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Review

Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: A review and roadmap for research

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Abstract

Disinfection by-products (DBPs) are formed when disinfectants (chlorine, ozone, chlorine dioxide, or chloramines) react with naturally occurring organic matter, anthropogenic contaminants, bromide, and iodide during the production of drinking water. Here we review 30 years of research on the occurrence, genotoxicity, and carcinogenicity of 85 DBPs, 11 of which are currently regulated by the U.S., and 74 of which are considered emerging DBPs due to their moderate occurrence levels and/or toxicological properties. These 74 include halonitromethanes, iodo-acids and other unregulated halo-acids, iodo-trihalomethanes (THMs), and other unregulated halomethanes, halofuranones (MX [3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone] and brominated MX DBPs), haloamides, haloacetonitriles, tribromopyrrole, aldehydes, and *N*-nitrosodimethylamine (NDMA) and other nitrosamines. Alternative disinfection practices result in drinking water from which extracted organic material is less mutagenic than extracts of chlorinated water. However, the levels of many emerging DBPs are increased by alternative disinfectants (primarily ozone or chloramines) compared to chlorination, and many emerging DBPs are more genotoxic than some of the regulated DBPs. Our analysis identified three categories of DBPs of particular interest. Category 1 contains eight DBPs with some or all of the toxicologic characteristics of human carcinogens: four regulated (bromodichloromethane, dichloroacetic acid, dibromoacetic acid, and bromate) and four unregulated DBPs (formaldehyde, acetaldehyde, MX, and NDMA). Categories 2 and 3 contain 43 emerging DBPs that are present at moderate levels (sub- to low- $\mu\text{g/L}$): category 2 contains 29 of these that are genotoxic (including chloral hydrate and chloroacetaldehyde, which are also a rodent carcinogens); category 3 contains the remaining 14 for which little or no toxicological data are available. In general, the brominated DBPs are both more genotoxic and carcinogenic than are chlorinated compounds, and iodinated DBPs were the most genotoxic of all but have not been tested for carcinogenicity. There were toxicological data gaps for even some of the 11 regulated DBPs, as well as for most of the 74 emerging DBPs. A systematic assessment of DBPs for genotoxicity has been performed for ~ 60 DBPs for DNA damage in mammalian cells and 16 for mutagenicity in *Salmonella*. A recent epidemiologic study found that much of the risk for bladder cancer associated with drinking water was associated with three factors: THM levels, showering/bathing/swimming (i.e., dermal/inhalation exposure), and

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genotype (having the *GSTT1-1* gene). This finding, along with mechanistic studies, highlights the emerging importance of dermal/inhalation exposure to the THMs, or possibly other DBPs, and the role of genotype for risk for drinking-water-associated bladder cancer. More than 50% of the total organic halogen (TOX) formed by chlorination and more than 50% of the assimilable organic carbon (AOC) formed by ozonation has not been identified chemically. **The potential interactions among the 600 identified DBPs in the complex mixture of drinking water to which we are exposed by various routes is not reflected in any of the toxicology studies of individual DBPs. The categories of DBPs described here, the identified data gaps, and the emerging role of dermal/inhalation exposure provide guidance for drinking water and public health research.**

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Keywords: *N*-Nitrosodimethylamine; Regulated and unregulated DBPs; Total organic halogen; Total organic carbon

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1. Introduction

Water disinfection is one of the most important public health advances of the last century; its introduction in the U.S. reduced cholera incidence by 90%, typhoid by 80%, and amoebic dysentery by 50% [1]. Millions of people worldwide receive quality drinking water every day from their public water

systems. However, chemical disinfection has also raised a public health issue: the potential for cancer and reproductive/developmental effects associated with chemical disinfection by-products (DBPs).

Chemical disinfectants are effective for killing harmful microorganisms in drinking water, but they are also powerful oxidants, oxidizing the organic matter, anthropogenic contaminants, and bromide/

iodide naturally present in most source waters (rivers, lakes, and many groundwaters). Chlorine, ozone, chlorine dioxide, and chloramines are the most common disinfectants in use today; each produces its own suite of DBPs in drinking water, with overlapping constituents [2]. Most developed nations have published regulations or guidelines to control DBPs and minimize consumers' exposure to potentially hazardous chemicals while maintaining adequate disinfection and control of targeted pathogens.

Scientists first became aware of DBPs only in the early 1970s. In 1974, Rook and others reported the identification of the first DBPs in chlorinated drinking water: chloroform and other trihalomethanes (THMs) [3,4]. In 1976, the U.S. Environmental Protection Agency (U.S. EPA) published the results of a national survey that showed that chloroform and the other THMs were ubiquitous in chlorinated drinking water [5]. In the same year, the National Cancer Institute published results showing that chloroform was carcinogenic in laboratory animals [6]. In addition, the first reports appeared in the late 1970s showing that organic extracts of drinking water were mutagenic in the *Salmonella* mutagenicity assay [7]. As a result of these observations, an important public health issue was recognized.

In the 30 years since the THMs were identified as DBPs in drinking water, significant research efforts have been directed toward increasing our understanding of DBP formation, occurrence, and health effects [2,8–17]. Although more than 600 DBPs have been reported in the literature [2,18], only a small number has been assessed either in quantitative occurrence or health-effects studies.

The DBPs that have been quantified in drinking water are generally present at sub- $\mu\text{g/L}$ (ppb) or low- to mid- $\mu\text{g/L}$ levels. However, more than 50% of the total organic halide (TOX) formed during the chlorination of drinking water [19] and more than 50% of the assimilable organic carbon (AOC) formed during ozonation of drinking water has not been accounted for as identified DBPs [20]; furthermore, nothing is known about the potential toxicity of many of the DBPs present in drinking water.

Here we review 30 years of results of occurrence, genotoxicity, and carcinogenicity studies of DBPs regulated by the U.S. Government and those that are not named specifically in regulations. The compounds in these two categories, and a qualitative assessment of the results, are shown in Table 1. Although most of the research has been performed on the regulated DBPs, there is a growing literature on the unregulated DBPs. The results of our analyses in this paper offer an

Table 1
Summary of occurrence, genotoxicity, and carcinogenicity of regulated and unregulated DBPs

DBP	Occurrence ^a	Genotoxicity ^b	Carcinogenicity
Regulated DBPs			
THMs			
Chloroform	*****	–	+
Bromodichloromethane	*****	+	+
Chlorodibromomethane	*****	+	+
Bromoform	*****	+	+
HAAs			
Chloroacetic acid	***	+	–
Bromoacetic acid	***	+	–
Dichloroacetic acid	*****	+	+
Dibromoacetic acid	*****	+	+
Trichloroacetic acid	*****	–	+
Oxyhalides			
Bromate	***	+	+
Chlorite	*****	–	– ^c
Unregulated DBPs			
Halonitromethanes			
Chloronitromethane	**	+	
Bromonitromethane	**	+	
Dichloronitromethane	**	+	
Dibromonitromethane	***	+	
Bromochloronitromethane	**	+	
Trichloronitromethane (chloropicrin)	*****	+	
Bromodichloronitromethane	***	+	
Dibromochloronitromethane	***	+	

Table 1 (Continued)

DBP	Occurrence ^a	Genotoxicity ^b	Carcinogenicity
Tribromonitromethane	***	+	
Iodo-acids			
Iodoacetic acid	***	+	
Bromoiodoacetic acid	***	+	
(Z)-3-Bromo-3-iodopropenoic acid	**		
(E)-3-Bromo-3-iodopropenoic acid	**		
2-Iodo-3-methylbutenedioic acid	***	+ ^d	
Other halo-acids			
Bromochloroacetic acid	****		+ ^e
Bromodichloroacetic acid	****		+ ^e
Dibromochloroacetic acid	****		+ ^e
Tribromoacetic acid	****	+	
Iodo-THMs and other unregulated THMs			
Dichloriodomethane	***		
Bromochloriodomethane	***		
Dibromiodomethane	***		
Chlorodiodomethane	***		
Bromodiodomethane	***		
Iodoform	***	+	
Dichloromethane	***	+	
Bromochloromethane	ND	+	
Dibromomethane	ND/**	+	
MX compounds			
MX	**	+	+
Red-MX	*	+	
Ox-MX	*	+	
EMX	*	+	
ZMX	*	+	
Mucochloric acid	**	+	
BMX-1	**	+	
BMX-2	*	+	
BMX-3	*	+	
BEMX-1	**	+	
BEMX-2	**	+	
BEMX-3	**	+	
Haloamides			
Chloroacetamide	***	+	
Bromoacetamide	***	+	
Iodoacetamide		+	
Dichloroacetamide	***	+	
Bromochloroacetamide	***	+	
Dibromoacetamide	***	+	
Bromoiodoacetamide	***	+	
Trichloroacetamide	***	+	
Bromodichloroacetamide	***	+	
Dibromochloroacetamide	***	+	
Tribromoacetamide	***	+	
Diiodoacetamide		+	
Chloroiodoacetamide		+	
Haloacetonitriles			
Chloroacetonitrile	***	+	
Bromoacetonitrile	***	+	
Iodoacetonitrile		+	
Dichloroacetonitrile	***	+	
Bromochloroacetonitrile	***	+	
Dibromoacetonitrile	***	+	On test

Table 1 (Continued)

DBP	Occurrence ^a	Genotoxicity ^b	Carcinogenicity
Trichloroacetonitrile	***	+	
Bromodichloroacetonitrile	***		
Dibromochloroacetonitrile	***		
Tribromoacetonitrile	***		
Halopyrroles			
2,3,5-Tribromopyrrole	**	+	
Nitrosamines			
NDMA	**	+	+ ^f
<i>N</i> -Nitrosopyrrolidine	*	+	+ ^f
<i>N</i> -Nitrosomorpholine	*	+	+ ^f
<i>N</i> -Nitrosopiperidine	*	+	+ ^f
<i>N</i> -Nitrosodiphenylamine	*	+	+ ^f
Aldehydes			
Formaldehyde	***	+	+
Acetaldehyde	***	+	+
Chloroacetaldehyde	***		+
Dichloroacetaldehyde	***		
Bromochloroacetaldehyde	***		
Trichloroacetaldehyde (chloral hydrate)	****	+	+
Tribromoacetaldehyde	***		
Other DBPs			
Chlorate	*****	+	+

^a Key to occurrence symbols: *low-ng/L levels; **ng/L to sub- μ g/L levels; ***sub- to low- μ g/L levels; ****low- μ g/L levels; *****low- to mid- μ g/L levels; *****high μ g/L levels; ND, non-detect; entries left blank have no occurrence data available; bromine-containing DBPs formed only when source waters contain natural bromide (occurrence lower than shown if low bromide levels in source waters).

^b Symbols represent weight of evidence for the genotoxicity data. In general, where a compound was genotoxic in several studies in the same assay or was genotoxic in several different assays, it was declared “+” in the table even if the compound was negative in other assays.

^c Based on 85-week studies.

^d M.J. Plewa, in preparation, personal communication.

^e A.B. DeAngelo, in preparation, personal communication.

^f Details of these studies are not given in the following tables because they have been reviewed extensively [230]. As noted in the text, most of these compounds are rodent carcinogens by various routes of exposure, including via the drinking water.

opportunity to assess the value and completeness of the current literature on the regulated DBPs and to consider how the emerging literature on the unregulated DBPs might inform future research needs and assessments of drinking water.

To provide a historical context for this work, we begin with an overview of U.S. DBP regulations, followed by a brief summary of the epidemiology of drinking water and cancer. We have not reviewed the literature on reproductive/developmental effects associated with DBPs or drinking water. We then review the occurrence, genotoxicity, and carcinogenicity literature for the regulated and then the unregulated DBPs, ending with our conclusions regarding research needs.

2. Overview of DBP regulations in the United States

Based on the discoveries of DBPs described in the Introduction, the U.S. EPA issued a regulation in 1979

to control total THMs at an annual average of 100 μ g/L (ppb) in drinking water; THMs here are defined as chloroform, bromodichloromethane, dibromochloromethane, and bromoform [21]. In 1998, the U.S. EPA issued the Stage 1 Disinfectants (D)/DBP Rule, which lowered permissible levels of total THMs to 80 μ g/L and regulated for the first time five haloacetic acids (HAAs) (60 μ g/L), bromate (10 μ g/L), and chlorite (1000 μ g/L) (Table 2) [22]. Stage 1 regulations required monitoring based on running annual averages, which represented averages of all samples collected in a utility's distribution system over a 1-year period. This Rule became effective on 1 January 2002 [23].

The Stage 2 D/DBP Rule (published in January 2006) maintained the Stage 1 Rule maximum contaminant levels (MCLs) for THMs and HAAs (Table 1) and required that MCLs be based on locational running annual averages; that is, *each location* in the distribution system needs to comply on a running annual average basis [24]. The reason for this change was that the

Table 2
DBP regulations and guidelines

DBP	
U.S. EPA regulations	MCL ^a (mg/L)
Total THMs	0.080
Five haloacetic acids	0.060
Bromate	0.010
Chlorite	1.0
World Health Organization (WHO) guidelines	
DBP	Guideline value (mg/L)
Chloroform	0.2
Bromodichloromethane	0.06
Chlorodibromomethane	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
European Union Standards	
DBP	Standard value (mg/L)
Total THMs	0.1
Bromate	0.01 ^c

^a The total THMs represent the sum of the concentrations of four trihalomethanes: chloroform, bromoform, bromodichloromethane, and chlorodibromomethane. They have been regulated in the United States since 1979 [21], but the maximum contaminant level (MCL) was lowered from 100 to 80 µg/L under the Stage 1 Disinfectants/DBP (D/DBP) Rule [22]. World Health Organization (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 [22]. 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 http://www.nucfilm.com/eu_water_directive.pdf.

^b Provisional guideline value.

^c Where 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 (3 November 1998); within 5 years of the Directive, a value of 25 µg/L must be met.

running annual averages (used with the Stage 1 D/DBP Rule) permitted some locations within a water distribution system to exceed the 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 maintains 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 are available at <http://www.epa.gov/safewater/stage2/index.html>). Other countries besides the United States have regulated DBPs, and there are World Health Organization (WHO) guidelines for DBPs as well as European Union DBP standards (Table 2).

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 so-called alternative disinfectants, including ozone, chlorine dioxide, and chloramines. In some cases, chlorine is used as a secondary disinfectant following primary treatment with an alternative disinfectant, particularly for ozone and chlorine dioxide. However, new issues and problems can result with changes in disinfection practice.

