

NEW DISINFECTION BY-PRODUCT ISSUES: EMERGING DBPS AND ALTERNATIVE ROUTES OF EXPOSURE

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ABSTRACT

This paper discusses current issues with drinking water disinfection by-products (DBPs), which include emerging (unregulated) DBPs that can be formed at greater levels with alternative disinfectants (as compared to chlorine) and routes of human exposure (which include inhalation and dermal exposure studies, in addition to ingestion). Health effects driving DBP research include the recently observed reproductive/developmental effects (including spontaneous abortion) observed in epidemiologic studies, as well as the discrepancy between the types of cancer observed in animal studies for regulated DBPs (mostly liver cancer) and the types of cancer observed in human epidemiologic studies (mostly bladder cancer). Emerging DBPs discussed in this paper include iodo-acids, bromonitromethanes, iodo-trihalomethanes (THMs), brominated forms of MX, bromoamides, a bromopyrrole, and nitrosodimethylamine (NDMA) and other nitrosamines. Recent toxicity studies have revealed that several of these DBPs are more genotoxic (in isolated cells) than many of the DBPs currently regulated, and new occurrence data have revealed that many of these DBPs can, in some cases, be present at levels comparable to regulated DBPs. Of the alternative disinfectants, chloramination appears to increase the formation of iodo-acids, iodo-THMs, and NDMA and other nitrosamines, relative to chlorine. Preozonation appears to increase the formation of halonitromethanes.

KEY WORDS: Disinfection by-products, DBPs, drinking water, emerging, exposure

1. INTRODUCTION

Providing microbially safe drinking water is an important public health issue, and the use of chemical disinfection in the 20th century is rightly regarded as a major public health triumph in that regard. However, chemical disinfection has also produced an unintended health hazard—the potential for cancer and other reproductive/developmental effects that may be linked to chemical disinfection by-products (DBPs) produced during disinfection. Chemical disinfectants are effective for killing harmful microorganisms in drinking water, but at the same time, disinfectants are also powerful oxidants and oxidize the organic matter and bromide/iodide naturally present in most source waters (rivers, lakes, and many groundwaters), forming DBPs. Chlorine, ozone, chlorine dioxide, and chloramines are the most common disinfectants in use today, and each produces its own suite of chemical DBPs in drinking water (Richardson, 1998). Most developed nations have created regulations or guidelines to control DBPs to minimize consumers' exposure to hazardous DBPs, while at the same time, maintaining adequate disinfection and control of targeted pathogens.

Despite much research on DBPs over the last several years, we have only been aware of them since the early 1970s. In 1974, Rook reported the identification of the first DBPs--

chloroform and the other trihalomethanes (THMs)--that are formed in chlorinated drinking water (Rook, 1974). In 1976, the U.S. Environmental Protection Agency (EPA) published the results of a national survey which showed that chloroform and the other THMs were ubiquitous in chlorinated drinking water (Kopfler *et al.*, 1976). In the same year (1976), the National Cancer Institute published results linking chloroform to cancer in laboratory animals (National Cancer Institute, 1976). As a result, an important public health issue was born. In 1979, the U.S. EPA issued a regulation to control THMs at $100 \mu\text{g l}^{-1}$ (ppb) in drinking water (U.S. EPA, 1979); and in 1998, the Stage 1 Disinfectants (D)/DBP Rule was promulgated, which lowered permissible levels of THMs to $80 \mu\text{g l}^{-1}$ and regulated five of the haloacetic acids (HAAs), bromate, and chlorite for the first time (Table 1) (U.S. EPA, 1998). Stage 1 regulations were based on running annual averages, which represented averages of all samples collected in a utility's distribution system over a one-year period. This Rule became effective on January 1, 2002 (3 years following its promulgation). Also listed in Table 1 are the 2004 World Health Organization (WHO) guidelines for DBPs and the European Union DBP standards.

In the United States, an upcoming Stage 2 D/DBP Rule is planned to be finalized in 2005. This Stage 2 Rule is an extension of the Stage 1 Rule, and will maintain the Stage 1 Rule maximum contaminant levels (MCLs) for THMs and HAAs (Table 1), but will require that MCLs be based on locational running annual averages (i.e., each location in the distribution system will need to comply on a running annual average basis). The reason for this change is that the running annual averages (used with the Stage 1 D/DBP Rule) permitted some locations within a water distribution system to exceed MCLs, as long as the average of all sampling points did not exceed the MCLs. As a result, consumers served by a particular section of the distribution system could receive water that regularly exceeded the MCLs. The Stage 2 D/DBP Rule is intended to target those higher DBP levels and reduce the variability of exposure for people served by different points in the distribution system. The Stage 2 D/DBP Rule will maintain the MCLs for bromate and chlorite, however the U.S. EPA plans to review the bromate MCL as part of their 6 year review process (additional details area available at www.epa.gov/safewater/stage2/index.html).

With stricter regulations for THMs and new regulations for HAAs, many drinking water utilities have changed their disinfection practices to meet the new regulations. Often, the primary disinfectant is changed from chlorine to alternative disinfectants (including ozone, chlorine dioxide, and chloramines), and in some cases, chlorine is used as a secondary disinfectant following primary treatment with an alternative disinfectant. However, new issues and problems can result. For example, the use of ozone (with chloramines) can significantly reduce (or eliminate) the formation of THMs and HAAs, but can result in the formation of bromate, especially when elevated levels of bromide are present in the source waters. Bromate is a concern because it has been shown to be a potent carcinogen in laboratory animals (Kurokawa *et al.*, 1986). As a result, the U.S. EPA has regulated bromate under the Stage 1 D/DBP Rule at an MCL of $10 \mu\text{g l}^{-1}$ to limit its occurrence (U.S. EPA, 1998). Nitrosodimethylamine (NDMA), which can form at higher levels with chloramination, is also a concern because it is recognized as a probable human carcinogen. Likewise, a recent U.S. Nationwide DBP Occurrence Study (that included drinking waters with source waters containing high bromide/iodide and natural organic matter levels) revealed that iodo-THMs and newly identified iodo-acids are increased in formation with chloramination, and bromonitromethanes are increased with preozonation (followed by post-chlorination or chloramination). Differences in source water conditions, including concentrations of bromide or iodide, concentrations of natural organic matter, and pH, can also have a dramatic effect on the DBP species (chlorine-, bromine-, or iodine-containing) and the levels formed.

Table 1. DBP Regulations/Guidelines^a

<i>U.S. EPA Regulations</i>	
DBP	MCL (mg l ⁻¹)
Total THMs	0.080
5 Haloacetic acids	0.060
Bromate	0.010
Chlorite	1.0
<i>World Health Organization (WHO) Guidelines</i>	
DBP	Guideline value (mg l ⁻¹)
Chloroform	0.2
Bromodichloromethane	0.06
Dibromochloromethane	0.1
Bromoform	0.1
Dichloroacetic acid	0.05 ^b
Trichloroacetic acid	0.2
Bromate	0.01 ^b
Chlorite	0.7 ^b
Chloral hydrate (trichloroacetaldehyde)	0.01 ^b
Dichloroacetonitrile	0.02 ^b
Dibromoacetonitrile	0.07
Cyanogen chloride	0.07
2,4,6-Trichlorophenol	0.2
Formaldehyde	0.9
<i>European Union Standards</i>	
DBP	Standard value (µg l ⁻¹)
Total THMs	100
Bromate	10 ^c

^aThe total THMs represent the sum of the concentrations of four THMs--chloroform, bromoform, bromodichloromethane, and dibromochloromethane. They have been regulated in the United States since 1979 (U.S. EPA, 1979), but their MCL was lowered from 100 to 80 µg l⁻¹ under the Stage 1 Disinfectants/DBP (D/DBP) Rule (U.S. EPA, 1998). WHO guidelines on THMs state that the sum of the ratio of the concentration of each THM to its respective guideline value should not exceed unity. The five haloacetic acids represent the sum of monochloro-, dichloro-, trichloro-, monobromo-, and dibromoacetic acid. These haloacetic acids, together with bromate and chlorite, were regulated for the first time in the United States under the Stage 1 D/DBP Rule (U.S. EPA, 1998). WHO guidelines can be found at http://www.who.int/water_sanitation_health/dwq/gdwq3/en. European Union drinking water standards can be found at www.nucfilm.com/eu_water_directive.pdf.

^bProvisional guideline value.

^cWhere possible, without compromising disinfection, EU member states should strive for a lower value. This value must be met, at the latest, 10 calendar years after the issue of Directive (November 3, 1998); within 5 years of the Directive, a value of 25 µg l⁻¹ must be met.

