectively reduces the consumption of precious freshwater resources, yet it introduces bromide and iodide ions into a wastewater treatment system, which may form bromo- and iodo-disinfection byproducts (DBPs) during chlorination of the wastewater effluent. Most of the newly identified DBPs in chlorinated wastewater effluents were halophenolic compounds. It has been reported that the newly identified bromo- and iodo-phenolic DBPs were generally significantly more toxic to a heterotrophic marine polychaete than the commonly known haloacetic acids and trihalomethanes. This has raised a concern over the discharge of chlorinated saline wastewater effluents into the marine ecosystem. In the study of Liu *et al.* (2015) the toxicity of new halophenolic DBPs and some haloaliphatic DBPs was tested against an autotrophic marine alga, Tetraselmis marina. The alga and polychaete bioassays gave the same toxicity orders for many groups of halo-DBPs. New halophenolic DBPs also showed significantly higher toxicity to the alga than the commonly known haloacetic acids, indicating that the emerging halophenolic DBPs deserve more attention. However, two bioassays did exhibit a couple of disparities in toxicity results, mainly because the alga was capable of metabolizing some (nitrogenous) halophenolic DBPs. A quantitative structure-toxicity relationship was developed for the halophenolic DBPs, by employing three physicochemical descriptors (log K(ow), pKa and molar topological index). This relationship presented the toxicity mechanism of the halophenolic DBPs to T. marina and gave a good prediction of the algal toxicity of the tested halophenolic DBPs.

The comparative toxicity of 25 halophenolic and haloaliphatic DBPs was evaluated against the marine alga T. marina. The algal toxicity data can help in prioritizing future research on wastewater DBPs. Halophenolic DBPs (except halosalicylic acids) were generally significantly more toxic to the alga than the commonly known haloacetic acids, indicating that the emerging halophenolic DBPs deserve more attention. Besides, T. marina was found to be capable of metabolizing some (nitrogenous) halophenolic DBPs. The metabolism of a halophenolic DBP in the alga might somewhat affect its toxicity to the alga. Polar narcosis might be the main toxicity mechanism of halophenolic DBPs to T. marina. A QSTR was developed for the halophenolic DBPs using log Kow, pKa and MTI as descriptors, and it can be used to estimate the algal toxicity of a new halophenolic DBP. Although the concentration of each individual DBP in the chlorinated wastewater effluent (Yang and Zhang, 2013) is below its LOEC, combinatorial effects of numerous DBPs in the wastewater effluent possibly occur, thus the overall toxicity of the complex DBP mixture in the effluent needs to be evaluated.

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