For example, the use of ozone can significantly reduce or eliminate the formation of THMs and HAAs, but it 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 carcinogen in laboratory animals [25]. As a result, the U.S. EPA regulated bromate under the Stage 1 D/DBP Rule at an MCL of 10 µg/L to limit its occurrence [22]. Nitrosodimethylamine (NDMA), which can form at higher levels with chloramination, is also a concern because there are data indicating that it is a carcinogen in several animal species. Under its 1986 *Guidelines for Carcinogen Risk Assessment* (http://www.epa.gov/ncea/raf/car2sab/guidelines_1986.pdf), the U.S. EPA classified NDMA as a probable human carcinogen [26].

Likewise, a recent U.S. Nationwide DBP Occurrence Study, which included drinking waters from source waters containing high bromide/iodide and natural organic matter levels, revealed that iodo-THMs and newly identified iodo-acids were increased in formation with chloramination; moreover, bromonitromethanes were increased with preozonation followed by post-chlorination or chloramination [9,27]. Differences in source water conditions, including concentrations of bromide or iodide, concentrations of natural organic matter, and pH, can have a dramatic effect on the formation of various DBPs (chlorine-, bromine-, or iodine-containing) and the levels formed [9,28,29].

3. Summary of epidemiology studies of cancer and drinking water

Some epidemiologic studies have shown that a lifetime exposure to chlorinated water is associated with an increased risk for cancer, especially of the urinary bladder and colorectum [17,30]. Besides DBPs, drinking water may contain other potential carcinogens, such as arsenic and radionuclides; however, the bladder cancer risk has generally been associated with THM levels [31,32]. One study showed that both bladder and kidney cancer risks were associated with the mutagenicity of the water, which may be related to levels of the chlorinated furanone, MX [33] or possibly other mutagenic DBPs. Risk for rectal cancer has recently been shown to be associated specifically with levels of the THM bromoform [34].

The first and only epidemiologic study to stratify risk by route of exposure has found that much of the bladder cancer risk associated with chlorinated water appears to be due to showering, bathing, and swimming rather than to drinking the water [32] and that the risk may be highest for people having the *GSTT1-1* gene [35]. Such observations indicate that genetic susceptibility may play a role in the cancer risk and that the risk may be especially related to dermal and inhalation exposure.

One study has shown that the risk for bladder cancer decreased as the duration of exposure to ozonated water increased [36]. Such an observation supports the shift from chlorination to modified treatments such as ozonation. Earlier studies had found that organic extracts of ozonated water were far less mutagenic than those of chlorinated water [37–39]; this has been confirmed recently for organic concentrates of ozonated water [40]. However, studies of water treated with alternative disinfectants are limited, and there has not been a systematic analysis carried out on drinking water prepared from various types of source waters, including high-bromide/iodide source waters.

Most of the DBPs tested for carcinogenicity in rodents cause primarily liver cancer rather than bladder or colorectal cancer [17,30]. As reviewed here, exceptions include renal tumors induced by bromodichloromethane, chloroform, and bromate; intestinal tumors induced by bromodichloromethane and bromoform; and thyroid tumors induced by bromate. The most striking exception is the variety of organ sites at which MX induced tumors in the rat, as well as the low doses at which these tumors were induced (relative to the doses of the other DBPs).

This general lack of correlation between site of tumors in animal cancer studies for individual DBPs and human epidemiological studies for drinking water has not yet been explained. However, in addressing the potential for

animal carcinogens to be hazardous to humans, most regulatory agencies do not presume that there is tumor site concordance between rodents and humans. Possible areas for exploration involve route of exposure. Most of the carcinogenicity studies of DBPs have involved administration of the DBP in the drinking water (oral exposures). However, the recent route-of-exposure study [32,41] indicated that much of the bladder cancer associated with chlorinated water may be due to showering, bathing, and swimming (dermal and inhalation exposures) rather than oral exposures. In addition, only a few of the newly identified DBPs discussed in this review have been tested for carcinogenicity, and perhaps some of these will cause bladder or colorectal cancer.

Although not reviewed here, recent epidemiologic studies have raised the issue of potential adverse reproductive and developmental effects, such as low birth weight, intrauterine growth retardation, and spontaneous abortion [8,42–56].

4. Occurrence, genotoxicity, and carcinogenicity of the regulated DBPs

4.1. Trihalomethanes (THMs)

4.1.1. Occurrence

The halomethanes make up one class of the approximately 600 drinking-water DBPs that have been identified. Within the halomethane class are the THMs (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane), which are currently regulated by the U.S. EPA at a level of 80 $\mu\text{g/L}$ for total trihalomethanes [24]. The THMs were the first DBPs identified [3,4]. Together, the THMs and HAAs are the two most prevalent classes of DBPs formed in chlorinated drinking water, accounting for approximately 25% of the halogenated DBPs [9]. They are also formed at significantly lower levels in chloraminated drinking water, and bromoform can be formed in high-bromide source waters treated with ozone [2,57]. Disinfection with chlorine dioxide does not form THMs; however, low THM levels can be present due to chlorine impurities in chlorine dioxide.

A National Organics Reconnaissance Survey (NORS) and National Organics Monitoring Survey (NOMS) conducted in the mid- to late-1970s collected the first substantial information on THMs in the United States [58]. Later, the U.S. EPA Information Collection Rule (ICR), which involved 500 large drinking-water plants in the United States, reported mean levels in the distribution system of 38 $\mu\text{g/L}$ and 90th percentage levels of 78 $\mu\text{g/L}$ for THM4 (the four regulated THMs summed together)

[23]. Chloroform was by far the most prevalent of the THMs measured, and it had the highest mean concentration of 23 µg/L. Brominated THMs (bromodichloromethane, chlorodibromomethane, and bromoform) can increase in formation relative to chloroform when elevated levels of natural bromide are present in source waters (often due to salt water intrusion). THM levels observed in the ICR were substantially lower (reduced by 50–60%) than levels observed in the earlier NORS study [23].

4.1.2. Genotoxicity

The THMs have been studied intensively over the past 30 years, and many *in vitro* techniques have been used to investigate their mutagenic and genotoxic properties [59] (Table 3). We have used the term “mutagenicity” to refer to assays that measure a change in DNA sequence (either gene or chromosomal mutation); we have used the term “genotoxicity” to refer to mutagenicity as well as DNA damage (DNA adducts, DNA strand breaks, etc.). Although many of the initial genotoxicity tests of the THMs resulted in negative responses, later studies (discussed below) showed that the brominated THMs were mutagenic after activation by glutathione S-transferase-theta (GSTT1-1).

The genotoxicity of chloroform (trichloromethane) has been reviewed extensively [59], and those reports not included in the IARC review are shown in Table 3.

With few exceptions, chloroform is not mutagenic or genotoxic in a wide array of systems and endpoints *in vivo* and *in vitro*. Although some weak positive responses have been observed, these are either in single studies, or the results have not been highly repeatable. Unlike some of the THMs, chloroform is not activated by GSTT1-1 to a mutagen in *Salmonella* [60]. As discussed in the carcinogenicity section below, chloroform is generally considered to be a nongenotoxic carcinogen whose mechanism of action involves cytotoxicity and regenerative cell proliferation [59] (<http://www.epa.gov/iris/subst/0025.htm>).

Bromodichloromethane, chlorodibromomethane, and bromoform have generally not induced gene mutations in the standard test systems; the few positive results are either in single studies or were not found in repeated studies [59] (Table 3). Nonetheless, some studies have found that chlorodibromomethane induced chromosomal aberrations or sister chromatid exchanges (SCEs) and that bromoform induced SCEs and micronuclei [61]. Recently these DBPs were evaluated for genotoxicity in CHO cells; they were refractory to concentrations of 5 mM. The rank order of chronic CHO cell cytotoxicity was bromoform > chlorodibromomethane > chloroform > bromodichloromethane [62]. However, unlike chloroform, these brominated THMs are activated to mutagens by GSTT1-1

Table 3
Comparative genotoxicity of halomethane DBPs

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References
Dibromomethane	<i>Salmonella</i> TA100	<i>his</i> reversion		[121]
		Preincubation –S9 +S9	279 revertants/µmol 551 revertants/µmol	
	<i>E. coli</i> TRG8 <i>Salmonella</i> TA1535 (+)GST5-5	<i>his</i> reversion <i>his</i> reversion	0.02–0.1 mM 0.1–1 mM	[140] [139]
Bromoform	Review			[61]
	<i>Salmonella</i> TA100	<i>his</i> reversion	Negative	[121]
		Preincubation –S9, +S9		
	Human lymphocytes	SCGE	Weakly +	[283]
	<i>S. typhimurium</i> RSJ100	<i>his</i> reversion		[77]
		–S9 +S9	44 revertants/µmol Negative	
	TA98	–S9 +S9	Negative 237 revertants/µmol	
	TA100	–S9 +S9	Negative 83 revertants/µmol	
Human lung epithelial cells		SCGE	100–1000 µM	[138]
<i>Salmonella</i> RSJ100	<i>his</i> reversion	1798 revertants/1600 ppm	[63]	
Dichloromethane	Review			[61]
	<i>Salmonella</i> TA100	<i>his</i> reversion		[121]

Table 3 (Continued)

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References	
Chloroform	Human lung epithelial cells <i>Salmonella</i> RSJ100 <i>Salmonella</i> TA100	Preincubation –S9 +S9	Negative 7.9 revertants/ μ mol		
		SCGE	Weakly +	[138]	
		<i>his</i> reversion <i>his</i> reversion	140 revertants/400 ppm 976 revertants/24,000 ppm	[63] [63]	
	Review <i>Salmonella</i> TA100	<i>his</i> reversion Preincubation –S9, +S9	Negative	[59] [121]	
	<i>Saccharomyces cerevisiae</i>	Deletion recombination Assay	5.59 mg/mL	[284]	
	Female B6C3F1 <i>lacI</i> transgenic mice <i>Salmonella</i> RSJ100 TA98 TA100	<i>lacI</i> mutation <i>his</i> reversion –S9, +S9	Negative Negative	[285] [77]	
	Human lung epithelial cells <i>Salmonella</i> TA1535 <i>Salmonella</i> TA98, TA100, TA1535, TA1537 <i>E. coli</i> WP2uvrA/pKM101 <i>E. coli</i> WP2/pKM101	SCGE <i>his</i> reversion Plate-incorporation –S9, +S9, Glutathione suppl. S9 \pm S9, Glutathione supplemented S9 +Glutathione supplemented S9	Weakly + 19,200 and 25,600 ppm Negative Negative Negative 0.5–2%	[138] [60] [286] [286]	
	Bromochloromethane	<i>Salmonella</i> TA100	<i>his</i> reversion Preincubation –S9 +S9	[121] 75 revertants/ μ mol 424.4 revertants/ μ mol	
		<i>Salmonella</i> TA1535 (+)GST5-5	<i>his</i> reversion	0.2–1.75 mM [139]	
	Chlorodibromomethane	Review <i>Salmonella</i> TA100	<i>his</i> reversion Preincubation –S9, +S9	Negative	[61] [121]
		Human lung epithelial cells <i>Salmonella</i> RSJ100 <i>Salmonella</i> RSJ100	SCGE <i>his</i> reversion <i>his</i> reversion	Negative 1364 revertants/400 ppm	[138] [63]
			–S9 +S9	1110 revertants/800 ppm 1018 revertants/800 ppm	[283]
		<i>Salmonella</i> TA1535	<i>umuDC-lacZ</i> –S9 +S9	Positive Negative	[287]
		Bromodichloromethane	Review <i>Salmonella</i> TA100	<i>his</i> reversion Preincubation –S9, +S9	Negative
	Human lung epithelial cells <i>Salmonella</i> RSJ100 <i>Salmonella</i> TA1535		SCGE <i>his</i> reversion <i>umuDC-lacZ</i> –S9,+S9	10–1000 μ M 831 revertants/plate Negative	[138] [60,63] [287]
Iodoform	<i>Salmonella</i> BA13		Ara	7371 mut/ μ mol –S9 1782 mut/ μ mol +S9	[306]
	SHE cells	Chrom. Ab.	Negative	[307]	

in a transgenic strain of *Salmonella* (RSJ100); their rank order of mutagenic potency was bromoform > bromodichloromethane > chlorodibromomethane [60,63]. Thus, the likely absence of GSTT1-1 in most (if not all) of the studies in which these compounds were not genotoxic may account for the general negative results in the standard test systems. The dependence of these compounds on GSTT1-1 to be activated to mutagens raises important limitations regarding the standard test systems and emphasizes the need for basic research of the sort that has been applied to these brominated THMs.

DeMarini et al. [63] proposed two possible pathways of metabolism of THMs that would result in the GC → AT transitions identified as the sole class of base substitutions induced by these THMs in strain RSJ100 of *Salmonella*. The authors demonstrated that GSTT1-1 had the ability to mediate the mutagenicity of bromine-containing THMs but not chloroform. They suggested that the difference in mutational mechanisms between the brominated THMs and chloroform is likely due to initial metabolism in which the bromine is removed via nucleophilic displacement of bromine or reductive dehalogenation. Data in humans and animals indicate that chloroform is metabolized chiefly to phosgene

except at high doses [63]. Pegram et al. [60] demonstrated that brominated THMs could be activated by GST-mediated transformation into mutagenic intermediates. Also, chloroform displayed a low affinity for the same pathway, indicating that the THMs as a chemical class do not share the same mode of action.

More recently, the biotransformation and genotoxicity of ¹⁴C-bromodichloromethane were studied. These *in vitro* experiments demonstrated that GSTT1-1 catalyzed the covalent binding of bromodichloromethane to DNA and the formation of guanine adducts [64]. The cancer target tissues in the rat had greater potential formation of bromodichloromethane-derived DNA adducts compared to the rat liver due to greater flux through the GSTT1-1 pathway [64].