In the almost 30 years since THMs were identified, DBPs have been actively investigated. Significant research efforts have been directed toward increasing our understanding of DBP formation, occurrence, and health effects (Richardson, 1998; Richardson *et al.*, 2002; Bull *et al.*, 2001; Plewa *et al.*, 2004a; Plewa *et al.*, 2004b). Although more than 500 DBPs have been reported in the literature (Richardson, 1998), only a small number have been addressed either in quantitative occurrence or health effects studies. The DBPs that have

been quantified in drinking water are generally present at sub- $\mu\text{g l}^{-1}$ (ppb) or low- to mid- $\mu\text{g l}^{-1}$ levels. However, more than 50% of the total organic halide (TOX) formed during the chlorination of drinking water (Krasner *et al.*, 1996), and more than 50% of the assimilable organic carbon (AOC) formed during ozonation of drinking water are still not accounted for (Stevens *et al.*, 1989), and nothing is known about the potential toxicity of many of the DBPs present in drinking water. Much of the previous health effects research directed toward understanding the effects of chronic exposure to DBPs has focused on cancer or mutagenicity. There are current, ongoing concerns that the types of cancer observed in animal studies (primarily liver cancer) for the DBPs that have been tested do not correlate with the types observed in human epidemiology studies (bladder, colon cancer). It is possible that 'new' DBPs that have been identified, but have not yet been tested (due to the high costs involved for animal studies) may be linked to the effects observed in humans. In addition, new concerns have been raised by epidemiology studies about potential adverse reproductive and developmental effects, such as low birth weight, intrauterine growth retardation, and spontaneous abortion (Richardson *et al.*, 2002; Waller *et al.*, 1998; Swan *et al.*, 1998; Nieuwenhuijsen *et al.*, 2000; Waller *et al.*, 2001; Kramer *et al.*, 1992; Aschengrau *et al.*, 1993; Bove *et al.*, 1995; Savitz *et al.*, 1995; Gallagher *et al.*, 1998; Magnus *et al.*, 1999; Klotz and Pyrch, 1999; Dodds *et al.*, 1999; King *et al.*, 2000; Dodds and King, 2001). A 2002 article summarized these new concerns and recent research and discusses new epidemiology and toxicology studies currently taking place (Richardson *et al.*, 2002). Also, because humans are exposed to mixtures of DBPs, rather than individual DBPs, and because the currently available single-chemical studies in experimental animals cannot by themselves explain the adverse health effects observed in some epidemiologic studies, toxicological investigations of DBP mixtures in experimental animals are needed (Simmons *et al.* 2002).

Finally, new human exposure research is revealing that ingestion is not the only important route of exposure--inhalation from showering and dermal absorption (from bathing and other activities) can provide equivalent exposures or increased exposures to certain DBPs. Therefore, these exposure routes are now being recognized in new epidemiologic studies that are being conducted. And, epidemiology studies are beginning to focus more on reproductive and developmental effects--which recent studies have been shown to be important.

As a result of all these new efforts, DBP research has entered an entirely new phase. Cancer is still important, but it is now not the only health endpoint detected in epidemiologic studies. Also, new DBPs besides the traditional regulated THMs (and HAAs) are beginning to be addressed in quantitative occurrence studies and toxicity/epidemiologic studies/risk assessments. As researchers continue to tackle this important public health issue, exciting new work is taking place. This article will discuss those new, emerging DBPs, as well as important alternative routes of exposure (beyond ingestion).

2. EMERGING DBPs

Emerging DBPs beyond those that are currently regulated are becoming important. In general, brominated DBPs are now being recognized as toxicologically important because there is indication that brominated DBPs may be more carcinogenic than their chlorinated analogs (WHO, 2000), and preliminary studies are indicating that iodinated compounds may be more toxic than their brominated analogs (Plewa *et al.*, 2004b). Brominated and iodinated DBPs form due to the reaction of the disinfectant (such as chlorine) with natural bromide or iodide present in source waters. Coastal cities, whose groundwaters and surface waters can be impacted by salt water intrusion, and some inland locations, whose surface waters can be impacted by natural salt deposits from ancient seas or oil-field brines, are examples of locations that can have high bromide and iodide levels. A significant proportion of the U.S. population and several other countries now live in coastal regions that are impacted by bromide and iodide; therefore, exposures to brominated and iodinated DBPs can be important. Early evidence in epidemiologic studies also gives indication that brominated DBPs may be associated with the new reproductive and developmental effects (Waller *et al.*,

2001), as well as cancer effects.

Specific DBPs that are of current interest include iodo-acids, bromonitromethanes, iodo-THMs, brominated forms of MX (MX is 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone), bromoamides, a bromopyrrole, and NDMA (which is not halogenated, but is classified as a probable carcinogen). In an extensive effort to prioritize all known DBPs (>500) according to predicted health effects (cancer), these specific DBPs were ranked a 'high priority' as more likely to cause an adverse health effect (Woo *et al.*, 2002). Subsequently, they were included in a U.S. Nationwide DBP Occurrence Study completed in 2002 (Weinberg *et al.*, 2002). This study was the first comprehensive effort where the selected DBPs to be measured were chosen because of potential adverse health effects. Table 2 lists the priority DBPs included in this study. Analytical standards were obtained for the priority DBPs (many of which were synthesized), rugged analytical methods were developed, and the DBPs were quantified in drinking waters from 12 locations across the United States. Waters treated with all four disinfectants commonly used (chlorine, ozone, chlorine dioxide, and chloramines) were included, as were high bromide waters. Results of this study revealed the presence of most of the priority DBPs in drinking waters sampled, with some at concentrations that were comparable to regulated DBPs. A particularly important finding was that while the use of alternative disinfectants (ozone, chlorine dioxide, chloramines) minimized the formation of the four regulated THMs, other priority DBPs were formed at significant concentrations. For example, iodo-THMs were highest at a plant using chloramines only (up to 15 ppb, individually); dihaloaldehydes were highest at a plant using chloramines and ozone (up to 16 ppb, individually); and halonitromethanes were highest at a plant using preozonation followed by chlorine-chloramine treatment (up to 3 ppb, individually). Also, MX analogs were found at increased concentrations at a chlorine dioxide-chlorine-chloramine plant (up to 1 ppb for combined MX analogs) relative to another plant using chloramines. For some priority DBPs—e.g., haloamides—this represented the first time they had ever been quantified (and they ranged individually up to 9.4 ppb).

Many of the high priority DBPs are also being measured as part of a large collaborative research effort involving scientists from the National Laboratories/Centers of the U.S. EPA's Office of Research and Development (ORD)—the National Health and Environmental Effects Research Laboratory, the National Exposure Research Laboratory, the National Risk Management Research Laboratory, and the National Center for Environmental Assessment (Simmons *et al.*, 2004). This effort (termed 'the Four Lab Study') involves the joint chemical and toxicological evaluation of mixtures of DBPs produced by different water treatment processes. In this study, treated drinking water is concentrated using reverse osmosis (RO), and a comprehensive chemical evaluation of the drinking waters is being made using gas chromatography (GC)/mass spectrometry (MS), including the quantitation of most of the high priority DBPs included in the Nationwide Occurrence Study. A battery of toxicological assays are being used to test the complex mixtures of DBPs. A nice feature of this work is that not only are identifiable DBPs being tested for toxicity, but the unidentified fraction is also being evaluated. The toxicological evaluation focuses on reproductive and developmental endpoints, with assays for other important endpoints and target organs, such as mutagenicity, carcinogenicity, hepatotoxicity, nephrotoxicity, immunotoxicity, neurotoxicity, developmental neurotoxicity, and pharmacokinetics (Simmons *et al.*, 2004).

An initial feasibility study was carried out to assess the ability of RO to successfully concentrate DBPs, determine whether the water concentrates are palatable for animals to drink, and determine whether this complex mixture can be evaluated chemically and toxicologically. This effort was a success, and the feasibility study is scheduled to be published in the *Journal of Toxicology and Environmental Health*. The expanded part of this effort is currently scheduled to begin in the summer of 2005.

Table 2. High priority DBPs included in Nationwide DBP Occurrence Study

MX and MX-Analogues:

3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX)
 3-Chloro-4-(dichloromethyl)-2-(5H)-furanone (red-MX)
 (E)-2-Chloro-3-(dichloromethyl)-butenedioic acid (ox-MX)
 (E)-2-Chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX)
 2,3-Dichloro-4-oxobutenoic acid (Mucochloric acid)
 3-Chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone (BMX-1)
 3-Chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-2)
 3-Bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-3)
 (E)-2-Chloro-3-(bromochloromethyl)-4-oxobutenoic acid (BEMX-1)^b
 (E)-2-Chloro-3-(dibromomethyl)-4-oxobutenoic acid (BEMX-2)^b
 (E)-2-Bromo-3-(dibromomethyl)-4-oxobutenoic acid (BEMX-3)^b

Haloacids:

3,3-Dichloropropenoic acid

Halomethanes:

Chloromethane
 Bromomethane (methyl bromide)^a
 Dibromomethane
 Bromochloromethane
 Bromochloroiodomethane
 Dichloroiodomethane
 Dibromoiodomethane^b
 Chlorodiiodomethane^b
 Bromodiiodomethane^b
 Iodoform^b
 Chlorotribromomethane
 Carbon tetrachloride

Halonitromethanes:

Bromonitromethane
 Chloronitromethane^b
 Dibromonitromethane
 Dichloronitromethane^b
 Bromochloronitromethane^b
 Bromodichloronitromethane^b
 Dibromochloronitromethane^b
 Tribromonitromethane (bromopicrin)^b

Haloacetonitriles:

Bromoacetonitrile
 Chloroacetonitrile
 Tribromoacetonitrile
 Bromodichloroacetonitrile
 Dibromochloroacetonitrile

Haloketones:

Chloropropanone
 1,3-Dichloropropanone
 1,1-Dibromopropanone
 1,1,3-Trichloropropanone
 1-Bromo-1,1-dichloropropanone
 1,1,1,3-Tetrachloropropanone
 1,1,3,3-Tetrachloropropanone
 1,1,3,3-Tetrabromopropanone^b
 1,1,1,3,3-Pentachloropropanone
 Hexachloropropanone

Haloaldehydes:

Chloroacetaldehyde
 Dichloroacetaldehyde
 Bromochloroacetaldehyde^b
 Tribromoacetaldehyde^b

Haloacetates:

Bromochloromethyl acetate

Haloamides:

Monochloroacetamide^b
 Monobromoacetamide^b
 Dichloroacetamide
 Dibromoacetamide^b
 Trichloroacetamide^b

Non-Halogenated Aldehydes and Ketones:

2-Hexenal
 5-Keto-1-hexanal^c
 Cyanoformaldehyde
 Methyl ethyl ketone (2-butanone)^c
 6-Hydroxy-2-hexanone^c
 Dimethylglyoxal (2,3-butanedione)

Volatile organic compounds (VOCs) and Miscellaneous DBPs:

1,1,1,2-Tetrabromo-2-chloroethane
 1,1,2,2-Tetrabromo-2-chloroethane^b
 Methyl-*tert*-butyl ether^a
 Benzyl chloride

^a Not a DBP, but included because it is an important source water contaminant.

^b DBP not originally prioritized (identified in drinking water after initial prioritization), but included due to similarity to other priority compounds.

^c DBP not given a high priority, but included for completeness sake to provide more representation to ozone DBPs for occurrence.

Iodo-Acids. Iodo-acids are a new, toxicologically significant class of DBP that was recently identified as part of a U.S. Nationwide Occurrence Study (Plewa *et al.*, 2004b; Weinberg *et al.*, 2002). Iodoacetic acid, one of five iodo-acids identified for the first time in chloraminated drinking water, has recently been shown to be more genotoxic and cytotoxic to mammalian cells than all DBPs that have been studied, including the regulated HAAs and bromate (Plewa *et al.*, 2004b). It is a factor of 2X more genotoxic than bromoacetic acid, which is the most genotoxic of the regulated HAAs. Low μM levels of iodoacetic acid caused these effects, which was similar to doses of iodoacetic acid that caused developmental effects (neural tube closures) in mouse embryos (Hunter and Tugman, 1995; Hunter *et al.*, 1996). Other iodo-acids identified—bromoiodoacetic acid, (*Z*)-3-bromo-3-iodopropenoic acid, (*E*)-3-bromo-3-iodopropenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid (Plewa *et al.*, 2004b) - have been synthesized and are currently under investigation for possible genotoxic and cytotoxic effects. Their structures are shown in Figure 1. They were initially discovered in chloraminated drinking water extracts using methylation with GC/high resolution-MS. In addition, analytical methods for the five iodo-acids are currently under development for an occurrence study to determine their concentrations in chloraminated drinking water. These iodo-acids are not only of concern for their potential health risks, but also because early research indicates that they may be formed at increased levels (along with iodo-THMs) in waters treated with chloramines. Chloramination has become a popular alternative to chlorination for plants that have difficulty meeting the regulations with chlorine, and its use is expected to increase with the advent of the new Stage 2 D/DBP Rule. Chloramines are generated from the reaction of chlorine with ammonia, and it appears that the length of free chlorine contact time (before ammonia addition to form chloramines) is an important factor in the formation of iodo-acids and iodo-THMs (Plewa *et al.*, 2004b). Because of chlorine's competing reaction to form iodate as a sink for the natural iodide, it is likely that plants with significant free chlorine contact time before the addition of ammonia will not produce substantial levels of iodo-acids or iodo-THMs (Plewa *et al.*, 2004b; Bichsel and von Gunten, 1999, 2000). More research is needed to understand the extent of iodo-acid and iodo-THM formation for different source water conditions and free chlorine conditions (dose/contact time) prior to ammonia addition.

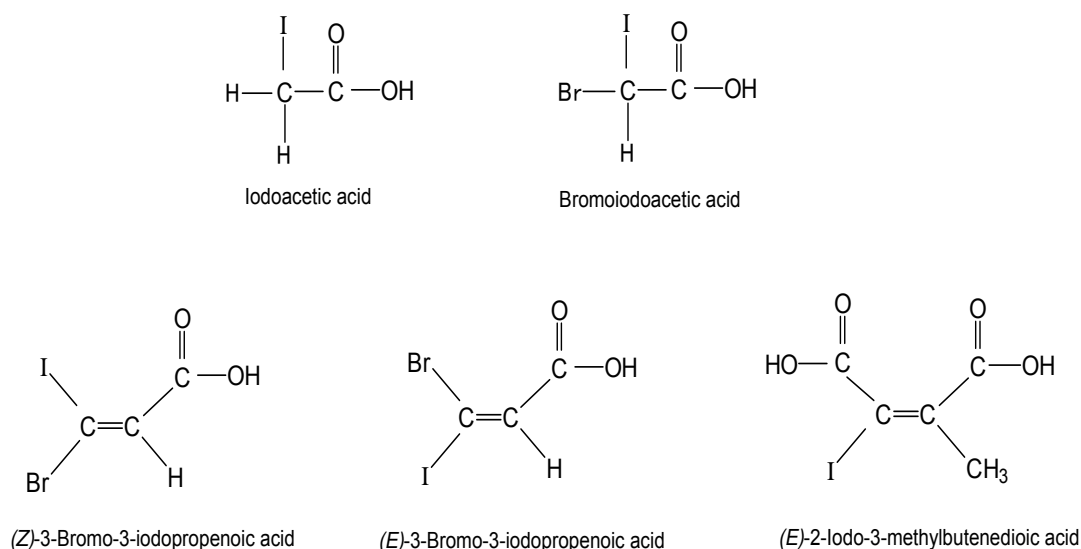


Figure 1. Structures of newly identified iodo-acids.

Halonitromethanes. Just as there are nine possible chloro-bromo haloacetic acids (HAA9) that can form in drinking water, nine halonitromethanes can be formed. Chloropicrin (trichloronitromethane) has been the most commonly measured example in this class, but has not been a concern for toxicity in drinking water (Bull and Kopfler, 1991). Recently, however, bromonitromethanes have been identified (Plewa *et al.*, 2004a; Richardson *et al.*,

1999a, 1999b; Krasner *et al.* 1991), and they have been found to be more cytotoxic and genotoxic than most DBPs currently regulated in drinking water (Plewa *et al.* 2004a). As a result, bromonitromethanes have become an important issue. In this new work, dibromonitromethane was found to be at least an order of magnitude more genotoxic to mammalian cells than MX, and is more genotoxic than all of the regulated DBPs, except for monobromoacetic acid.

Other brominated forms were also potent in this assay. The halonitromethanes have also recently been shown to be mutagenic in the *Salmonella* bacterial cell assay (Kundu *et al.* 2004a), with mutagenic potencies greater than that of the regulated THMs (Kundu *et al.*, 2004b). The halonitromethanes were also at least 10X more cytotoxic than the THMs, and the greater cytotoxic and mutagenic activities of the halonitromethanes was indicated to be likely due to the greater intrinsic reactivity conferred by the nitro group (Kundu *et al.*, 2004b).

In both the mammalian cell and the bacterial cell work, the bromonitromethanes were more potent than the chloronitromethanes, which is similar to previous findings with the THMs and HAAs.

The study by Plewa *et al.* also reported the identification of new halonitromethanes using GC/high resolution-MS (Plewa *et al.*, 2004a). Following this investigation, nine chloro/bromonitromethanes (equivalent to the nine chloro/bromo-HAAs) were characterized as DBPs from chlorine and chloramines, and, as mentioned earlier, have been shown to be increased in formation when pre-ozonation is used before chlorine or chloramine treatment.

Results of the U.S. Nationwide DBP Occurrence Study revealed a range of concentrations for individual halonitromethanes of 0.1 to 3 $\mu\text{g l}^{-1}$, with dichloronitro-, bromochloronitro-, bromodichloronitro-, and dibromochloronitromethane being the most prevalent forms observed. New laboratory-scale formation studies also indicate that nitrite may also play a role in the formation of the nitro group in these DBPs (Choi and Richardson, 2004).

Tribromonitromethane (bromopicrin) and other trihalonitromethanes (which include bromodichloro- and chlorodibromonitromethane) require particular analytical conditions for their analysis. These compounds are thermally unstable and decompose under commonly used injection port temperatures during GC or GC/MS analysis (Chen *et al.*, 2003). The major decomposition products are haloforms (such as bromoform), which result from the abstraction of a hydrogen atom from the solvent by thermally generated trihalomethyl radicals. A number of other products formed by radical reactions with the solvent and with other radicals are also formed. In addition, these trihalonitromethanes can decompose in a hot GC/MS transfer line and exhibit unusual mass spectra, due to H/Br exchanges by some of their fragment ions. In order to successfully detect and quantify these compounds in drinking water, a GC injection temperature, 170°C and a GC/MS transfer line, 225°C should be used.