4.1.3. Carcinogenicity

All four of the regulated THMs are carcinogenic in rodents (Table 4) [59,61,65]. Only two have been administered in the drinking water, bromodichloromethane and chloroform, and both were negative in the mouse via this route. However, in the rat, bromodichloromethane produced liver tumors, and chloroform produced renal tumors when exposure was via the drinking water (Table 4). When administered by

Table 4
Carcinogenicity of regulated disinfection by-products in rodents based on 2-year dosing studies

Chemical (RfD)	Species	Route and dose	Tumor diagnoses	References
Trihalomethanes				
Bromodichloromethane (20 µg/(kg day))	Mouse	Drinking water: 0, 9, 18, 36 mg/(kg day)	Drinking water: no evidence of carcinogenicity	[288]
		Gavage male mice: 0,25, 50 mg/(kg day)	Gavage: male mice renal tumors 1/49, 2/50, 10/50	
	Rat	Gavage female mice: 0, 75, 150 mg/(kg day)	Gavage: female mice hepatocellular tumors 3/50, 18/48, 33/50	
		Drinking water: 8.1, 27.2, 43.4 mg/(kg day)	Drinking water: no evidence of carcinogenicity	[290]
Bromoform (20 µg/(kg day))	Rat	Drinking water: 0, 6, 12, 25 mg/(kg day)	Drinking water: no evidence of carcinogenicity	[288–290]
		Gavage: 0, 50, 100 mg/(kg day)	Gavage: male rats renal tumors 0/50, 1/50, 13/50; intestinal carcinoma 0/50, 11/50, 38/50	
		Feed: 0, 6.1, 25.5, 138 mg/(kg day)	Gavage: female rats renal tumors 0/50, 1/50, 15/50; intestinal carcinoma 0/46, 0/50, 6/47	
		Drinking water 2: 0, 8.1, 27.2, 43.4 mg/(kg day)	Drinking water 2: male rat liver tumors 2/45, 8/45, 7/48, 4/49	
		Drinking water 2: 0, 8.1, 27.2, 43.4 mg/(kg day)	Drinking water 2: male rat liver tumors 2/45, 8/45, 7/48, 4/49	
Bromoform (20 µg/(kg day))	Mouse	Gavage: 0, 50, 100 mg/(kg day)	Gavage: no evidence of carcinogenicity	[291]
	Rat	Gavage: 0, 100, 200 mg/(kg day)	Gavage: male rats intestinal tumors 0/50, 0/50, 3/50	[291]
			Gavage: female rats intestinal tumors 0/50, 1/50, 8/50	

Table 4 (Continued)

Chemical (RfD)	Species	Route and dose	Tumor diagnoses	References
Chlorodibromomethane (20 µg/(kg day))	Mouse	Gavage: 0, 50, 100 mg/(kg day)	Gavage: male mice hepatocellular tumors 23/50, 27/50 Gavage: female mice hepatocellular tumors 6/50, 10/49, 19/50	[292]
	Rat	Gavage: 0, 40, 80 mg/(kg day)	Gavage: no evidence of carcinogenicity	[292]
Chloroform (10 µg/(kg day))	Mouse	Gavage: males 0, 138, 277 mg/(kg day); females 0, 238, 477 mg/(kg day)	Gavage: male mice hepatocellular tumors 3/50, 18/50, 49/50 Gavage: female mice hepatocellular tumors 0/50, 40/50, 48/50	[6,293]
	Rat	Drinking water: 0, 34, 65, 130, 263 mg/(kg day)	Drinking water: no evidence of carcinogenicity	[6,293]
		Gavage: males 0, 90, 180 mg/(kg day); females 0, 100, 200 mg/(kg day)	Gavage: male rats renal tumors 0/50, 4/50, 12/50	
		Drinking water: 0, 19, 38, 81, 160 mg/(kg day)	Drinking water: male rat renal tumors 4/301, 4/313, 4/148, 3/48, 7/50	
		Inhalation males (0, 25, 50, 100 ppm, 6 h/day, 5 day/week) combined with drinking water (1000 ppm): total dose was 0, 73, 93, 135, mg/(kg day)	Combined exposure: renal tumors 0/50, 4/50, 4/50, 18/50	[66]
Haloacetic acids				
Chloroacetic acid (not listed on IRIS)	Mouse	Gavage: 0, 50, 100 mg/(kg day)	Gavage: no evidence of carcinogenicity	[87]
	Rat	Gavage: 0, 15, 30 mg/(kg day)	Gavage: no evidence of carcinogenicity	[86,87]
		Drinking water: 0, 3.5, 26.1, 59.9 mg/(kg day)	Drinking water: no evidence of carcinogenicity	
Bromoacetic acid (not listed on IRIS)	Mouse	No data	No data	No cancer studies performed
	Rat	No data	No data	No cancer studies performed
Dibromoacetic acid (not listed on IRIS)	Mouse	Drinking water: 0, 50, 500, 1000 mg/L	Male hepatocellular tumors 28/49, 41/50, 42/50, 47/50; male lung tumors 12/49, 12/50, 22/50, 47/50 Female hepatocellular tumors 22/49, 28/50, 37/50, 37/49	[88]
	Rat	Drinking water: 0, 50, 500, 1000 mg/L	Male mesothelioma 3/50, 1/50, 0/50, 10/50; male leukemia 17/50, 31/50, 24/50, 13/50 Female mesothelioma 11/50, 13/50, 16/50, 22/50	[88]
Dichloroacetic acid (4 µg/(kg day))	Mouse	Drinking water 52 weeks: 0, 1, 2 g/L Drinking water: 0, 8, 84, 168, 315, 429 mg/(kg day)	Drinking water 52 weeks: male mouse liver tumors 0/35, 0/11, 7/24 Drinking water: male mouse hepatocellular tumors 13/50, 11/33, 12/24, 23/32, 13/14, 8/8	[294,295]
	Rat	Drinking water: 0, 3.6, 40.2, 139.1 mg/(kg day)	Drinking water: male rat hepatocellular tumors 1/33, 0/26, 7/29, 8/28	[296]

Table 4 (Continued)

Chemical (RfD)	Species	Route and dose	Tumor diagnoses	References
Trichloroacetic acid (no RfD)	Mouse	Drinking water 52 weeks: 0, 1, 2 g/L	Drinking water 52 weeks: male mouse hepatocellular tumors 0/35, 4/11, 5/24	[294]
	Rat	Drinking water: 0, 3.6, 32.5, 363.8 mg/(kg day)	Drinking water: no evidence of carcinogenicity	[86]
Other				
Bromate (4 µg/(kg day))	Mouse	Drinking water: 0, 9.1, 42.4, 77.8 mg/(kg day)	Drinking water: mouse renal tumors 0/40, 5/38, 3/41, 1/44	[101]
	Rat	Drinking water: males 0, 12.5, 27.5; females 0, 12.5, 25.5 mg/(kg day)	Drinking water: male rat renal tumors 3/53, 32/53, 46/52; male rat mesothelioma 6/53, 17/52, 28/46; female rat renal tumors 0/47, 28/50, 39/49	[25,100, 101,297]
		Drinking water 2: 0, 0.9, 1.7, 3.3, 7.3, 16.0, 43.4 mg/(kg day)	Drinking water 2: male rat renal tumors 0/19, 0/19, 0/20, 1/24, 5/24, 5/20, 9/20; male rat thyroid follicular cell tumor 0/16, 0/19, 3/20, 4/24, 2/24, 3/20, 15/19; male rat mesothelioma 0/19, 0/20, 3/20, 4/24, 2/24, 3/20, 15/20	
Drinking water 3: 0, 1.5, 7.9, 16.9, 37.5 mg/(kg day)	Drinking water 3: male rat renal tumors 1/45, 1/43, 6/47, 3/39, 12/32; male rat thyroid follicular cell tumor 0/36, 4/39, 1/43, 4/35, 14/30; male rat mesothelioma 0/47, 4/49, 5/49, 10/47, 27/43			
Chlorite (30 µg/(kg day)) (85-week studies)	Mouse	Drinking water: 0, 0.025, 0.05%	Drinking water: no evidence of carcinogenicity	[100,298]
		Drinking water 2: 0, 250, 500 ppm	Drinking water 2: no evidence of carcinogenicity	
Chlorite (30 µg/(kg day)) (85-week study)	Rat	Drinking water: 0, 300, 600 ppm	Drinking water: no evidence of carcinogenicity	[100]

gavage, bromodichloromethane produced renal and liver tumors in the mouse, and renal and intestinal tumors in the rat. Chloroform also produced liver tumors in the mouse and renal tumors in the rat (Table 4). A combined exposure of rats to chloroform via both the drinking water and inhalation produced renal tumors [66].

The other two regulated THMs, bromoform and chlorodibromomethane, have been administered only by gavage, and both were negative in one species (bromoform in mouse and chlorodibromomethane in rat) (Table 4). However, bromoform induced intestinal tumors in the rat, and chlorodibromomethane induced liver tumors in the mouse (Table 4). All but bromoform produced liver tumors. Chloroform and bromodichloromethane also produced renal tumors, and bromodichloromethane and bromoform produced intestinal tumors. Only two of the four regulated THMs produced tumors at multiple organ sites (chloroform and bromodichloromethane), and these same two are the

only ones that are carcinogenic in both mouse and rats, i.e., are trans-species carcinogens.

With two notable exceptions, the regulated THMs did not produce urinary bladder or colorectal tumors, which are the primary tumors associated with drinking-water exposure in epidemiological studies (Section 3). The exceptions were bromodichloromethane and bromoform, which produced tumors of the large intestine in the rat, and these tumors are anatomically and functionally analogous to the colon in humans. Mechanistic studies have also shown that bromoform and bromodichloromethane induce aberrant crypt foci (ACF) primarily in the rectal segment of the colon of rats (not in mice) when administered either via drinking water or gavage [66a,66b]. A high-fat diet had no influence on the ACF frequency induced by bromodichloromethane; however, it increased by twofold the frequency of ACF induced by bromoform [66c]. A diet lacking folate significantly increased the frequency of ACF induced by bromoform relative to that of a normal

diet in rats [66d]. These studies provide an important mechanistic link to a type of cancer associated with drinking-water exposure in humans.

IARC has found bromoform [61] and chlorodibromomethane [65] to be group 3, which is not classifiable as to their human carcinogenicity. In contrast, both chloroform [59] and bromodichloromethane [61] have been classified by IARC as 2B, possibly carcinogenic to humans. The U.S. EPA's Integrated Risk Information System (IRIS) describes bromodichloromethane as B2, probable human carcinogen (<http://www.epa.gov/iris/subst/0213.htm>).

Chloroform is the only regulated THM for which there is enough evidence to develop a risk assessment based on its mode of action [67]. Numerous studies have shown that chloroform is not genotoxic and that tumors, when they arise, develop only at doses that produce significant cellular toxicity, cell death, and regenerative proliferation [68–70]. The IRIS discussion of chloroform (<http://www.epa.gov/iris/subst/0025.htm>) indicates that three different types of quantitative assessments are possible. The weight-of-evidence assessment concludes that “chloroform is likely to be carcinogenic to humans by all routes of exposure under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues. However, chloroform is not likely to be carcinogenic to humans by any route of exposure under exposure conditions that do not cause cytotoxicity and cell regeneration.”

Chloroform has induced kidney tumors in male rats and liver tumors in male and female mice only at doses that resulted in cytotoxicity. The tumors were postulated to be secondary to sustained or repeated oxidative metabolism-mediated cytotoxicity and secondary regenerative hyperplasia. This oxidative pathway can produce the electrophilic metabolite phosgene, which can lead to tissue injury and cell death by reaction with tissue proteins and cellular macromolecules as well as phospholipids, glutathione, free cysteine, histidine, methionine, and tyrosine. Persistent cell proliferation could lead to increased mutation, increased conversion of spontaneous DNA damage into mutations, and subsequent cancer. The weight of the evidence indicates that a mutagenic mode of action via DNA reactivity is not significant.

Although there is insufficient information for the other regulated THMs to develop a specific mode of action, mutational events and cellular death and regeneration may be necessary for the carcinogenicity of the brominated THMs. Recent data on the pharmacokinetics of bromodichloromethane in humans showed that the maximum blood concentrations of bromodichloromethane were 25–130 times higher from

dermal exposure compared to oral exposure [71], emphasizing the importance of route of exposure in risk assessment of the brominated THMs [64,72,73].

4.2. Haloacetic acids (HAAs)

4.2.1. Occurrence

Currently, five haloacetic acids are regulated by the U.S. EPA. The maximum contaminant level (MCL) is 60 $\mu\text{g/L}$ for the sum of bromoacetic acid, dibromoacetic acid, chloroacetic acid, dichloroacetic acid, and trichloroacetic acid. HAAs can be formed by disinfection with chlorine, chloramines, chlorine dioxide, and ozone, but they are generally formed at highest levels with chlorination [2]. Chloramines form substantially lower levels of HAAs, which is one of the reasons it has become a popular alternative disinfectant for public water systems that cannot meet the regulation with chlorination [74]. Because chlorine dioxide disinfection significantly reduces the levels of THMs and HAAs relative to chlorine, it is not generally well known that chlorine dioxide can form HAAs. However, studies have shown that chlorine dioxide can form HAAs, primarily dichloro-, bromochloro-, and dibromoacetic acid [9,23,29,74–76].

The Information Collection Rule (ICR) data revealed that water-treatment systems using chlorine dioxide had higher haloacetic acid levels for the nine bromo-chloro-HAAs than those using chlorine or chloramine only [23]. Water-treatment systems using chlorine dioxide also used chlorine or chloramines (mostly as post-disinfectants), but this is further evidence that chlorine dioxide can contribute to the formation of HAAs. Increased formation of dihaloacetic acids was also observed in a recently conducted Nationwide Occurrence Study [9,27]. Overall, the ICR found mean concentrations of the five regulated HAAs at 23 $\mu\text{g/L}$ and a 90th percentile of 47.5 $\mu\text{g/L}$ at all water-treatment systems measured [23].

Like chloramines and chlorine dioxide, ozone used in water treatment is well known for lowering the levels of THMs and HAAs formed, relative to chlorine. However, when source waters contain elevated levels of natural bromide, dibromoacetic acid has been shown to form [2,57].