Because of their potency in mammalian and bacterial cells and because of their occurrence in drinking waters, selected bromonitromethanes are now being studied further in animal studies. Preliminary work is revealing that dibromonitromethane produces DNA adducts in rat liver (*in vivo*) after only 30 days of exposure (DeAngelo, 2005). In addition, dibromonitromethane has been found to be a direct-acting mutagen with calf thymus DNA (*in vitro*), and produces DNA adducts (DeAngelo, 2005). Further work is underway to determine whether dibromonitromethane (and other bromonitromethanes) produces aberrant crypts that can lead to bladder or colon cancer. In addition, experiments are being conducted in transgenic medaka fish to determine whether bromonitromethanes cause tumors.

Iodo-THMs. Iodinated THMs have been identified as DBPs in chlorinated drinking water (Glaze *et al.*, 1975; Brass *et al.*, 1977; Cancho *et al.*, 2000; Bichsel and von Gunten, 2000; Richardson *et al.*, 2003; Weinberg *et al.*, 2002) and in chloraminated drinking water (Weinberg *et al.*, 2002) in several locations, with reports as early as 1975 (Glaze *et al.*, 1975); however, they are not widely measured and are not regulated. Iodo-THMs identified and measured include dichloroiodomethane, bromochloroiodomethane, dibromoiodomethane, chlorodiiodomethane, bromodiiodomethane, and iodoform. Previous

studies of iodo-THMs were conducted mainly because of taste and odor problems (due to a low threshold concentration of medicinal tastes and odors in drinking water - as low as 0.02 to 5 $\mu\text{g l}^{-1}$) (Cancho *et al.*, 2000). However, there is new concern that iodinated compounds may be more toxic than brominated and chlorinated compounds. This prediction stems from evidence that brominated DBPs are, in general, more toxic (and carcinogenic) than their corresponding chlorinated analogs, and that iodine is expected to be more biologically reactive than bromine. Mammalian cell cytotoxicity and genotoxicity results for iodoacetic acid mentioned earlier (Plewa *et al.*, 2004b) support this hypothesis. Therefore, future concern over iodinated compounds may be more than just for taste and odor reasons; it is expected that toxicological studies will continue for additional iodinated DBPs, including the iodo-THMs and other iodo-acids identified in the U.S. Nationwide Occurrence Study.

Iodo-THMs can form in drinking water treated with chlorine or chloramines when natural iodide is present in the source waters, and have been found as DBPs in drinking water in many countries. Levels reported are generally sub- $\mu\text{g l}^{-1}$, however, levels of iodo-THMs were consistently at $\mu\text{g l}^{-1}$ levels and as high as 15 $\mu\text{g l}^{-1}$ at one location in the Nationwide Occurrence Study (that used chloramines for primary disinfection). The total iodo-THMs were 81% of the THM4 (total of four regulated THMs) in one sampling from this location (Weinberg *et al.*, 2002). In the nationwide study, dichloriodomethane was the most common of the iodo-THMs found (found in all states sampled), and it was even observed in waters that were not extremely high in bromide (where iodide levels would be expected to be low).

Controlled laboratory studies, carried out by Bichsel and von Gunten (2000), showed that chloramination (with ammonia addition before chlorine addition) increased the formation of iodo-THMs, whereas pre-chlorination favored the formation of bromochloro-THMs. Chlorination produced both iodate and iodo-THMs; increased chlorine doses lowered iodo-THM levels and raised iodate levels. In contrast, no iodo-THMs were formed by ozonation. Alternatively, in the U.S. Nationwide Occurrence Study (Weinberg *et al.*, 2002), iodoform and other iodo-THMs were observed after ozonation and chloramination. Their research suggested that when a lower ratio of ozone to natural organic matter was used in the Nationwide Occurrence Study (as compared to that used in laboratory-scale tests by Bichsel and von Gunten (2000)), that there was less conversion of iodide to iodate.

MX and BMX Compounds. Before it was discovered to be a drinking water DBP, MX was originally identified in pulp mill effluent; subsequently, it was found in chlorinated drinking water from a number of samples taken around the world. MX has both an open and closed form that is dependent on pH; the ring-opened, oxo-butenoic acid form is present at the pH of drinking water (ZMX, Figure 2). Other analogs of MX were also later identified in chlorinated drinking water, including its geometric isomer (EMX) (Kronberg and Vartiainen, 1988), oxidized and reduced forms of MX (ox-MX and red-MX), as well as brominated analogs (the so-called BMXs) (Suzuki and Nakanishi, 1995). Structures of several of these analogs are shown in Figure 2. Bacterial mutagenicity tests were the original cause of concern for MX, as MX was found to be a potent mutagen in the *Salmonella* Ames assay, and MX can account for as much as 20-50% of the total mutagenicity in chlorinated drinking water (Kronberg *et al.*, 1988). At the time it was identified, MX was the most mutagenic DBP ever identified in drinking water, and in 1997, it was found to be a carcinogen in rats (Komulainen *et al.*, 1997). However, the genotoxic effects in mammalian cells are relatively moderate, and the concentration of MX required to produce a genotoxic effect *in vivo* is usually very high, around 100 mg/kg mouse oral administration (Sasaki *et al.*, 1997). Recent mutagenicity studies with transgenic medaka fish showed that MX did not induce mutations in the liver (for 96 hr exposures) (Geter *et al.*, 2004).

In the few occurrence studies that had been previously carried out, measured concentrations of MX were generally 60 ng l^{-1} or lower. However, in 2002, Wright *et al.* reported levels as high as 80 ng l^{-1} of MX found in drinking waters from Massachusetts (Wright *et al.*, 2002), and in the U.S. Nationwide Occurrence Study--which specifically focused on waters high in natural organic matter and/or bromide--Weinberg *et al.* found much higher levels of MX

(frequently $>100 \text{ ng l}^{-1}$ and as high as $0.85 \text{ } \mu\text{g l}^{-1}$) in finished drinking waters across the United States (Weinberg *et al.*, 2002). Highest levels for total halogenated furanones occurred at a plant that disinfected with chlorine-chloramines ($2.38 \text{ } \mu\text{g l}^{-1}$ in plant effluent drinking water) and at a plant that disinfected with chlorine dioxide-chlorine-chloramines ($1.02 \text{ } \mu\text{g l}^{-1}$ in the distribution system). In drinking water plant effluents, a maximum level of $0.31 \text{ } \mu\text{g l}^{-1}$ was observed for MX; maximum levels of brominated MX analogs included measurements of 0.72 and $0.81 \text{ } \mu\text{g l}^{-1}$ for BEMX-1 and BEMX-2, respectively. MX levels reached a high of $0.85 \text{ } \mu\text{g l}^{-1}$ in the average distribution system sample from the chlorine dioxide-chlorine-chloramine plant. It is also interesting to note that the halogenated furanones were often stable in the distribution system and in simulated distribution system tests. Previous controlled laboratory studies had suggested that halogenated furanones, particularly MX, may not be stable in distribution systems. In at least five instances, MX levels actually increased in concentration from the plant effluent to the distribution system point sampled. Occasionally, MX levels decreased in the distribution system, but in these instances, it was still generally present at detectable levels.

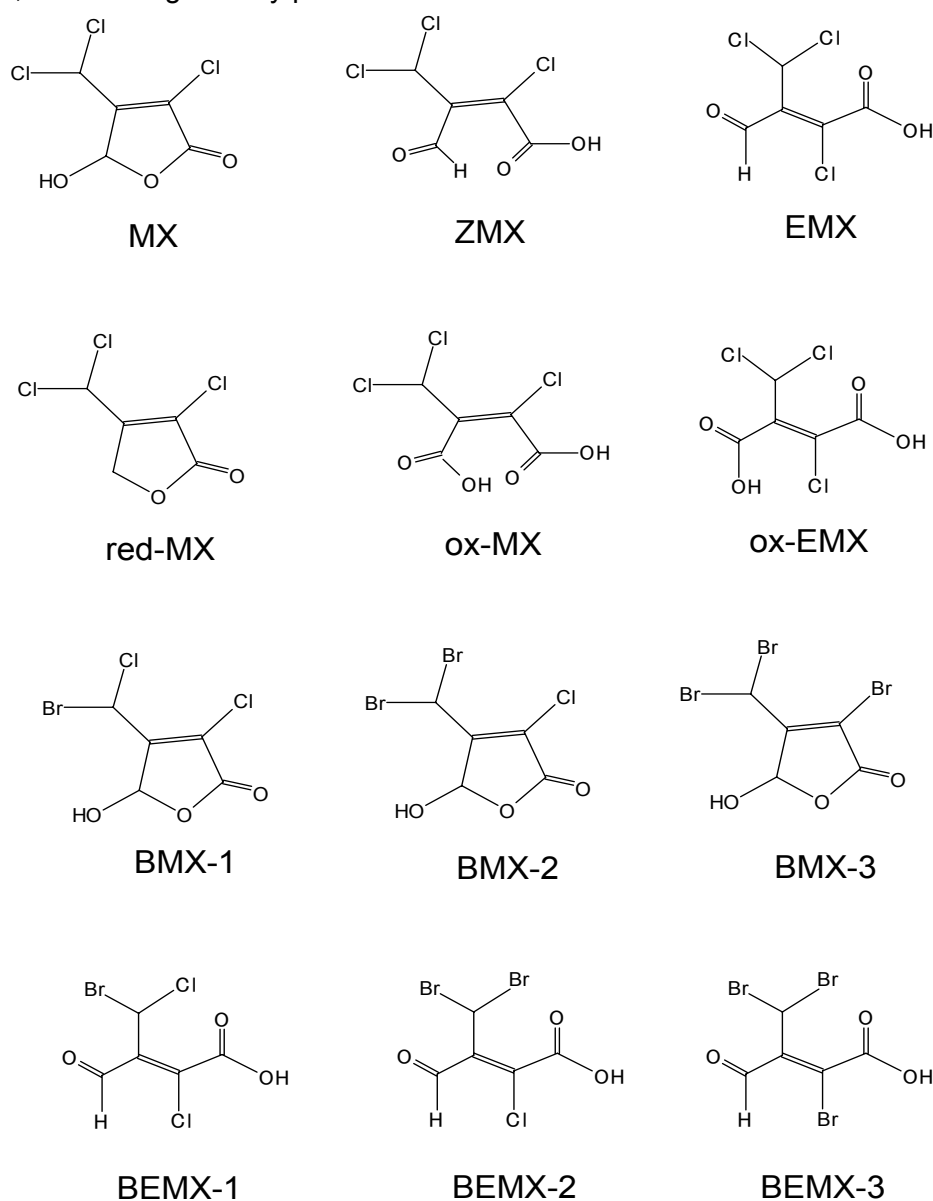


Figure 2. Structures of MX analogs

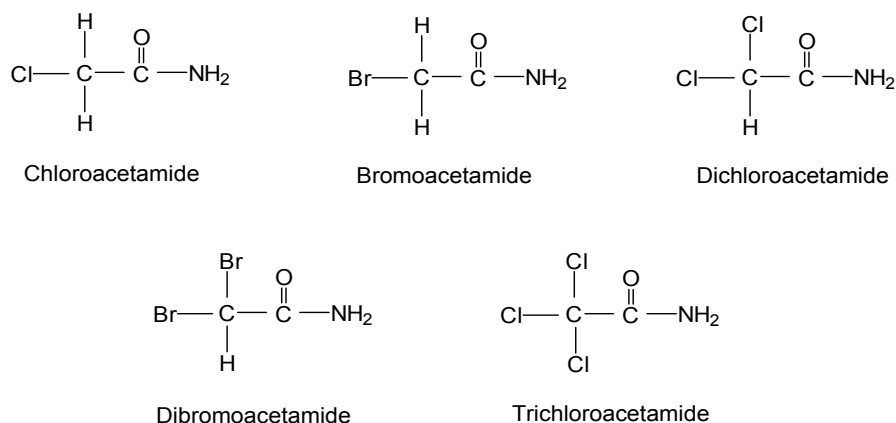


Figure 3. Structures of haloamides recently measured

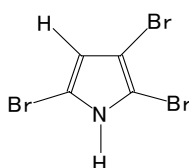


Figure 4. Structure of 2,3,5-tribromopyrrole (representing a new class of DBP identified)

Tribromopyrrole. In 2003, a new halogenated pyrrole--2,3,5-tribromopyrrole (Figure 4) - was identified in drinking water (Richardson *et al.*, 2003). This represents the first time that a halogenated pyrrole has been observed as a drinking water DBP for any disinfectant. This halopyrrole was found in finished drinking water from a full-scale drinking water treatment plant in Israel that used pre-chlorination (at an initial reservoir) followed by primary treatment with combined chlorine dioxide-chlorine or combined chlorine dioxide-chloramine to treat a high bromide source water (approximately 2 ppm). This identification resulted from the first study of chlorine dioxide DBPs formed under high bromide/iodide conditions. Bromide levels in U.S. source waters generally range up to a maximum of approximately 0.5 ppm, and so to-date, this tribromopyrrole has not been identified in drinking waters from the United States. GC with low and high resolution-MS was used for DBP identification in the Israel drinking water. Mammalian cell toxicity testing revealed tribromopyrrole to be 8X more cytotoxic than dibromoacetic acid (a regulated DBP) and to have about the same genotoxic potency as MX. When the formation of tribromopyrrole was investigated using isolated humic and fulvic acid fractions collected from the source waters (as natural organic matter precursors), tribromopyrrole was found to be formed primarily from humic acid, whereas the THMs, HAAs, and aldehydes were mostly formed from fulvic acid. It is interesting to note that a soil humic model proposed by Schulten and Schnitzer (1993) that was based on ¹³C NMR, pyrolysis, and oxidative degradation data, includes a pyrrole group in its structure. In addition, the elementary analysis (C, H, N, X) for these natural humic and fulvic acids showed a greater contribution from N in the humic acid as compared to that in the fulvic acid. The finding of 2,3,5-tribromopyrrole at significant levels only when humic acid was reacted with a mixture of both chlorine dioxide and chlorine supports the observation of 2,3,5-tribromopyrrole in full-scale treatments involving combinations of chlorine dioxide and chlorine or chlorine dioxide and chloramines, as well as a controlled laboratory reaction of chlorine dioxide and chlorine with the same source water. In none of the samplings from this research was tribromopyrrole found in pre-chlorinated waters (with chlorine treatment only). Thus, the combination of chlorine dioxide and chlorine (or chloramines) may be necessary for its formation. It is also possible that chloramination alone may also be important for its formation.

NDMA and Other Nitrosamines. Nitrosodimethylamine (NDMA) is recognized as a probable human carcinogen, and until recently, concerns about NDMA primarily stemmed from its presence in food, beverages, consumer products, contaminated groundwater (from the use of rocket fuel), and polluted air (e.g., tobacco smoke) (Mitch *et al.*, 2003). However, it has recently become evident that NDMA is also a drinking water DBP, which could make human exposure more widespread (Jobb *et al.*, 1992; Graham *et al.*, 1995; Kohut and Andrews, 2002; Andrews and Taguchi, 2000; Najm and Trussell, 2001). NDMA has primarily been found in chloraminated drinking water, where the nitrogen in monochloramine (NH_2Cl) is incorporated into the structure of the NDMA by-product formed (Choi and Valentine, 2002). Chlorination can also form NDMA to some extent, when there are nitrogen precursors present (e.g., natural ammonia in the source water or nitrogen-containing coagulants used in the water treatment process) (Mitch *et al.*, 2003; Wilczak *et al.*, 2003). NDMA was initially discovered in chlorinated drinking waters from Ontario, Canada (Jobb *et al.*, 1995), and has recently been found in other locations and in laboratory studies (Choi and Valentine, 2002; Mitch and Sedlak, 2002). The observation of NDMA in U.S. waters is largely due to improved analytical techniques that have allowed its determination at low nanogram per liter concentrations. Recent measurements have shown it is generally present at ng l^{-1} levels in chloraminated/chlorinated drinking water, and it can be formed at much higher levels in wastewater treated with chlorine. Following its discovery in California well water, the State of California issued an action level of $0.002 \mu\text{g l}^{-1}$ (2 parts per trillion) for NDMA, which was subsequently revised to $0.01 \mu\text{g l}^{-1}$, due to the analytical difficulty in measuring it at the original proposed level. The California Department of Health Services has a website that provides further background and details about NDMA (www.dhs.ca.gov/ps/ddwem/chemicals/NDMA/NDMAindex.htm). This site also provides a link to the 2002 U.S. National Toxicology Program (NTP) report on NDMA. NDMA is not currently regulated in the United States for drinking water. It has been considered for the Contaminant Candidate List (CCL), but is currently not listed. Canada (as a country) does not regulate NDMA, but Ontario has issued an interim maximum acceptable concentration for NDMA at 9 ng l^{-1} (www.ene.gov.on.ca/envision/gp/4449e.pdf).

Mitch *et al.* published a review in late 2003 that discusses issues with NDMA as a drinking water contaminant, including potential approaches for removing organic nitrogen precursors and the use of UV treatment to minimize/eliminate NDMA in drinking water (Mitch *et al.*, 2003). This review article also discusses analytical methods used for the analysis of NDMA and the sources and occurrence of NDMA.

New research is expanding beyond NDMA, the first nitrosamine discovered as a DBP to other nitrosamines. In fact, a new EPA method has been created for measuring NDMA and six additional nitrosamines in drinking water (EPA Method 521, Determination of Nitrosamines in Drinking Water by Solid-Phase Extraction and Capillary Column Gas Chromatography with Large Volume Injection and Chemical Ionization Tandem Mass Spectrometry (MS/MS) (www.epa.gov/nerlcwww/m_521.pdf). This method enables the measurement of NDMA and six other nitrosamines (*N*-nitrosomethylethylamine, *N*-nitrosodiethylamine, *N*-nitroso-di-*n*-propylamine, *N*-nitroso-di-*n*-butylamine, *N*-nitrosopyrrolidine, and *N*-nitrosopiperidine) in drinking water at detection limits ranging from 1.2 to 2.1 ng l^{-1} .