4.2.2. Genotoxicity

The genotoxicity data for the HAAs are summarized in [17] and Table 5. As shown in Table 5, limited data are available for iodoacetic acid, bromoacetic acid, dibromoacetic acid, tribromoacetic acid, and chloroacetic acid. However, in general, all five were mutagenic in *Salmonella* and induced DNA damage (SCGE assay) in CHO cells in the absence of S9. Thus, these HAAs

Table 5
Comparative genotoxicity of haloacetic acid DBPs

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References
Iodoacetic acid	<i>Salmonella</i>	<i>his</i> reversion	14129 revertants/ μ mol	[11,13]
	CHO cells	SCGE	8.7 μ M (GP)	[11,13]
	CHO and TK6 cells	Chromosome aberrations	20–50 μ M	[78]
Bromoacetic acid	<i>Salmonella</i>	Fluctuation test	20–75 μ g/mL	[80]
	<i>Salmonella</i>	<i>his</i> reversion	5465 revertants/ μ mol	[77]
	<i>E. coli</i> PQ37	SOS chromotest	Negative	[80]
	<i>Pleurodeles</i> newt	Micronucleus test	Negative	[80]
	CHO cells	SCGE	17.0 μ M (GP)	[13,15,123]
Dibromoacetic acid	<i>Salmonella</i>	Fluctuation test –S9, +S9	0–750 μ g/mL; 30–3000 μ g/mL	[80]
	<i>Salmonella</i>	<i>his</i> reversion	148 revertants/ μ mol	[77]
	<i>E. coli</i> PQ37	SOS chromotest, –S9, +S9	200–750 μ g/mL; 100–3000 μ g/mL	[80]
	<i>Pleurodeles</i> newt	Micronucleus test	Negative	[80]
	CHO cells	SCGE	1.76 mM (GP)	[15]
Tribromoacetic acid	<i>Salmonella</i>	<i>his</i> reversion	Negative	[77]
	<i>Salmonella</i>	Fluctuation test –S9, +S9	2000–3000 μ g/mL; 5000–10000 μ g/mL	[80]
	<i>E. coli</i> PQ37	SOS chromotest, –S9, +S9	750–1500 μ g/mL; 100–3000 μ g/mL	[80]
	<i>Pleurodeles</i> newt	Micronucleus test	Negative	[80]
	CHO cells	SCGE	2.46 mM (GP)	[15]
Chloroacetic acid	<i>Salmonella</i>	<i>his</i> reversion	27 revertants/ μ mol	[77]
	<i>Salmonella</i>	Fluctuation test	Negative	[80]
	<i>E. coli</i> PQ37	SOS chromotest	Negative	[80]
	<i>Pleurodeles</i> newt	Micronucleus test	Negative	[80]
	CHO cells	SCGE	411 μ M (GP)	[15]
	L5178Y/ <i>Tk</i> ^{+/-} cells	<i>Tk</i> ^{+/-}	400 μ g/mL	[299]
Dichloroacetic acid	See review and text			[17]
Trichloroacetic acid	See review and text			[17]

GP: the SCGE genotoxic potency for SCGE analysis which is the concentration at the midpoint of the concentration–response curve [10–13].

were mutagenic in bacteria and induced DNA damage in mammalian cells *in vitro* [11,13,15,77]. In addition, iodoacetic acid induced chromosomal aberrations in mammalian cells *in vitro* [78].

More extensive data (15 studies) have been reported for dichloroacetic acid [17]. Although most initial studies were negative for mutagenic and genotoxic effects in bacteria and mammalian cells *in vitro*, subsequent studies in which cells were exposed to dichloroacetic acid in closed systems or to dichloroacetic acid vapors generally produced weakly positive results at high concentrations. Thus, dichloroacetic acid was weakly mutagenic in *Salmonella* TA100 \pm S9 [79,80], with a mutagenic potency of 35 revertants/ μ mol [77]. It was weakly positive with S9 activation in a prophage-induction assay in *E. coli* [79] and in the *E. coli* SOS chromotest [80]. Dichloroacetic acid did not induce micronuclei in newts, rat bone marrow, or in mouse lymphoma cells [80–82]. However, dichloroacetic acid was weakly positive for induction of micronuclei in mouse bone marrow *in vivo*, and the micronuclei were kinetochore-negative, indicating that dichloroacetic acid induced chromosome

breakage [83]. In this study, dichloroacetic acid also induced apparent DNA cross-links in peripheral blood lymphocytes based on results with the comet assay *in vivo*. At high concentrations (800 μ g/mL), dichloroacetic acid induced small-colony *Tk*^{+/-} mutants, indicative of chromosomal mutation, and it also induced chromosome aberrations in mouse lymphoma cells [81]. Dichloroacetic acid did not induce DNA damage in CHO cells [15] or in rodent liver *in vivo* [84]. In transgenic Big Blue mice, dichloroacetic acid was mutagenic at the *lacI* gene (2.3-fold increased mutation frequency relative to the control), and 33% of the mutations were at AT sites compared to only 19% of those in the controls [85]. The majority of the base substitutions (33%) were GC to AT transitions, which were also the primary class of mutations (80%), induced in *Salmonella* TA100 [79]. As discussed below, the weak genotoxicity of dichloroacetic acid, which is exhibited only at high concentrations, is not considered to play a primary role in its carcinogenicity.

Trichloroacetic acid also has been studied extensively, with more than 20 reports on the genotoxicity of

this compound [17]. Unlike dichloroacetic acid, trichloroacetic acid has given generally negative results for gene mutation in bacteria and mammalian cells and for DNA damage *in vitro*, even when tested in closed systems. Single studies have reported that trichloroacetic acid induced DNA damage (SCGE assay) or chromosomal aberrations *in vivo* [17].

Two studies evaluated six haloacetic acids for mutagenicity in *Salmonella* [77,80]. Although they produced slightly different rankings for the compounds in terms of cytotoxic and mutagenic potency, the brominated acetic acids were more toxic than their chlorinated analogues, and toxicity decreased with an increase in the number of halogen atoms per molecule. Brominated haloacetic acids also were more mutagenic than the chlorinated acids. Likewise, based on the induction of DNA damage (SCGE assay) in CHO cells, the brominated haloacetic acids were more genotoxic and cytotoxic than the chlorinated acids [15].

4.2.3. Carcinogenicity

Among the five regulated HAAs, bromoacetic acid has not been tested for carcinogenicity, and chloroacetic acid gave no evidence of carcinogenicity in rodent 2-year bioassays after either gavage or drinking-water exposure [86,87] (Table 4). The remaining three regulated HAAs (dibromoacetic acid, dichloroacetic acid, and trichloroacetic acid) have produced tumors after drinking-water exposures (Table 4), with dibromoacetic acid inducing liver tumors in male mice at an average daily dose of 50 mg/L, which is equivalent to an average daily dose of ~ 4 mg/kg [88]. All three carcinogenic HAAs produced liver tumors; in addition, dibromoacetic acid also produced leukemias and abdominal cavity mesotheliomas in rats, and liver and lung tumors in mice. A recent study has confirmed the induction of liver tumors in the mouse by trichloroacetic acid (A.B. DeAngelo, personal communication).

There is not sufficient information for the three carcinogenic regulated HAAs to develop a mode of action for their carcinogenicity. However, the weak genotoxicity of dichloroacetic acid and the lack of reproducible genotoxicity of trichloroacetic acid indicate that genotoxic mechanisms probably are not a primary mode of action for the carcinogenicity of these two regulated HAAs [17,89]. Some of the HAAs appear to produce significant metabolic disturbances which, in part, result in intrahepatic glycogen accumulation [90–92]. An assessment by IARC found dichloroacetic acid to be a possible (2B) human carcinogen; however, trichloroacetic acid was not classifiable in terms of its

carcinogenicity to humans [17]. The U.S. EPA's IRIS (<http://www.epa.gov/iris/subst/0654.htm>) describes dichloroacetic acid as B2, probable human carcinogen and considers nongenotoxic mechanisms as the basis for its carcinogenicity.

4.3. Bromate

4.3.1. Occurrence

Bromate (BrO_3^-) is produced primarily by ozone disinfection when source waters contain high levels (>50 $\mu\text{g/L}$) of natural bromide [2]. However, there are a few studies that have shown bromate formation following chlorine dioxide treatment [14], particularly when chlorine dioxide disinfection is conducted in the presence of sunlight [93]; it is also possible to have bromate contamination from the use of hypochlorite, a form of chlorine [94]. Bromate is currently regulated at 10 $\mu\text{g/L}$ in the U.S.

In the ICR, bromate was detected in ozonated drinking water at levels ranging from <0.2 to 25.1 $\mu\text{g/L}$, and in 11% of the chlorine dioxide-treated drinking-water samples, at levels ranging from below detection to 2.4 $\mu\text{g/L}$ [23]. Because none of the 28 participating chlorine dioxide water treatment plants used hypochlorite solutions for post-treatment, the bromate observed was attributed to chlorine dioxide disinfection and not to bromate contamination. In the ozonated drinking waters treated with post-hypochlorite disinfection, bromate levels increased on average by 0.84 $\mu\text{g/L}$ (from bromate contamination in the hypochlorite) over the level formed by ozone only.

4.3.2. Genotoxicity

The genotoxicity of potassium bromate has been reported in 18 studies that were reviewed by IARC [59]; additional studies have been reviewed by Moore [95] and are included in Table 6. Potassium bromate is a base-substitution mutagen that required S9 in *Salmonella* TA100; it was not a frameshift mutagen in TA98 [59]. Its ability to induce 8-hydroxydeoxyguanosine has been well documented [59], and consistent with bromate being an oxidative mutagen, it was mutagenic in strains TA102 and TA104 of *Salmonella*, which are sensitive to oxidative mutagens [96]. Such activity would indicate that potassium bromate might be a clastogen (i.e., break chromosomes), and three studies have shown that it induced chromosomal aberrations in mammalian cells *in vitro*. Potassium bromate's clastogenicity has also been confirmed *in vivo*, with five studies in mice and one with rats showing the induction of micronuclei in bone marrow [59].

Table 6
Comparative genotoxicity of bromate

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References
Potassium bromate	See reviews			[59,95]
	<i>Salmonella</i>	<i>his</i> reversion +S9	3–5 mg/plate	[96]
	V79 cells	SCGE	5–20 mM	[98]
		MCN	2.5–10 mM	
		Chromosome aberrations	5–20 mM	
		<i>Hprt</i> mutation	10–20 mM	
CHO AS52 cells	SCGE	7.2 mM (GP)	[15]	
CHO K1 cells	SCGE	2.5–10 mM	[97]	

GP: the SCGE genotoxic potency for SCGE analysis which is the concentration at the midpoint of the concentration–response curve [10–13].

Recent studies have shown that potassium bromate induced DNA damage (SCGE assay) in mammalian cells [15,97] and chromosomal aberrations and *Hprt* mutations (deletions and G to T base substitutions) in V79 cells, indicative of oxidative damage [98]. The relationship between DNA damage and 8-hydroxy-deoxyguanosine was also indicated by a modified SCGE assay in V79 cells [98]. Although potassium bromate (500 ppm in drinking water) was not mutagenic at the *gpt* locus *in vivo* in rat kidney, it was mutagenic using the *Spi*⁻ (insensitive P2 interference) selection system, which detects deletions [99]. Thus, potassium bromate is a clastogen, causing oxidative damage and chromosomal mutations at the target organ, which is the kidney (see below).

4.3.3. Carcinogenicity

Of the regulated DBPs, bromate is the most potent carcinogen in laboratory animals, inducing renal and thyroid follicular cell tumors in rats [25,100,101] and renal tumors in mice [101] (Table 4). There is a significant body of work that is highly suggestive that bromate causes DNA damage secondary to oxidative stress from intracellular bromate within the kidney cells where tumors arise [102–105]. A recent microarray study *in vivo* also supports a role for oxidative stress in bromate-induced kidney tumors [106]. Bromate produced an increased incidence of tumors after 1.5 mg/(kg day) (1500 µg/(kg day)). Under the old *Guidelines for Carcinogen Risk Assessment* (<http://www.epa.gov/iris/subst/0025.htm>), bromate was classified as B2, probable human carcinogen. According to the IRIS entry, “under the *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996) [now final U.S. EPA 2005], bromate should be evaluated as a likely human carcinogen by the oral route of exposure. Insufficient data are available to evaluate the human carcinogenic potential of bromate by the inhalation route” (IRIS,

<http://www.epa.gov/iris/subst/1002.htm#carc>). IARC considers potassium bromate as a group 2B, possible human carcinogen [59]. The U.S. EPA’s reference dose is 4 µg/(kg day) from the IRIS based on urothelial hyperplasia (<http://www.epa.gov/iris/subst/1002.htm>).

4.4. Chlorite

4.4.1. Occurrence

Chlorite is a DBP formed with chlorine dioxide treatment, and it is currently regulated at 1.0 mg/L in the United States [22,24]. Dilute solutions of chlorine dioxide are stable under low or zero oxidant-demand conditions, but when chlorine dioxide is in contact with naturally occurring organic and inorganic matter, chlorine dioxide rapidly degrades to chlorite (ClO₂⁻), chlorate (ClO₃⁻), and chloride (Cl⁻) [93,107–109]. Overall, chlorite levels can vary between 30 and 70% of the chlorine dioxide dose, depending on oxidant demand, temperature, competitive side reactions with other chemicals or processes, and generator efficiency [108,110].

Probably the richest data set on chlorite comes from the ICR, which included 28 water-treatment plants using chlorine dioxide (among the 500 large treatment plants sampled [23,111]). The median level of chlorite was 0.29 mg/L at these facilities using chlorine dioxide for disinfection. Recent measurements of chlorite included a study of full-scale treatment plants in Israel using chlorine dioxide [14] in which chlorite was found at levels up to 0.58 mg/L; a full-scale treatment plant in Virginia [112], where chlorite was found at a median level of 0.29 mg/L, following initial treatment with chlorine dioxide; full-scale treatment plants in Quebec, where chlorite was found at a maximum level of 1.1 mg/L [113]. Korn et al. [114] recently developed a model that can be used to predict chlorite formation in drinking waters treated with chlorine dioxide. This model

includes non-purgeable organic carbon (NPOC) and UV_{254} as key parameters in the prediction of chlorite and chlorate levels.

4.4.2. Genotoxicity

Few data exist on the genotoxicity of chlorite. In a study that measured differential cell killing in *E. coli* strains deficient in oxygen-scavenging enzymes, chlorite ($NaClO_2$) was highly cytotoxic, indicating the formation of active oxygen species. However, no direct measurements on the mutagenicity of chlorite were conducted [115].

4.4.3. Carcinogenicity

Chlorite showed no evidence of carcinogenicity in two studies in mice and one using rats when the animals were exposed via the drinking water; however, these were 85-week studies (Table 4). No 2-year carcinogenicity bioassays have been performed with chlorite.

5. Summary of the occurrence, genotoxicity, and carcinogenicity of the regulated DBPs

5.1. Summary of the occurrence of the regulated DBPs

In chlorinated drinking water, the THMs and HAAs are generally present at the highest levels of the DBPs measured (mid-ppb levels), with chloroform generally being the dominant THM (mean of 23 $\mu\text{g/L}$ in the ICR, with some samples above 100 $\mu\text{g/L}$) and dichloroacetic acid and trichloroacetic acid being the dominant HAAs (mean of 11 and 10 $\mu\text{g/L}$ in the ICR, respectively). The sum of the four regulated THMs (THM4) are generally present at levels higher than the sum of the five regulated HAAs (HAA5); however, the sum of the nine total chloro-bromo-HAAs (HAA9, i.e., the five regulated plus four unregulated HAAs) can be present at levels comparable to THM4. Chloramination and ozonation generally produce much lower levels of THMs and HAAs relative to chlorine, although ozonation can produce ppb levels of bromoform and dibromoacetic acid in high-bromide source waters. Chlorine dioxide can also produce HAAs (mean of 23 $\mu\text{g/L}$ in the ICR for the five regulated HAAs). With the U.S. Stage 1 and Stage 2 D/DBP regulations, the current mean levels will likely be lower in the United States than when the ICR data were collected (1997–1998). However, other countries that have higher regulatory limits or no regulatory limits for DBPs will likely have DBPs at levels higher than these means.

Bromate is formed as a DBP primarily when high-bromide source waters are treated with ozone, but it can also be formed by chlorine dioxide when the disinfection is carried out in the presence of sunlight. Bromate can also be introduced into chlorinated drinking water from an impurity in hypochlorite solutions. Chlorite is a DBP formed by chlorine dioxide treatment and is often the limiting factor in the dose of chlorine dioxide that is applied for disinfection. Chlorite levels can vary between 30 and 70% of the chlorine dioxide dose, and chlorite has the highest concentration of any regulated DBP—a median of 0.29 mg/L was observed in the ICR, but levels above 1 mg/L have been observed.

5.2. Summary of the genotoxicity of the regulated DBPs

Of the four regulated THMs, chloroform was generally not mutagenic or genotoxic. Chloroform may be considered a nongenotoxic carcinogen as discussed in the following section. Bromodichloromethane, chlorodibromomethane, and bromoform generally did not induce mutations in various organisms unless the capacity of activation by GSTT1-1 was included. This is a common enzyme in mammalian cells (including humans); thus, the capability to activate brominated THMs to mutagenic intermediates is clearly present in humans. Some studies also uncovered other genotoxic damage, including chromosomal aberrations, SCEs, and micronuclei. Data suggest that bromodichloromethane is likely a genotoxic carcinogen (see following section).

Of the five regulated HAAs, bromoacetic acid, dibromoacetic acid, and chloroacetic acid were mutagenic in bacteria and induced DNA damage in mammalian cells. Dichloroacetic acid was weakly mutagenic only at high concentrations, whereas trichloroacetic acid gave negative results. In two comparative studies, the brominated HAAs were more genotoxic than the chlorinated HAAs.

There are very few data on chlorite; however, it may form active oxygen species. Bromate is widely mutagenic and genotoxic *in vivo* and *in vitro*. Its clastogenic ability and production of oxidative damage likely play a role in its carcinogenicity.

5.3. Summary of the carcinogenicity of the regulated DBPs

As summarized in Table 7, all of the regulated DBPs, except for bromoacetic acid, have been subjected to 2-year rodent carcinogenicity bioassays. Of those tested in 2-year studies, all showed evidence of carcinogenicity

Table 7

Summary of the 2-year carcinogenicity studies of the regulated DBPs

DBP	Route of administration and target organ of tumors					
	Gavage		Drinking water		Feed (Rat)	Inhal+water (Rat)
	Mouse	Rat	Mouse	Rat		
THMs						
Bromodichloromethane	Renal, liver	Renal, intestine	Negative Negative	Liver	Negative	
Bromoform	Negative	Intestine				
Chlorodibromomethane	Liver	Negative				
Chloroform	Liver	Renal	Negative	Renal		Renal
HAAs						
Chloroacetic acid	Negative	Negative		Negative		
Bromoacetic acid						
Dibromoacetic acid			Liver, lung	Leukemia, mesothelioma		
Dichloroacetic acid			Liver	Liver		
Trichloroacetic acid			Liver	Negative		
Bromate			Renal	Renal, thyroid, mesothelioma		
Chlorite ^a			Negative	Negative		
			Negative			

^a This was an 85-week study.

except for chloroacetic acid. All four of the regulated THMs were carcinogenic in rodents; of the two that have been tested via the drinking water (bromodichloromethane and chloroform), both were negative in the mouse but carcinogenic in the rat. All four regulated THMs have been tested by gavage, but two were carcinogenic in only one species via this route (bromoform in rat and chlorodibromomethane in mouse). Chloroform produced renal tumors when rats were exposed via a combination of inhalation and drinking water.

Only two of the regulated THMs produced tumors at multiple organ sites (chloroform and bromodichloromethane), and these same two were the only ones that were carcinogenic in both mouse and rats, i.e., were trans-species carcinogens. Both have also been classified as either probable or possible human carcinogens by various organizations. Data in experimental organisms as well as humans suggest that bromodichloromethane is likely a genotoxic carcinogen, whereas chloroform may be a nongenotoxic carcinogen with the ability to induce cancer in humans only under conditions of considerable cytotoxicity and repeated cell proliferation.

The three carcinogenic HAAs (dibromoacetic acid, dichloroacetic acid, and trichloroacetic acid) and bromate have been tested only in drinking water. By this route of exposure, all three carcinogenic haloacids produced liver tumors, and dibromoacetic acid also produced lung tumors, leukemias, and abdominal cavity mesotheliomas. Bromate produced kidney and thyroid tumors as well as mesotheliomas. Two of the three

carcinogenic HAAs, dibromoacetic acid and dichloroacetic acid, were carcinogenic in both mice and rats; trichloroacetic acid was carcinogenic only in the mouse. Dichloroacetic acid is considered a possible or probable human carcinogen, and its weak genotoxicity at only high doses has suggested that genotoxicity is not the primary basis for its carcinogenicity. Trichloroacetic acid was neither genotoxic nor classifiable with regard to its carcinogenicity to humans.

Bromate is the most potent carcinogen among the regulated DBPs, it is clearly mutagenic and genotoxic *in vivo* and *in vitro*, and it is considered a possible human carcinogen by some organizations. Its ability to produce oxidative stress, resulting in damage to DNA, may play a role in its ability to induce renal tumors. Chlorite showed no evidence of carcinogenicity in two studies in mice and one in rat when administered via the drinking water for 85 weeks.

Among all of the regulated DBPs, bromodichloromethane, dichloroacetic acid, bromoacetic acid, and bromate most clearly exhibit the features of most IARC-declared human carcinogens, i.e., they are mutagenic, trans-species carcinogens. At the end of this review (Section 12), we discuss the implications of these findings for future research.

5.4. Overall summary of the regulated DBPs

Table 8 shows a qualitative summary of the occurrence, genotoxicity, and carcinogenicity (both rodent and human) of the regulated DBPs. All occur at

Table 8
Summary of occurrence, genotoxicity, and carcinogenicity of regulated DBPs

DBP	Occurrence ^a	Genotoxicity						Carcinogenicity			
		Gene mutation		Chrom. mutation		DNA damage		Rodent		Human ^b	
		Bacteria	MC ^c	<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>	Mouse	Rat	IARC	EPA
Bromodichloromethane	****	+		–		+	–	+, –, –	+, +, –	2B	B2
Bromoform	****	+	+	+	+	+	–	–	+	3	B2
Chlorodibromomethane	****	+	+	+		–		+	–	3	C
Chloroform	*****	–	–	–	–	+		+, –	+, +, +	2B	
Chloroacetic acid	***	–	+			–	–	–	–, –		
Bromoacetic acid	***	+				+					
Dibromoacetic acid	*****	+				+		+	+		
Dichloroacetic acid	*****	+	+	+	+	–	–	+	+	2B	B2
Trichloroacetic acid	*****	–	–			–	–	+	–	3	
Bromate	***	+	+	+	+	+	+	+	+	2B	B2
Chlorite	*****							–, –	–		

^a *Low-ng/L levels; **ng/L to sub- μ g/L levels; ***sub- to low- μ g/L levels; ****low- μ g/L levels; *****low- to mid- μ g/L levels; ***** high μ g/L levels.

^b IARC: 1 2B, possibly carcinogenic to humans; 3, not classifiable as to its carcinogenicity in humans. EPA: B2, probably carcinogenic to humans as evaluated using the U.S. EPA's 1986 *Guidelines for Carcinogen Risk Assessment*; C, possibly carcinogenic to humans as evaluated using the U.S. EPA's 1986 *Guidelines for Carcinogen Risk Assessment*.

^c MC, mammalian cells.

high to moderate levels, which distinguish them from many of the other DBPs evaluated in this review. However, two of the regulated DBPs (chloroacetic acid and chlorite) are not carcinogenic in two species; another (bromoacetic acid) has not been tested for carcinogenicity. Although chlorite has been tested for carcinogenicity in two species in three long-term bioassays and is not carcinogenic in any of the three studies, it has never been tested for genotoxicity. Two of the regulated DBPs are generally not genotoxic: chloroform and trichloroacetic acid, although both are carcinogenic. Almost half of the 11 DBPs have considerable data gaps for genotoxicity, and as noted, bromoacetic acid has never been tested for carcinogenicity. The most complete data sets, at least for genotoxicity and carcinogenicity, exist for bromoform, chlorodibromomethane, dichloroacetic acid, and bromate. However, even for some of these, additional mechanistic studies are needed to characterize their mode of action. Six of these DBPs, bromodichloromethane, bromate, bromoform, chloroform, and dichloroacetic acid, have been assessed as probable or possible human carcinogens by either IARC or the U.S. EPA or both.

6. Emerging unregulated DBPs

Although more than 600 DBPs have been reported in the literature, only 11 are currently regulated in the

United States. Some of the unregulated chemicals are similar to those that are regulated, such as the haloacetic acids, whereas others are unique. Most of these unregulated DBPs have been found in chlorinated drinking water, but many of them are also formed by alternative disinfectants, such as chlorate from chlorine dioxide treatment or formaldehyde from ozonation. For the most part, there are few carcinogenicity studies for unregulated DBPs. However, there is a growing database of genotoxicity data for many of these emerging unregulated DBPs, and these data are introduced in the following sections on emerging DBP classes.

6.1. Halonitromethanes

6.1.1. Occurrence

Just as there are nine possible chloro-bromo haloacetic acids that can form in drinking water, nine halonitromethanes can be formed from chlorine, chloramine, ozone-chlorine, or ozone-chloramine disinfection. Chloropicrin (trichloronitromethane) has been the most commonly measured example in this class. Recently, however, bromonitromethanes have been identified [12,28,116,117], which are a potential concern for toxicity [12]. Moreover, research indicates that the halonitromethanes may be increased in formation when pre-ozonation is used before chlorine or chloramine treatment [9,19,27,118,119].

Results of the U.S. Nationwide DBP Occurrence Study revealed a range of concentrations for individual halonitromethanes of 0.1–5 µg/L, with bromopicrin (tribromonitromethane) found at the highest levels of this class of DBP [9,27]. New laboratory-scale formation studies also indicate that nitrite may also play a role in the formation of the nitro group in these DBPs [119].

Tribromonitromethane (bromopicrin) and other trihalonitromethanes (which include bromodichloro- and chlorodibromonitromethane) require particular analytical conditions for their detection and/or identification. These compounds are thermally unstable and decompose under commonly used injection-port temperatures during gas chromatography (GC) or GC/mass spectrometry (MS) analysis [120]. The major decomposition products are haloforms (such as bromoform), which result from the abstraction of a hydrogen atom from the solvent (e.g., ethyl acetate, acetone, or methylene chloride) 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 of 170 °C and a GC/MS transfer line temperature of 225 °C should be used.

6.1.2. Genotoxicity

Recent studies on the halonitromethanes have defined the genotoxicity of this emerging class of DBPs [12,121–123] (Table 9). Nine halonitromethanes were assessed for mutagenicity in *Salmonella* TA100 with and without S9 under preincubation conditions. The mutagenic potency was calculated from the slope of the linear regression over the linear portion of the concentration–response curve. The rank order of mutagenic potency in TA100 was dibromonitromethane ≈ bromochloronitromethane > tribromonitromethane = chloronitromethane > bromonitromethane = dichloronitromethane = bromodichloronitromethane > dibromochloronitromethane ≈ trichloronitromethane. Based on these results, the halonitromethanes were classified as weak mutagens in

Table 9
Comparative genotoxicity of halonitromethane DBPs

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References
Chloronitromethane	<i>Salmonella</i>	<i>his</i> reversion +GST	1.8 revertants/nmol	[124]
	<i>Salmonella</i>	<i>his</i> reversion	1156 revertants/µmol	[122]
	CHO cells	SCGE	2.15 mM (GP)	[12]
Dichloronitromethane	<i>Salmonella</i>	<i>his</i> reversion	0.56 revertants/nmol	[124]
	<i>Salmonella</i>	<i>his</i> reversion	263.1 revertants/µmol	[122]
	CHO cells	SCGE	421 µM (GP)	[12]
Trichloronitromethane (chloropicrin)	<i>Salmonella</i>	Fluctuation test	3–10 µg/mL	[127]
	<i>Salmonella</i>	<i>his</i> reversion +GST	0.56 revertants/nmol	[124]
	<i>Salmonella</i>	<i>his</i> reversion	40.5 revertants/µmol	[122]
	<i>E. coli</i> PQ37	SOS chromotest	0.3–100 µg/mL	[127]
	<i>Pleurodeles newt</i>	Micronucleus test	Negative	[127]
Bromonitromethane	CHO cells	SCGE	93.4 µM (GP)	[12]
	<i>Salmonella</i>	<i>his</i> reversion	856.5 revertants/µmol	[122]
Dibromonitromethane	CHO cells	SCGE	136 µM (GP)	[12,123]
	<i>Salmonella</i>	<i>his</i> reversion	5571 revertants/µmol	[122]
Tribromonitromethane (bromopicrin)	CHO cells	SCGE	26.2 µM (GP)	[12,123]
	<i>Salmonella</i>	<i>his</i> reversion	1149 revertants/µmol	[122]
Bromochloronitromethane	CHO cells	SCGE	69.9 µM (GP)	[12]
	<i>Salmonella</i>	<i>his</i> reversion	1980 revertants/µmol	[122]
Dibromochloronitromethane	CHO cells	SCGE	165 µM (GP)	[12]
	<i>Salmonella</i>	<i>his</i> reversion	269.5 revertants/µmol	[122]
Bromodichloronitromethane	CHO cells	SCGE	143 µM (GP)	[12]
	<i>Salmonella</i>	<i>his</i> reversion	748 revertants/µmol	[122]
Bromodichloronitromethane	CHO cells	SCGE	63.2 µM (GP)	[12]
	<i>Salmonella</i>	<i>his</i> reversion	748 revertants/µmol	[122]

GP: the SCGE genotoxic potency for SCGE analysis which is the concentration at the midpoint of the concentration–response curve [10–13].