Probably the most significant study of NDMA and nitrosamines in the last two years was the discovery of nitrosamines beyond NDMA in finished drinking water. Charrois *et al.* (2004) discovered two new nitrosamines—*N*-nitrosopyrrolidine and *N*-nitrosomorpholine—in finished drinking water (both at the plant and in the distribution system) from two cities in Canada that use chloramination for treatment. This represents the first report of other nitrosamines besides NDMA in drinking water. Levels of *N*-nitrosopyrrolidine ranged from 2–4 ng l^{-1} , and *N*-nitrosomorpholine was found in drinking water from one city at 1 ng l^{-1} . NDMA was also found in drinking water from these cities and ranged from 2–180 ng l^{-1} . The structures of these nitrosamines are shown in Figure 5. The 180 ng l^{-1} level found for NDMA, which was measured in the distribution system of one city, is the highest to-date concentrations that has

been observed for NDMA in drinking water. The data in this study indicate that NDMA (and other nitrosamines) can continue to form in the distribution system and show dramatically increased levels in the distribution system as compared to the drinking water treatment plant (e.g., from an initial 67 ng l⁻¹ of NDMA at the plant to 180 ng l⁻¹ in the distribution system). This suggests that previous measurements of NDMA at the treatment plant may substantially underestimate the public's exposure to this probable carcinogen.

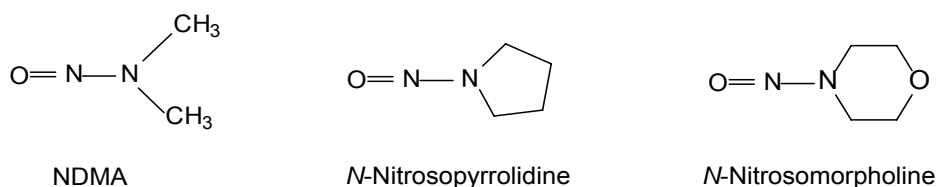


Figure 5. Structures of NDMA and two other nitrosamines identified as DBPs in drinking water

In another important study, Wilczak *et al.* (2003) investigated the effect of a popular nitrogen-containing coagulant on the formation of NDMA in drinking water. For this research, controlled laboratory studies were carried out by reacting the diallyldimethylammonium chloride (DADMAC) polymer with chlorine and chloramines in pure water; pilot plant studies were carried out by using the DADMAC polymer in a pilot plant that utilized chlorine, chloramines, and ozone, and their combinations; and full-scale drinking water treatment plants using DADMAC and chlorine/chloramine disinfection were investigated. Results showed that chloramine was necessary to form significant levels of NDMA with DADMAC; much lower levels were observed with free chlorine. The levels of NDMA observed strongly depended on the amount of DADMAC used; NDMA concentrations in the distribution system decreased with decreasing DADMAC doses. The length of free chlorine contact time before ammonia addition (to form chloramines) was also an important component—a free chlorine contact time of 1-4 hr before ammonia addition resulted in much lower NDMA levels. Further, it appeared that recycled filter backwash water was a significant source of NDMA precursors, likely due to recycling of residual DADMAC polymer.

Gerecke and Sedlak (2003) recently investigated precursors of NDMA in natural waters. For this study, samples from lakes, reservoirs, groundwaters, and isolated natural organic matter were reacted with monochloramine. A compound that had been previously suggested to be an important precursor of NDMA—dimethylamine—was found to be responsible for only a small fraction of the NDMA produced. Results showed that natural organic matter (NOM) accounts for a significant fraction of the precursors. However, NOM could not account completely for the amount of NDMA formed in drinking water treatment. As a result, it is suggested that nitrogen-containing coagulants (like DADMAC mentioned above) are probably also significant precursors. Unplanned wastewater reuse was also suggested as a source of NDMA, as wastewater typically contains 50-500 nM of dimethylamine, which would be enough to contribute to increased NDMA formation. In an investigation of NDMA precursors in wastewater treatment plants, Mitch and Sedlak measured NDMA after extended chloramination in advanced wastewater treatment plants and in reactions of model precursors (115). Of the model precursors investigated, only dimethylamine, tertiary amines with dimethylamine functional groups, and dimethylamides formed significant NDMA levels upon chloramination. In samples from municipal wastewater treatment plants, dissolved NDMA precursors were always present in primary and secondary effluents. Biological treatment was found to remove dimethylamine, but it was not effective for removing the other NDMA precursors.

3. HUMAN EXPOSURE STUDIES

Interesting human exposure studies continue to be conducted for DBPs. These human

exposure studies are not only useful to demonstrate exposure/uptake of DBPs in the human body, but they can also ultimately provide more accurate information about an individual's exposure, as compared to using water consumption questionnaires and quarterly water treatment plant data, which have been traditionally used in epidemiologic studies. Previous research has revealed that showering and bathing can result in higher blood levels of THMs than ingesting 1 L of water, and other recent research has demonstrated the permeability of certain DBPs across the skin. For example, THMs have been measured in the blood of human volunteers following drinking, showering, and bathing (Backer *et al.*, 2000). Among the different exposure routes (10-min shower, 10-min bath, ingesting 1 L of water), the 10-min shower produced the highest levels of THMs in the blood (from inhalation), with ingestion yielding only a slight increase in blood levels. THM internal dose levels (through the analysis of blood samples) have been compared for women of reproductive age from different states in the United States that have substantial differences in DBP speciations in their household water (specifically, one state had a higher level of brominated THMs, the other more chlorinated THMs) (Miles *et al.*, 2002). The differences in speciation in the tap water were also observed in the blood samples from the women.

DBPs have also been measured in the exhaled breath of people exposed to DBPs through drinking, showering, and bathing (Benoit *et al.*, 1997; Weisel *et al.*, 1999), in blood and exhaled breath of swimmers (Levesque *et al.*, 2000; Aggazzotti *et al.*, 1998), and in the urine of people exposed to DBPs (HAAs) through drinking water (Froese *et al.*, 2002).

In a new study published in 2005, Xu and Weisel conducted a controlled human study on six subjects to determine the respiratory uptake of haloketones and chloroform (as a reference compound) during showering. Breath and air concentrations of the DBPs were measured using GC-electron capture detection (ECD) during and following the inhalation exposures. A lower percentage of the haloketones (10%) was released from shower water to air than was chloroform (56%), which is more volatile. Breath concentrations were elevated during the inhalation exposure, but declined rapidly afterwards. Approximately 85-90% of the inhaled haloketones were absorbed, as compared to only 70% of the chloroform.

The permeation of THMs, HAAs, and haloketones across human skin was the focus of another study by Xu *et al.* (2002). This study showed that compounds in these three classes had different permeabilities--indicating that DBPs can have different dermal absorptions. Of the THMs, bromoform had the highest permeation, chloroform the least; THMs were 10 times more permeable than haloketones; and the permeability of HAAs was very low. It was proposed that ionization may be the most significant factor limiting the permeability of the HAAs (since they are anions at neutral pH). The dose of THMs by dermal absorption was estimated to be 40-70% of the dose from the ingestion of drinking water, while for haloketones, it was 10%, and insignificant for HAAs. Significant dermal exposures for chloroform have also been reported by other researchers.

4. CONCLUSIONS

Given the cytotoxic and genotoxic potencies of some of these emerging DBPs, further health effects research and/or occurrence information is warranted. *In vivo* work in progress for the bromonitromethanes will be informative to determine whether these DBPs may be associated with the bladder cancer observed in human populations. Also, it will be interesting to know whether any of these emerging DBPs may be associated with newly found reproductive and developmental effects. More research is needed to determine whether any of these emerging DBPs are a potential human health risk. New mixtures research (as with the Four Lab Study) will be informative in our understanding of the toxicologic role that complex mixtures play as compared to single-chemical toxicities (are the effects additive, less than additive, or greater than additive?). Finally, alternative routes of human exposure (in addition to ingestion) are now being recognized as important. It is expected that this information will support future epidemiologic studies in the understanding of DBP risks.

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policies and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. EPA.