Salmonella [122]. The halonitromethanes induced primarily GC to AT base substitutions, which was similar to their halomethane analogues [121].

Earlier work [124] found that the chlorinated nitromethanes were mutagenic in *Salmonella* TA100 with S9 and that the addition of glutathione to the preincubation mixture did not alter the mutagenic potency of the halonitromethanes; it did reduce the cytotoxicity, which allowed for the analysis of a wider concentration range. Trichloronitromethane is metabolized to dichloronitromethane and chloronitromethane by reductive dechlorination [125,126]. Schneider et al. [124] indicated that trichloronitromethane was metabolized by GSH into mutagenic compounds, suggesting the conversion of trichloronitromethane to dichloronitromethane. Kundu et al. [122] reported the mutagenic potency for trichloro-, dichloro-, and chloronitromethane as 560, 560, and 1800 revertants/ μmol in *Salmonella* TA100. These studies in *Salmonella* indicate that as a class, the halonitromethanes are weak mutagens that are not activated by GSTT1-1, and they confirm previous observations on trichloronitromethane using a *Salmonella* fluctuation mutagenicity assay [127].

In contrast to the bacterial assays, a quantitative comparative analysis of nine halonitromethanes demonstrated them to be potent genotoxicants in mammalian cells [12]. All of the halonitromethanes directly induced genomic DNA damage in CHO cells as measured by SCGE. The rank order of genotoxic potency for the halonitromethanes was dibromonitromethane > bromodichloronitromethane > tribromonitromethane > trichloronitromethane > bromonitromethane > dibromochloronitromethane > bromochloronitromethane > dichloronitromethane > chloronitromethane. The SCGE genotoxic potencies for each halonitromethane are presented in Table 9. The SCGE genotoxic potency is the concentration at the midpoint of the SCGE concentration–response curve at low acute cytotoxicity, derived from a regression analysis. The data indicated that the halonitromethanes were potent genotoxicants, with the brominated and mixed bromo-chloro-nitromethanes being more genotoxic than the chlorinated nitromethanes. A structure–activity analysis predicted that the brominated nitromethanes and the mixed bromo-chloro-nitromethanes were more genotoxic than the chlorinated nitromethanes. Consistent with this prediction, the actual data showed that the mono-, di-, and tribrominated nitromethanes were 16 \times , 16 \times , and 1.3 \times more genotoxic than the mono-, di-, and trichlorinated nitromethanes, respectively [12].

No significant correlations were found between the rank order of these compounds for mutagenicity in

Salmonella TA100 + S9 and DNA damage (SCGE assay) in CHO cells or for cytotoxicity in either cell type [12,122]. Notably, dibromonitromethane was the most cytotoxic and mutagenic halonitromethane in both cell types. All of the halonitromethanes were genotoxic in both *Salmonella* and CHO cells, but quantitatively, there was a limited relationship between the results in the two systems.

6.1.3. Carcinogenicity

Two cancer studies have been carried out on trichloronitromethane (chloropicrin) in rats and in mice [128] (Table 10). However, these studies were inadequate because of the short survival time of the treated rats, and the results were inconclusive for carcinogenicity in mice. For rats, the doses were 20 or 25 mg/(kg day); for mice they were 33 or 66 mg/(kg day). These doses were not excessively high compared to those required for some DBPs to be carcinogenic (Table 4).

6.2. Iodo-acids and other unregulated halo-acids

6.2.1. Occurrence

Iodo-acids are a new, and potentially toxicologically significant class of DBP that was identified as part of the recent U.S. Nationwide Occurrence Study [9,11,13,27,129]. Five have been identified in finished drinking water: iodoacetic acid, bromiodoacetic acid, (*Z*)-3-bromo-3-iodopropenoic acid, (*E*)-3-bromo-3-iodopropenoic acid, and (*E*)-2-iodo-3-methylbutenedioic acid [13]. Their structures are shown in Fig. 1. They were initially discovered in chloraminated drinking-water extracts using methylation with GC/high resolution-mass spectrometry (MS), and a new occurrence study is nearing completion to determine their concentrations in chloraminated drinking water [129]. These iodo-acids are of concern not only 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. These iodo-acids have been found in chloraminated drinking waters from several cities, at maximum levels of low ppb.

Chloramination has become a popular alternative to chlorination for water-treatment systems that have difficulty meeting the regulations with chlorine, and its use is expected to increase with the advent of the Stage 2 D/DBP Rule [24]. 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

Table 10
Carcinogenicity of unregulated disinfection by-products in rodents based on 2-year dosing studies

Chemical (RfD)	Species	Route and dose	Tumor diagnoses	References	
Haloacetic acids					
Bromodichloroacetic acid ^a (not listed on IRIS)	Mouse	Drinking water: 0, 250, 500, or 1000 mg/L	No data yet	NTP ^b	
	Rat	Drinking water: 0, 250, 500, or 1000 mg/L	No data yet	NTP ^b	
Bromochloroacetic acid ^a	Mouse				
Dibromochloroacetic acid ^a	Mouse				
Other					
Acetaldehyde (not listed on IRIS)	Mouse	No data	No data	No cancer studies performed [225]	
	Rat	Inhalation: 0, 750, 1500, 3000 ppm	Inhalation: male nasal tumors 1/49, 17/52, 41/53, 37/49 Inhalation: female nasal tumors 0/50, 6/48, 34/53, 43/53		
Chloral hydrate (100 µg/(kg day))	Mouse	Drinking water 1: 0, 166 mg/(kg day)	Drinking water 1: liver tumors, 3/20, 17/24	[227,300,301]	
		Drinking water 2: 0, 13.5, 65.0, 146.6 mg/(kg day) Gavage 1: 0, 25, 50, 100 mg/(kg day) Gavage 2: 0, 25, 50, 100 mg/(kg day)	Drinking water 2: liver tumors, 27/42, 36/46, 31/39, 29/32 Gavage 1: adenoma in the pars distalis of pituitary Gavage 2: liver tumors, 11/48, 11/48, 14/48, 18/48		
	Rat	Drinking water 1: 0, 15, 45, 135 mg/(kg day) Drinking water 2: 0, 7.4, 37.4, 162.6 mg/(kg day)	Drinking water 1: negative Drinking water 2: negative		[300,302]
Chlorate (not listed on IRIS)	Mouse	Drinking water: male 0, 40, 80, 160; female 0, 30, 60, 120 mg/(kg day)	Drinking water: no evidence of carcinogenicity	[229]	
	Rat	Drinking water male: 0, 5, 35, 75; female 0, 5, 45, 95 mg/(kg day)	Drinking water: male rat thyroid follicular cell tumors 1/47, 0/44, 0/43, 6/47; female rat thyroid follicular cell tumors 1/47, 0/47, 1/43, 4/46	[229]	
Chloropicrin (not listed on IRIS)	Mouse	Gavage: males 0, 66; females 0, 33 mg/(kg day)	Inadequate study	[128]	
	Rat	Gavage: males 0, 25, 26; females 0, 20, 22 mg/(kg day) modified exposures	Inadequate study	[128]	
Dibromoacetonitrile (not listed on IRIS)	Mouse	Drinking water: 0, 50 100, 200 mg/L	No data yet	NTP ^b	
	Rat	Drinking water: 0, 50 100, 200 mg/L	No data yet	NTP ^b	
Formaldehyde (200 µg/(kg day))	Review			[208]	
	Mouse	Inhalation: 0, 2, 5.6, 14.3 ppm	Inhalation: male mice nasal tumors 0/68, 0/67, 0/71, 2/72 (2/17 at 24 months) Inhalation: female mice, no evidence of carcinogenicity	[221]	
	Rat	Inhalation: 0, 2, 5.6, 14.3 ppm Drinking water 1: males 0, 1.2, 15, 82 mg/(kg day); females 0, 1.8, 21, 109 mg/(kg day)	Inhalation: male rats nasal squamous cell carcinoma 0/118, 0/118, 1/119, 51/117; female rats nasal squamous cell carcinoma 0/114, 0/118, 1/116, 52/115 Drinking water 1: no evidence of carcinogenicity	[221,222,303]	

Table 10 (Continued)

Chemical (RfD)	Species	Route and dose	Tumor diagnoses	References
		Drinking water 2: 0, 10, 50, 100, 500, 1000, 1500 mg/L	Drinking water 2: male rat testicular interstitial cell tumors 6/50, 6/50, 12/50, 12/50, 20/50, 24/50, 18/50; male rat lymphoma/leukemia combined 20/50, 8/50, 20/50, 26/50, 24/50, 22/50, 46/50 Drinking water 2: female rat no evidence of carcinogenicity compared to vehicle control	
Chloroacetaldehyde	Mouse	Drinking water: 17 mg/(kg day)	Male mouse liver carcinoma or adenoma 3/20, 10/26	[227]
3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) (not listed on IRIS)	Mouse	No data	No data	No cancer studies performed
	Rat	Drinking water male rats: 0, 0.4, 1.3, 5.0 mg/(kg day) Drinking water female rats: 0, 0.6, 1.9, 6.6 mg/(kg day)	Drinking water: male hepatocellular tumors 0/50, 1/50, 3/50, 5/50; male biliary tumors 0/50, 1/50, 1/50, 4/50; male adrenal cortical tumors 5/50, 2/50, 7/50, 14/50; male thyroid gland follicular tumors 2/49, 20/50, 38/50, 44/49; male pulmonary tumors 3/50, 1/50, 1/50, 7/50 Drinking water: female mammary gland tumors 3/50, 2/50, 7/50, 12/50; female hepatocellular tumors 2/50, 2/50, 4/50, 10/50; female biliary tumors 1/50, 4/50, 10/50, 34/50; female adrenal cortical tumors 5/50, 10/50, 12/50, 16/50; thyroid follicular tumors 5/50, 18/49, 38/50, 47/50; lymphoma and leukemia 1/50, 1/50, 2/50, 4/50	[154]
Nitrosamines				See reviews [189,203]

Studies are currently underway at the U.S. National Toxicology Program; see <http://ntp.niehs.nih.gov/index.cfm?objectid=071A51D3-F87E-D33F-530336DDD0ADCF98>.

^a A.B. DeAngelo, Carcinogenic in mouse, in preparation, personal communication.

^b Studies are currently underway at the U.S. National Toxicology Program; see <http://ntp.niehs.nih.gov/index.cfm?objectid=071A3D02-D098-1D7B-AA6056974321C23A>.

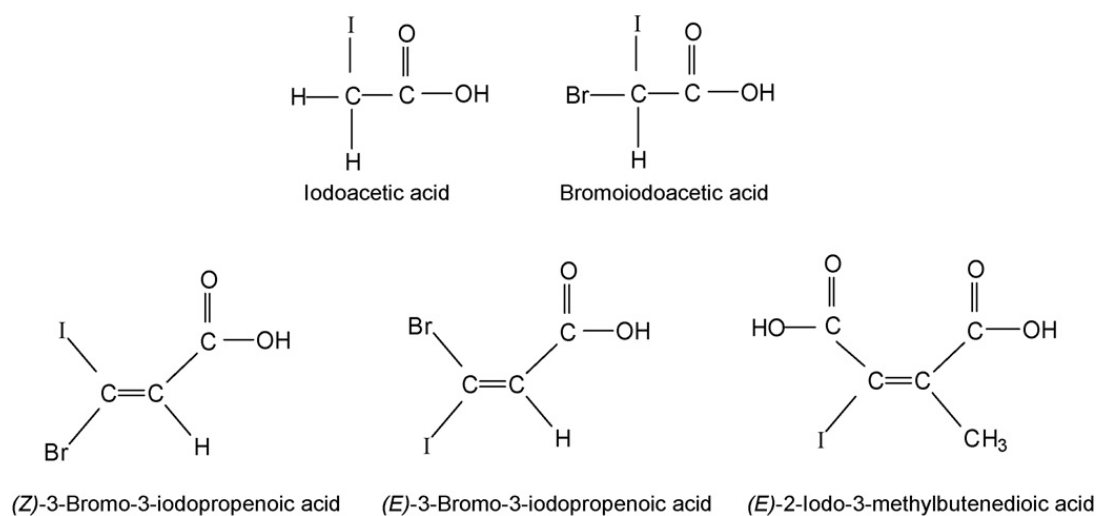


Fig. 1. Structures of iodo-acids.

[13]. Because of chlorine's competing reaction to form iodate as a sink for the natural iodide, it is likely that treatment with significant free chlorine contact time before the addition of ammonia will not produce substantial levels of iodo-acids or iodo-THMs [13,130,131]. 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.

There are four bromo-chloro-HAAs that are not currently regulated in the United States, bromochloroacetic acid, bromodichloroacetic acid, dibromochloroacetic acid, and tribromoacetic acid. They can hardly be considered "emerging" because many laboratories have been measuring them routinely as part of the nine total bromo-chloro-HAAs. A recent study by Singer and colleagues [132] makes the case that measuring all nine bromo-chloro HAAs is important because measuring only the five regulated ones can significantly underestimate the total exposure, especially for water systems that contain appreciable levels of bromide in their source waters. The additional four unregulated HAAs are bromine-containing species that can be found at increased levels in drinking waters that have high bromide in their source waters, and their concentrations can be similar to the five regulated HAAs. Also, because bromine-containing DBPs are generally more toxic than chlorine-containing DBPs, knowing their concentrations can be important.

Two-carbon HAAs (longer chain acids) can also be formed in drinking water, mostly with chlorine and chloramine. One of these, 3,3-dichloropropenoic acid, was included in the priority DBPs measured in the U.S. Nationwide Occurrence Study [9,27]. It was found at a maximum of 4.7 $\mu\text{g/L}$ and was present in all of the water-treatment plants studied. The corresponding brominated acid, 3,3-dibromopropenoic acid, has also been identified as a DBP in drinking water, as well as several other 3-carbon, 4-carbon, and 5-carbon acids and di-acids [9]. Two of the more unusual bromo-acids include the bromo-oxo-acids 3,3-dibromo-4-oxopentanoic acid and 3-bromo-3-chloro-4-oxopentanoic acid [9]. So far, there are no quantitative data on these other brominated acids, but preliminary toxicity data indicate that they may be toxicologically important [133].

6.2.2. Genotoxicity

Iodoacetic acid is a potent mutagen in *Salmonella* TA100, inducing 14,129 revertants/ μmol [13]. Under preincubation conditions, iodoacetic acid was more mutagenic in TA100 than bromoacetic acid or

chloroacetic acid ($2.6\times$ and $523.3\times$, respectively). Iodoacetic acid was $2.0\times$ more genotoxic than bromoacetic acid and $47.2\times$ more genotoxic than chloroacetic acid in CHO cells. In a comparison of the chronic cytotoxicity and acute genotoxicity in CHO cells, the rank order of toxic potency was iodoacetic acid > bromoacetic acid > chloroacetic acid [13]. These results in *Salmonella* and CHO cells were correlated with the estimated ability of the HAA to cross cell membranes. The toxicity results were correlated with their log P values and to a lesser extent with the pKa values. The monohaloacetic acids express an S_N2 -type alkylating function. Their relative reactivity is related primarily to the carbon-halogen bond dissociation energy. The CHO cell genotoxicity was correlated with the lowest unoccupied molecular orbital (E_{LUMO}) of the monohaloacetic acids [13]. The impact of the halogens as a leaving group was highly correlated to the toxicity of these HAAs. In addition, for iodoacetic acid the induction of genotoxicity in *Salmonella* and CHO cells was via an oxidative stress mechanism [11].

Tribromoacetic acid was negative in one study in *Salmonella* [77] but was mutagenic in both *Salmonella* and *E. coli* at high concentrations in another study [80]. It was negative in a newt micronucleus assay [80] and was a relatively weak inducer of DNA damage (SCGE assay) in CHO cells, with a genotoxic potency of 2.46 mM [15].

6.2.3. Carcinogenicity

No carcinogenicity studies were available for the iodo-acids.

6.3. Iodo-THMs and other unregulated halomethanes

6.3.1. Occurrence

Iodinated THMs have been identified as DBPs in chlorinated drinking water [9,27,29,130,134–136] and in chloraminated drinking water [9,27] in several locations, with reports as early as 1975 [134]; however, they are not widely measured and are not regulated. Iodo-THMs identified and measured included dichloriodomethane, bromochloriodomethane, dibromiodomethane, chlorodiodomethane, bromodiodomethane, and iodoform. Previous studies of iodo-THMs were conducted mainly because of taste and odor problems; there is a low threshold for detection of medicinal tastes and odors in drinking water (as low as 0.02–5 $\mu\text{g/L}$) [136].

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 analogues and that iodine is expected to be more biologically reactive than bromine or chlorine. Mammalian cell cytotoxicity and genotoxicity data for iodoacetic acid mentioned earlier [11,13,129] 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 [9].

Iodo-THMs can form in drinking water treated with chlorine or chloramines when natural iodide is present in the source waters, and they have been found as DBPs in drinking water in many countries. Levels reported are generally sub- $\mu\text{g/L}$; however, levels of iodo-THMs were consistently at $\mu\text{g/L}$ and as high as 15 $\mu\text{g/L}$ at one location in the Nationwide Occurrence Study that used chloramines for primary disinfection. The total iodo-THMs were 81% of the total of the four regulated THMs in one sampling from this location [9,27]. In the nationwide study, dichloriodomethane was the most common of the iodo-THMs 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 [130] 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; addition of increased amounts of chlorine 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 [27], iodo-THMs were observed after ozonation and chloramination. This research suggested that when a lower ratio of ozone to natural organic matter was used in the Nationwide Occurrence Study, compared to that used in laboratory-scale tests [130], that there was less conversion of iodide to iodate.

Other unregulated halomethanes have also been identified, including bromochloromethane and dibromomethane [2]. These were included in the U.S. Nationwide Occurrence Study as priority DBPs [9,27]; however, there was only one instance where one of these was detected in this study. Dibromomethane was detected once in a simulated distribution system sample at 0.13 $\mu\text{g/L}$. This detection was likely due to the lower

detection limit (0.11 $\mu\text{g/L}$) during this period of the study; other sampling events had higher detection limits of 0.5 $\mu\text{g/L}$. It may be that these two halomethanes can be formed in drinking water, but at levels lower than 0.1 $\mu\text{g/L}$.

6.3.2. Genotoxicity

Iodo-THMs have been predicted to cause cancer based on quantitative structure–activity relationships [137], but until now, there have been no toxicity studies conducted. This is likely due to the lack of commercial availability of the pure standards (until recently, only iodoform could be purchased commercially). There are current efforts underway to investigate the mammalian cell genotoxicity and cytotoxicity of the iodo-THMs. Preliminary data indicate that iodoform is highly cytotoxic but not genotoxic to mammalian cells [133]. Iodoform is mutagenic in bacteria [306] but does not induce chromosome aberrations in SHE cells *in vitro* [307].

There are also some genotoxicity data available on three unregulated bromochloromethanes (dichloromethane, bromochloromethane, and dibromomethane) (Table 3). Dichloromethane was mutagenic in *Salmonella* TA100 using a pre-incubation assay with S9 activation, resulting in a mutagenic potency of 7.9 revertants/ μmol ; no mutagenicity was observed without S9 activation [121]. In *Salmonella* RSJ100 (a strain transfected with *GSTT1-1*), at a dose of 400 ppm, dichloromethane was mutagenic, causing 140 revertants/plate. TA100 exposed to a dose of 24,000 ppm resulted in 976 revertants/plate. Dichloromethane also produced two different mutation spectra, suggesting several different mutagenic pathways [63]. Dichloromethane (100–1000 μM) was a weak inducer of DNA damage (SCGE assay) in primary human lung epithelial cells [138].

Bromochloromethane was mutagenic in *Salmonella* TA100 using a pre-incubation assay with and without S9 activation, resulting in mutagenic potencies of 424.4 and 75 revertants/ μmol , respectively [121]; it was also mutagenic in TA1535 (+GST5-5) [139], indicating activation by GSTT1-1.

Dibromomethane was mutagenic in *Salmonella* TA100 using a pre-incubation assay with and without S9 activation, resulting in mutagenic potencies of 551 and 279 revertants/ μmol respectively [121]. Dibromomethane induced primarily GC to TA base substitutions in TA100 [121]. Liu et al. [140] employed a strain expressing human *O*⁶-alkylguanine-DNA alkyltransferase (AGT) with the goal of increasing the toxicity and mutagenicity of dibromomethane. In *E. coli* TRG8

(expressing AGT), dibromomethane was mutagenic in a *his* reversion assay at concentrations up to 0.1 mM. AGT was a target for the binding of dibromomethane due to the reaction of a Cys¹⁴⁵ residue. When this reaction occurs, AGT is inactivated, which can impair the cell's ability to repair an alkylation site. The resulting AGT-Cys¹⁴⁵S-CH₂Br complex can react with guanine residues in DNA, causing mutations and reducing survival [140].

Bromoform, chloroform, iodoform, bromochloroiodomethane, bromodichloromethane, dibromochloro-

methane, and dibromoiodomethane were not genotoxic in CHO cells. However, the iodo-THMs were the most cytotoxic of the group. The rank order of their CHO cell chronic cytotoxicity was iodoform > dibromoiodomethane > bromochloroiodomethane > bromoform > chlorodibromomethane > chloroform > bromodichloromethane [133].

6.3.3. Carcinogenicity

No 2-year carcinogenicity studies were available for this class of DBPs.

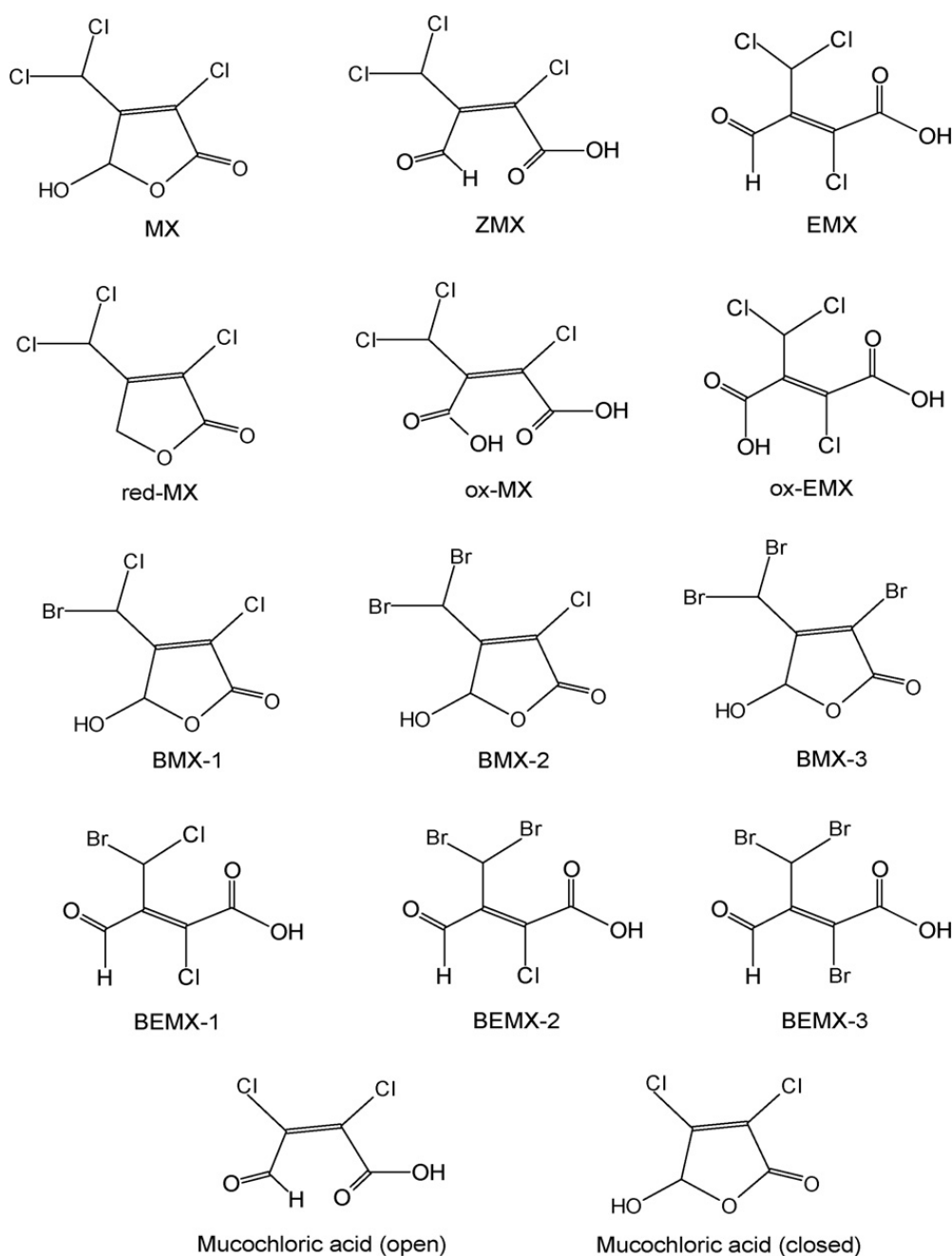


Fig. 2. Structures of MX analogues.

6.4. MX and BMX compounds (halofuranones)

6.4.1. Occurrence

3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) was originally identified as a chlorination by-product in pulp mill effluent [141]; subsequently, it was found in chlorinated drinking water. MX has both an open and closed form; the ring-opened, oxo-butenic acid form is present at the pH of drinking water (ZMX, Fig. 2). Other analogues of MX have also been identified in chlorinated drinking water, including its geometric isomer (EMX) [142,143], oxidized and reduced forms of MX (ox-MX and red-MX), as well as brominated analogues (the so-called BMXs) [144] and mucochloric acid, which has an open and closed form like MX [9,27]. Structures of several of these analogues are shown in Fig. 2. Results of bacterial mutagenicity tests were the initial cause of concern for MX because it was found to be a potent mutagen in the *Salmonella* mutagenicity assay, accounting for as much as 20–50% of the total bacterial mutagenicity in some tests of chlorinated drinking-water extracts [143].

In the few early drinking-water occurrence studies, concentrations of MX were generally 60 ng/L or lower. However, in 2002, Wright et al. reported MX levels as high as 80 ng/L in drinking waters from Massachusetts [145]. Later, 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 ng/L and as high as 850 ng/L) in finished drinking waters across the United States [9,27]. The highest levels of total halogenated furanones occurred in a water system that disinfected with chlorine–chloramines (2380 ng/L in drinking-water treatment-plant effluent) and at a treatment plant that disinfected with chlorine dioxide–chlorine–chloramines (1020 ng/L in the distribution system). In drinking-water treatment-plant effluents, a maximum level of 310 ng/L was observed for MX; maximum levels of brominated MX analogues included 720 and 810 ng/L for BEMX-1 and BEMX-2, respectively. MX levels reached a high of 850 ng/L in the average distribution system sample from a chlorine dioxide–chlorine–chloramine treatment plant.

The halogenated furanones are 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 finished water leaving the water treatment plant 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 [9,27].

6.4.2. Genotoxicity

The halofuranones, especially MX, have been the most extensively studied group of unregulated DBPs for their genotoxicity (Table 11). MX has two open-ring tautomeric forms that exist at physiological pH: (Z)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (ZMX) and (E)-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX). All are potent genotoxicants in a wide variety of genetic assays and endpoints *in vitro*. Recently, comprehensive reviews on the genotoxicity and carcinogenicity of MX and some related halofuranone DBPs were published by McDonald and Komulainen [146] and IARC [17]. We have not included the more than 80 papers on the genotoxicity of MX reviewed in these two reports; instead we have noted in Table 11 the few papers published since these reviews as well as papers on MX-related furanones. The halofuranones are direct-acting genotoxicants. MX directly generates abasic sites and DNA strand breaks. In *E. coli*, MX induces DNA damage, forward mutation, reverse mutation, and prophage λ induction. MX was mutagenic in frameshift and base-substitution strains of *Salmonella*. MX is among the most potent direct-acting mutagens (and DBPs) in *Salmonella* TA100, producing ~4700 revertants/nmol (~22 revertants/ng) [37], and under preincubation conditions adjusted for cytotoxicity, the mutagenic potency was 981 revertants/nmole in TA100 and 116 revertants/nmole in TA98 [77].