REFERENCES

- Aggazzotti, G., Fantuzzi, G., Righi, E. and Predieri, G. (1998), Blood and breath analyses as biological indicators of exposure to trihalomethanes in indoor swimming pools, *Sci. Total Environ.*, **217**, 155-163.
- Andrews, S.A. and Taguchi, V.Y. (2000), NDMA – Canadian issues, *Proceedings of the American Water Works Association Water Quality Technology Conference*.
- Aschengrau, A., Zierler, S. and Cohen, A. (1993), Quality of community drinking-water and the occurrence of late adverse pregnancy outcomes, *Arch. Environ. Health*, **48**, 105-113.
- Backer, L.C., Ashley, D.L., Bonin, M.A., Cardinali, F.L., Kieszak, S.M. and Wooten, J.V. (2000), Household exposures to drinking water disinfection by-products: whole blood trihalomethanes levels, *J. Expos. Anal. Environ. Epidemiology*, **10**, 321-326.
- Benoit, F.M., Nicolidakis, H., Cardinali, C., Alleyne, C. and Mori, B. (1997), Characterization of chloroform levels in the breathing zone of showers by GC/MS, *Proceedings of the American Society for Mass Spectrometry*, p. 212, American Society for Mass Spectrometry, Santa Fe, NM.
- Bichsel, Y. and von Gunten, U. (1999), Oxidation of iodide and hypiodous acid in the disinfection of natural waters, *Environ. Sci. Technol.*, **33**, 4040-4045.
- Bichsel, Y. and von Gunten, U. (2000), Formation of iodo-trihalomethanes during disinfection and oxidation of iodide containing waters, *Environ. Sci. Technol.*, **34**, 2784-2791.
- Bove, F.J., Fulcomer, M.C., Klotz, J.B., Esmart, J., Dufficy, E.M. and Savrin, J.E. (1995), Public drinking-water contamination and birth outcomes, *Am. J. Epidemiology*, **141**, 850-862.
- Brass, H.J., Feige, M.A., Halloran, T., Mello, J.W., Munch, D. and Thomas, R.F. (1977), The national organic monitoring survey: samplings and analysis for purgeable organic compounds, In: *Drinking Water Quality Enhancement Through Source Protection*, Pojasek, R.B. (Ed.), Ann Arbor Science, Ann Arbor, MI, pp. 393-416.
- Bull, R. J. and Kopfler, F.C. (1991), *Health Effects of Disinfectants and Disinfection By-products*, Report, American Water Works Association Research Foundation, Denver, CO.
- Bull, R. J., Krasner, S.W., Daniel, P.A. and Bull, R.D. (2001), *Health Effects and Occurrence of Disinfection By-Products*, American Water Works Association Research Foundation and American Water Works Association, Denver, CO.
- Cancho, B., Ventura, F., Galceran, M., Diaz, A. and Ricart, S. (2000), Determination, synthesis and survey of iodinated trihalomethanes in water treatment processes, *Water Res.*, **34**, 3380-3390.
- Charrois, J.W.A., Arend, M.W., Froese, K.L. and Hrudey, S.E. (2004), Detecting *N*-nitrosamines in drinking water at nanogram per liter levels using ammonia positive chemical ionization. *Environ. Sci. Technol.*, **38** (18), 4835-4841.
- Chen, P.H., Richardson, S.D., Majetich, G. and Glish, G.L. (2002), Hydrogen abstraction and decomposition of tribromonitromethane and other trihalo compounds by GC/MS, *Environ. Sci. Technol.*, **36**, 3362-3371.
- Choi, J.H. and Valentine, R.L. (2002), Formation of *N*-nitrosodimethylamine (NDMA) from reaction of monochloramine: a new disinfection by-product, *Water Res.*, **36** (4), 817-824.
- Choi, J.H. and Richardson, S.D. (2004), Formation of halonitromethanes in drinking water, *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association, Denver, CO.
- DeAngelo, A.B. (2005), Emerging halonitromethanes health effects, *Proceedings of the American Water Works Association Annual Conference*, American Water Works Association, Denver, CO.
- Dodds, L., King, W.D., Woolcoot, C. and Pole, J. (1999), Trihalomethanes in public water supplies and adverse birth outcomes, *Epidemiology*, **10** (3), 233-237.
- Dodds, L. and King, W.D. (2001), Relation between trihalomethanes compounds and birth defects, *Occup. Environ. Medicine*, **58**, 443-446.
- Froese, K.L., Sinclair, M.I. and Hrudey, S.E. (2002), Trichloroacetic acid as a biomarker of exposure to disinfection by-products in drinking water: a human exposure trial in Adelaide, Australia, *Environ. Health Perspect.*, **110**, 679-687.
- Gallagher, M.D., Nuckols, J.R., Stallones, L. and Savitz, D.A. (1998), Exposure to trihalomethanes and adverse pregnancy outcomes, *Epidemiology*, **9** (5), 484-489.

- Gerecke, A.C. and Sedlak, D.L. (2003), Precursors of *N*-nitrosodimethylamine in natural waters, *Environ. Sci. Technol.*, **37** (7), 1331-1336.
- Geter, D.R., Winn, R.N., Fournie, J.W., Norris, M.B., DeAngelo, A.B. and Hawkins, W.E. (2004), MX [3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone], a drinking water carcinogen, does not induce mutations in the liver of cll transgenic medaka (*Oryzias latipes*), *J. Toxicol. Environ. Health, Part A*, **67**, 373-383.
- Glaze, W.E., Henderson, J.E. and Smith, G. (1975), Analysis of new chlorinated organic compounds formed by chlorination of municipal wastewater, In: *Water Chlorination: Environmental Impact and Health Effects*, Vol. 1, Jolley, R.J. (Ed.), Ann Arbor Science, Ann Arbor, MI, pp. 139-159.
- Graham, J.E., Meresz, O., Farquhar, G.J. and Andrews, S.A. (1995), Factors affecting NDMA formation during drinking water treatment, *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association, Denver, CO.
- Hunter, E.S., III and Tugman, J.A. (1995), Inhibitors of glycolytic metabolism affect neurulation-staged mouse conceptuses *in vitro*, *Teratology*, **52**, 317-323.
- Hunter, E.S., III, Rogers, E.H., Schmid, J.E. and Richard, A. (1996), Comparative effects of haloacetic acids in whole embryo culture, *Teratology*, **54**, 57-64.
- Jobb, D.B., Hunsinger, R.B., Meresz, O. and Taguchi, V.Y. (1992), A study of the occurrence and inhibition of formation of *N*-nitrosodimethylamine (NDMA) in the Ohsweken water supply, *Proceedings of the American Water Works Association Water Quality Technology Conference*, pp. 103-132, American Water Works Association, Denver, CO.
- King, W.D., Dodds, L. and Allen, A.C. (2000), Relation between stillbirth and specific chlorination by-products in public water supplies, *Environ. Health Perspect.*, **108**, 883-886..
- Klotz, J.B. and Pynch, L.A. (1999), Neural tube defects and drinking water disinfection by-products, *Epidemiology*, **10**, 383-390.
- Kohut, K.D. and Andrews, S.A. (2002), *N*-Nitrosodimethylamine formation in drinking water due to amine-based polyelectrolytes, *Proceedings of the American Water Works Association Water Quality Technology Conference*, American Water Works Association, Denver, CO.
- Komulainen, H., Kosma, V.-M., Vaitinen, S.-L., Vartiainen, T., Kaliste-Korhonen, E., Lotjonen, S., Tuominen, R. K. and Tuomisto, J. (1997), Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H) furanone in the rat, *J. Nat. Cancer Instit.*, **39** (12), 848-855.
- Kopfler, F.C., Melton, R.G., Lingg, R.D. and Coleman, W.E. (1976), GC/MS determination of volatiles for the national organics reconnaissance survey (NORS) of drinking water, In: *Identification and Analysis of Organic Pollutants in Water*, Keith, L.H., (Ed.), Ann Arbor Science, Ann Arbor, MI, pp. 87-104.
- Kramer, M.D., Lynch, C.F., Isacson, P. and Hanson, J.W. (1992), The association of waterborne chloroform with intrauterine growth-retardation, *Epidemiology*, **3** (5), 407-413.
- Krasner, S.W., Chinn, R., Hwang, C.J. and Barrett, S.E. (1991), Analytical methods for brominated organic disinfection by-products, *Proceedings of the 1990 American Water Works Association Water Quality Technology Conference*, American Water Works Association, Denver, CO.
- Krasner, S.W., Coffey, B.M., Hacker, P.A., Hwang, C.J., Kuo, C.-Y., Mofidi, A.A. and Scilimenti, M.J. (1996), *Proceedings of the 4th International BOM Conference, International Working Group on Biodegradable Organic Matter in Drinking Water*, Waterloo, Ontario, Canada.
- Kronberg, L. and Vartiainen, T. (1988), Ames mutagenicity and concentration of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and of its geometric isomer *E*-2-chloro-3-(dichloromethyl)-4-oxo-butenoic acid in chlorine-treated tap waters, *Mutat. Res.*, **206**, 177-182.
- Kronberg, L., Holmbom, B., Reunanen, M. and Tikkanen, L. (1988), Identification and quantification of the ames mutagenic compound 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and of its geometric isomer (*E*)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid in chlorine-treated humic water and drinking water extracts. *Environ. Sci. Technol.* **22**, 1097-1103.
- Kundu, B., Richardson, S.D., Swartz, P.D., Matthews, P.P., Richard, A.M. and DeMarini, D.M. (2004), Mutagenicity in Salmonella of halonitromethanes: a recently recognized class of disinfection by-product in drinking water, *Mutat. Res.*, **562**, 39-65.
- Kundu, B., Richardson, S.D., Granville, C.A., Shaughnessy, D.T., Hanley, N.M., Swartz, P.D., Richard, A.M. and DeMarini, D.M. (2004), Comparative mutagenicity of halomethanes and halonitromethanes in Salmonella TA100: structure-activity analysis and mutation spectra, *Mutat. Res.*, **554**, 335-350.
- Kurokawa, Y., Aoki, S., Matsushima, Y., Takamura, N., Imazawa, T. and Hayashi, Y. (1986), Dose-

- response studies on the carcinogenicity of potassium bromate in F344 rats after long-term oral administration, *J. Natl. Cancer Inst.*, **77**, 977-982.
- Levesque, B., Ayotte, P., Tardif, R., Charest-Tardif, G., Dewailly, E., Prud'Homme, D., Gingras, G., Allaire, S. and Lavoie, R.J. (2000), Evaluation of the health risk associated with exposure to chloroform in indoor swimming pools, *Toxicol. Environ. Health, Pt. A*, **61**, 225-243.
- Magnus, P., Jaakkola, J.J.K. and Skrondal, A. (1999), Water chlorination and birth defects, *Epidemiology*, **10**, 513-517.
- Miles, A.M., Singer, P.C., Ashley, D.L., Lynberg, M.C., Mendola, P., Langlois, P.H. and Nuckols, J.R. (2002), Comparison of trihalomethanes in tap water and blood, *Environ. Sci. Technol.*, **36**, 1692-1698.
- Mitch, W.A., Sharp, J.O., Trussell, R.R., Valentine, R. L., Alvarez-Cohen, L. and Sedlak, D.L. (2003), *N*-nitrosodimethylamine (NDMA) as a drinking water contaminant: a review, *Environ. Engin. Sci.*, **20** (5), 389-404.
- Mitch, W.A. and Sedlak, D.L. (2002), Formation of *N*-nitrosodimethylamine (NDMA) from dimethylamine during chlorination, *Environ. Sci. Technol.*, **36**, 588-595.
- Najm, I. and Trussell, R.R. (2001), NDMA formation in water and wastewater, *J. Am. Water Works Assoc.*, **93** (2), 92-99.
- National Cancer Institute (1976), Report on Carcinogenesis Bioassay of Chloroform, Carcinogenesis Program, Division of Cancer Cause and Prevention, Bethesda, MD.
- Nieuwenhuijsen, M.J., Toledano, M.B., Eaton, N.E., Fawell, J. and Elliott, P. (2000), Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review, *Occup. Environ. Med.* **57** (2), 73-85.
- Plewa, M.J., Wagner, E.D., Jazwierska, P., Richardson, S.D., Chen, P.H. and McKague, A.B. (2004), Halonitromethane drinking water disinfection byproducts: chemical characterization and mammalian cell cytotoxicity and genotoxicity, *Environ. Sci. Technol.*, **38** (1), 62-68.
- Plewa, M.J., Wagner, E.D., Richardson, S.D., Thruston, A.D., Jr., Woo, Y.-T. and McKague, A.B. (2004), Chemical and biological characterization of newly discovered iodoacid drinking water disinfection byproducts, *Environ. Sci. Technol.*, **38** (18), 4713-4722.
- Plewa, M.J. (2005), Toxicity concerns for disinfection byproducts from alternative disinfectants, *Proceedings of the American Water Works Association Annual Conference*, American Water Works Association, Denver, CO.
- Richardson, S.D. (1998), Drinking water disinfection by-products, In: *The Encyclopedia of Environmental Analysis and Remediation*, Vol. 3, Meyers, R.A. (Ed.), John Wiley & Sons, pp. 1398-1421.
- Richardson, S.D., Thruston, A.D., Jr., Caughran, T.V., Chen, P.H., Collette, T.W., Floyd, T.L., Schenck, K.M., Lykins, B.W., Jr., Sun, G.-R. and Majetich, G. (1999), Identification of New Ozone Disinfection By-Products in Drinking Water, *Environ. Sci. Technol.*, **33**, 3368-3377.
- Richardson, S.D., Thruston, A.D., Jr., Caughran, T.V., Chen, P.H., Collette, T.W., Floyd, T.L., Schenck, K.M., Lykins, B.W., Jr., Sun, G.-R. and Majetich, G. (1999), Identification of new drinking water disinfection byproducts formed in the presence of bromide, *Environ. Sci. Technol.*, **33**, 3378-3383.
- Richardson, S.D., Simmons, J.E. and Rice, G. (2002), Disinfection byproducts: the next generation, *Environ. Sci. Technol.*, **36** (9), 198A-205A.
- Richardson, S.D., Thruston, A.D., Jr., Rav-Acha, C., Groisman, L., Popilevsky, I., Juraev, O., Glezer, V., McKague, A.B., Plewa, M.J. and Wagner, E.D. (2003), Tribromopyrrole, brominated acids, and other disinfection byproducts produced by disinfection of drinking water rich in bromide, *Environ. Sci. Technol.*, **37** (17), 3782-3793.
- Rook, J.J. (1974), Formation of haloforms during chlorination of natural waters, *Water Treat. Exam.*, **23**, 234-243.
- Sasaki, Y.F., Nishidate, E., Izumiyama, F., Watanabe-Akanuma, M., Kinase, N., Matsusaka, N. and Tsuda, S. (1997), Detection of *in vivo* genotoxicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) by the alkaline single cell gel electrophoresis (comet) assay in multiple mouse organs, *Mutat. Res.*, **393**, 47-53.
- Savitz, D.A., Andrews, K.W. and Pastore, L.M. (1995), Drinking-water and pregnancy outcome in central North Carolina—source, amount, and trihalomethanes levels, *Environ. Health Perspect.*, **103** (6), 592-596.

- Schulten, H.-R. and Schnitzer, M. (1993), A state-of-the-art structural concept for humic substances, *Naturwissenschaften*, **80** (1), 29-30.
- Simmons, J.E., Richardson, S.D., Speth, T.F., Miltner, R.J., Rice, G., Schenck, K., Hunter, E.S., III and Teuschler, L.K. (2002), Development of a research strategy for integrated technology-based toxicological and chemical evaluation of complex mixtures of drinking water disinfection byproducts, *Environ. Health Perspect.*, **110** (Suppl. 6), 1013-1024.
- Simmons, J.E., Teuschler, L.K., Gennings, C., Speth, T.F., Richardson, S.D., Miltner, R.J., Narotsky, M.G., Schenck, K.D., Hunter, E.S., Hertzberg, R.C. and Rice, G. (2004), Component-based and whole-mixture techniques for addressing the toxicity of drinking water disinfection byproducts mixtures, *J. Toxicol. Environ. Health, Pt. A*, **67** (8-10), 741-754.
- Stevens, A.A., Moore, L.A., Slocum, C.J., Smith, B.L., Seeger, D.R. and Ireland, J.C. (1989), In: *Disinfection By-Products: Current Perspectives*, American Water Works Association, Denver, CO.
- Suzuki, N. and Nakanishi, J. (1995), Brominated analogues of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone) in chlorinated drinking water, *Chemosphere*, **30** (8), 1557-1564.
- Swan, S.H., Waller, K., Hopkins, B., Windham, G., Fenster, L., Shaefer, C., Neutra, R.R. (1998), A prospective study of spontaneous abortion: relation to amount and source of drinking water consumed in early pregnancy, *Epidemiology*, **9** (2), 126-133.
- U.S. EPA (1979), National Interim Primary Drinking Water Regulations, *Fed. Registr.* **44**, 68624.
- U.S. EPA (1998), National Primary Drinking Water Regulations: Disinfectants and Disinfection By-products; Final Rule, *Fed. Registr.* **63** (16 Dec 1998), 69389.
- Waller, K., Swan, S.H., DeLorenze, G. and Hopkins, B. (1998), Trihalomethanes in drinking water and spontaneous abortion, *Epidemiology*, **9** (2), 134-140.
- Waller, K., Swan, S.H., Windham, G.C. and Fenster, L.J. (2001), Influence of exposure assessment methods on risk estimates in an epidemiologic study of total trihalomethanes exposure and spontaneous abortion, *J. Exp. Anal. Environ. Epidemiology*, **11**, 522-531.
- Weinberg, H.S., Krasner, S.W., Richardson, S.D. and Thruston, A.D., Jr. (2002), *The Occurrence of Disinfection By-Products (DBPs) of Health Concern in Drinking Water: Results of a Nationwide DBP Occurrence Study*, EPA/600/R02/068, U.S. Environmental Protection Agency, National Exposure Research Laboratory, Athens, GA. This report is also available on the web at: www.epa.gov/athens/publications/DBP.html.
- Weisel, C.P., Kim, H., Haltmeier, P. and Klotz, J.B. (1999), Exposure estimates to disinfection by-products of chlorinated drinking water, *Environ. Health Perspect.*, **107**, 103-110.
- Wilczak, A., Assadi-Rad, A., Lai, H.H., Hoover, L.L., Smith, J.F., Berger, R., Rodigari, F., Beland, J.W., Lazzelle, L. J., Kinicannon, E.G., Baker, H. and Heaney, C.T. (2003), Formation of NDMA in chloraminated water coagulated with DADMAC cationic polymer, *J. Am. Water Works Assoc.*, **95** (9), 94-106.
- World Health Organization (2000), Disinfectants and Disinfectant By-products, Environmental Health Criteria 216, International Programme on Chemical Safety.
- Woo, Y.-T., Lai, D., McLain, J.L., Manibusan, M.K. and Dellarco, V. (2002), Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products, *Environ. Health Perspec.*, **110** (Suppl. 1), 75-87.
- Wright, J.M., Schwartz, J., Vartiainen, T., Maki-Paakkanen, J., Altshul, L., Harrington, J.J. and Dockery, D.W. (2002), 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and mutagenic activity in Massachusetts drinking water, *Environ. Health Perspect.*, **110**, 157-164.
- Xu, X., Mariano, T.M., Laskin, J.D. and Weisel, C.P. (2002), Percutaneous absorption of trihalomethanes, haloacetic acids, and haloketones, *Toxicol. Appl. Pharmacol.*, **184**, 19-26.
- Xu, X. and Weisel, C.P. (2005), Human respiratory uptake of chloroform and haloketones during showering, *J. Exp. Anal. Environ. Epidemiol.*, **15** (1), 6-16.