As reviewed [17,146], MX is also a potent genotoxicant in mammalian cells, inducing unscheduled DNA synthesis, SCEs, micronuclei, chromosome aberrations, forward mutation at several loci, and DNA strand breaks. Although the results were generally negative for micronucleus induction *in vivo* in rodents, MX consistently induced DNA strand breaks/DNA damage and SCEs *in vivo* [17]. The departure between the positive mammalian cell assays and the *in vivo* assays is associated with treatment conditions. Under single, acute exposure regimens, MX was generally not positive in test animals, but it was generally positive when animals were exposed repeatedly to MX [17,146]. Thus, the *in vivo* results appeared to be minimized if the animals had an opportunity to repair the MX-induced DNA damage [146].

Of special interest is the generation of bromine-substituted furanones (BMXs) and their mutagenic potencies compared to MX in *Salmonella* TA100. LaLonde and his colleagues synthesized and determined the mutagenic potency (revertants/nmol in strain

Table 11
Comparative genotoxicity of MX-related compounds

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References
MX	See reviews			[17,146]
	<i>gpt</i> delta reporter gene transgenic mice	6-TG and Spi selection	Negative	[148]
	Wistar rat	SCGE		[149]
		Blood	Negative	
	Liver	Negative		
	Brain		19 µg/mL continuous in water	
3-Chloro-4-(bromochloromethyl)-5-hydroxy-2(5H)-furanone (BMX-1)	<i>Salmonella</i> TA100	<i>his</i> reversion	35 revertants/ng	[144]
3-Chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-2)	<i>Salmonella</i> TA100	<i>his</i> reversion	38 revertants/ng	[144]
	<i>Salmonella</i> TA100	<i>his</i> reversion	5470 revertants/nmol	[147]
3-Bromo-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-3)	<i>Salmonella</i> TA100	<i>his</i> reversion	27 revertants/ng	[144]
	<i>Salmonella</i> TA100	<i>his</i> reversion	2880 revertants/nmol	[147]
3-Bromo-4-(bromomethyl)-5-hydroxy-2(5H)-furanone (BMBF)	<i>Salmonella</i> TA100	<i>his</i> reversion	420 revertants/nmol	[147]
3-Bromo-4-(dibromomethyl)-2(5H)-furanone	<i>Salmonella</i> TA100	<i>his</i> reversion	129 revertants/nmol	[147]
3-Chloro-4-(dibromomethyl)-2(5H)-furanone	<i>Salmonella</i> TA100	<i>his</i> reversion	181 revertants/nmol	[147]
3-Bromo-4-(bromomethyl)-2(5H)-furanone	<i>Salmonella</i> TA100	<i>his</i> reversion	8.2 revertants/nmol	[147]
3-Bromo-4-(chloromethyl)-2(5H)-furanone	<i>Salmonella</i> TA100	<i>his</i> reversion	3.9 revertants/nmol	[147]
3-Chloro-4-(bromomethyl)-2(5H)-furanone	<i>Salmonella</i> TA100	<i>his</i> reversion	3.9 revertants/nmol	[147]
2,3-Dichloro-5-hydroxy-2(5H)-furanone (mucochloric acid)	<i>Salmonella</i> TA100	<i>his</i> reversion	2 µg/plate	[150]
	<i>Salmonella</i> TA100	<i>his</i> reversion	Positive	[151]
	CHO/Hprt	Gene mutation	23.7 µM	[152]
	<i>Salmonella</i> TA1535	<i>his</i> reversion	2.5 nmol/plate	
	<i>E. coli</i> DNA repair in mouse-mediated assay	DNA repair	10 µg/mL <i>in vitro</i>	[153]
			200 mg/kg <i>in vivo</i>	

TA100) of 12 bromine-, chlorine-, and mixed halogen-substituted 4-methyl-2(5H)-furanones [147]. The mutagenic potencies of the bromine-substituted furanones are presented in Table 11. The most mutagenic compounds were the trihalo-, followed by the dihalo-4-methyl-5-hydroxy-2(5H)-furanones, irrespective of bromine or chlorine substitutions. Trihalides and dihalides lacking the C-5 hydroxyl group expressed lower mutagenic potencies. The bromine-substituted furanones were substantially less mutagenic than MX, except for 3-chloro-4-(dibromomethyl)-5-hydroxy-2(5H)-furanone (BMX-2), which induced a 140% increase in mutagenicity compared to MX [147].

Since the publication of the MX reviews [17,146], a few additional papers were published on the genotoxicity of this class of DBP. In a 12-week study, MX was given to *gpt* delta mice at concentrations of 10, 30, or 100 ppm. No induction of mutation in the reporter gene

(*gpt*) was detected nor were deletions >1 kb observed using a phage-insensitive selection system (Spi⁻). However, MX inhibited gap junctions in rat WB cells [148]. To compare the possible interaction of genotoxic damage by MX and radiofrequency electromagnetic fields, Wistar rats were chronically exposed to 19 µg/mL in the drinking water and to whole body absorption rates of 0.3 W/kg or 0.9 W/kg of 900 MHz electromagnetic radiation. No genotoxic interaction between MX and the radiofrequency fields was observed. MX induced DNA strand breaks in brain cells of the treated rats but failed to cause damage in blood and liver cells [149].

Another MX-related DBP, (Z)-2,3-dichloro-4-oxobutenoic acid (mucochloric acid, Fig. 2), was also mutagenic in *Salmonella*, inducing primarily GC to AT base substitutions, which is different from the spectrum of mutations induced by MX, which is primarily GC to

TA [37,150,151]. Mucochloric acid also induced mutations at the *Hprt* locus in CHO cells [152] as well as induced DNA damage in indicator *E. coli* K-12 cells injected into mice who were then exposed to the compound [153].

6.4.3. Carcinogenicity

Most unregulated DBPs that result in rodent tumors induce them at doses of 75 mg/(kg day) or greater. MX is the exception; it has been shown to induce rat tumors in multiple organs at doses as low as 0.4 mg/(kg day) [154]. MX was classified as possibly carcinogenic to humans (group 2B) by IARC [17]. MX induced tumors in multiple organs in male and female Wistar rats receiving MX for 104 weeks in the drinking water [154] (Table 10). In the male, MX induced increases in thyroid gland follicular cell adenomas and carcinomas at all doses (Table 10), and the combined incidence of the adenomas and carcinomas reached 90% [154]. Combined liver adenoma and carcinoma were also increased, as were adrenal gland cortical adenoma in the high-dose group only. Dose-related effects were seen for lung and liver adenoma, combined liver adenoma and carcinoma, liver cholangioma, pancreas Langerhans' cell adenoma as well as adenoma and carcinoma combined, adrenal gland cortical adenoma, and thyroid follicular cell adenoma and carcinoma—separately and combined.

In the male, MX induced thyroid gland follicular cell adenoma and carcinoma—as well as the two combined, as well as liver cholangioma and cholangioma combined with carcinoma. The combined incidence of thyroid gland follicular cell adenoma and carcinoma reached 94%. MX also induced mammary gland adenocarcinoma, fibroadenoma, and combined adenoma and adenocarcinoma. It also induced liver adenoma and adenoma and carcinoma combined. MX induced adrenal gland cortical adenoma in the high-dose group only. Dose-related increases were found for

mammary gland adenocarcinoma, fibroadenoma, and combined adenoma and adenocarcinoma. Dose-related increases were also found for lymphoma and leukemia combined, liver adenoma, liver adenoma and carcinoma combined, liver cholangioma, liver cholangioma and cholangiocarcinoma, adrenal gland cortical adenoma, thyroid follicular cell adenoma, thyroid follicular cell carcinoma, and thyroid follicular cell adenoma and carcinoma combined. A portion of this study was repeated, and similar organ-site carcinogenicity was found [155].

The mechanistic features of MX, as well as related effects beyond carcinogenicity and genotoxicity, have been reviewed [146]. In addition to its well-characterized genotoxicity, recent data have confirmed earlier studies indicating that MX has promoter activity [148,156] and induces oxidative stress [157]. MX also has been shown to be an indicator of the ability of drinking water to induce chromosomal aberrations and mammalian cell transformation [158].

McDonald and Komulainen [146] calculated a mean cancer potency estimate for MX using either a linearized multistage model or a benchmark dose model, and similar results were obtained by either model, which was 2.3 per mg/(kg day) and an upper 95 percentile estimate of 4.5 per mg/(kg day). As shown next, this value was then used to calculate a population cancer risk. Assuming an MX concentration of 310 ng/L, which is clearly present in some distribution systems (see Section 6.4.1), and consumption of 2 L of tap water/day, the authors show that this would correspond to a daily intake of 9×10^{-6} mg/(kg day) for a 70-kg person. Multiplying the mean and upper-bound cancer potency estimates for MX, 3.2–4.5 (mg/(kg day)), gives a lifetime cancer risk estimate of 40 excess cases per million people exposed. Using an attributable-risk approach, the authors showed that MX may produce 700 excess cases per million people exposed [146].

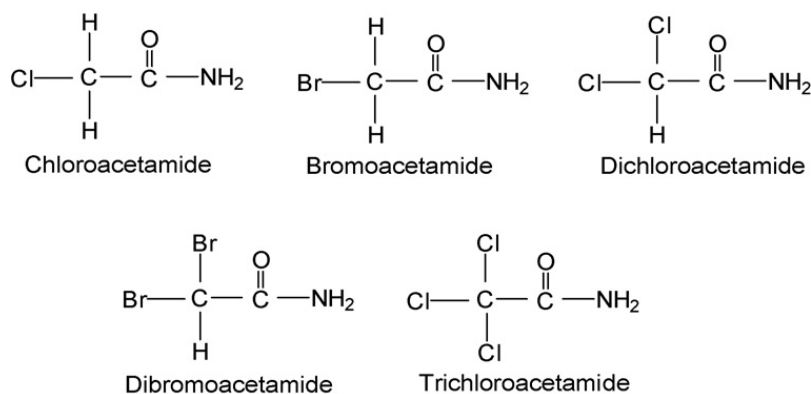


Fig. 3. Structures of haloamides.

6.5. Haloamides

6.5.1. Occurrence

Haloamides have only recently been identified as drinking-water DBPs, and they were first quantified as part of the U.S. Nationwide DBP Occurrence Study (Fig. 3). Chloro-, bromo-, dichloro-, dibromo-, and trichloroacetamide were found in finished drinking waters from several U.S. States in this study, with individual concentrations ranging up to 9.4 µg/L in a simulated distribution system sample and up to 7.6 µg/L in an actual distribution system sample from one of the drinking-water systems [9,27]. They were found in drinking waters treated with chlorine or chloramines. There is some preliminary indication that haloamides may be increased with chloramination, and new studies are underway to investigate this.

6.5.2. Genotoxicity

Limited research has been conducted on the genotoxicity of haloamides (Table 12). In a recent comprehensive CHO cell toxicity study and analysis of structure–activity relationship (SAR), Plewa et al. [159] analyzed CHO cell cytotoxicity and genotoxicity, and the mechanisms of reactivity were correlated for 13 haloacetamides. These chemicals are alkylating agents that react with protein thiols and induce a multitude of biological responses, including apoptosis and necrosis [160]. The rank order for CHO cell cytotoxicity was diiodoacetamide > iodoacetamide > bromoacetamide > tribromoacetamide > bromoiodoacetamide > dibromochloroacetamide > chloroiodoacetamide > bromodichloroacetamide > dibromoacetamide > bromochloroacetamide > chloroaceta-

mid > dichloroacetamide > trichloroacetamide. The rank order of their genotoxicity in CHO cells was tribromoacetamide > diiodoacetamide ≈ iodoacetamide > bromoacetamide > dibromochloroacetamide > bromoiodoacetamide > bromodichloroacetamide > chloroiodoacetamide > bromochloroacetamide > dibromoacetamide > chloroacetamide > trichloroacetamide. Dichloroacetamide was not genotoxic. A new iodinated DBP, bromoiodoacetamide, was also identified in finished drinking waters [159].

The SAR analysis indicated that the haloacetamides participate in several electrophilic reactive processes: (i) S_N2-mediated alkylation for the monohaloacetamides involving the displacement of a halogen atom at the α carbon; (ii) the potential generation of reactive α-halothioether electrophilic intermediates by cellular glutathione GSH or –SH compounds for the dihaloacetamides; (iii) for the trihaloacetamides, nucleophilic attack at the electrophilic carbonyl carbon to yield trihalomethyl carbanions. Log *P* increased with the degree of halogenation and with the size of the halogen.

The order of CHO cell toxicity for the monohaloacetamides (cytotoxicity and genotoxicity) was I > Br > > Cl. The rank order and relative activity of the monohaloacetamides were related to their S_N2 reactivity with increasing bond length and decreasing dissociation energy. The leaving tendency of the halogen in alkyl halides followed the order I > Br > > Cl. CHO cell cytotoxicity of iodoacetamide was 1.3 × greater than bromoacetamide, which was 78 × greater than chloroacetamide. Iodoacetamide induced more genomic DNA damage than bromoacetamide, which was 38 × more

Table 12
Comparative genotoxicity of haloacetamide DBPs

Chemical	Biosystem	Genetic endpoint	SCGE genotoxic potency ^a	References
Iodoacetamide	CHO cells	SCGE	34.1 µM	[159]
Diiodoacetamide	CHO cells	SCGE	33.9 µM	[159]
Bromoiodoacetamide	CHO cells	SCGE	72.1 µM	[159]
Chloroiodoacetamide	CHO cells	SCGE	302.0 µM	[159]
Bromoacetamide	CHO cells	SCGE	36.8 µM	[159]
Dibromoacetamide	CHO cells	SCGE	744.0 µM	[159]
Tribromoacetamide	CHO cells	SCGE	32.5 µM	[159]
Bromochloroacetamide	CHO cells	SCGE	583.0 µM	[159]
Dibromochloroacetamide	CHO cells	SCGE	69.4 µM	[159]
Bromodichloroacetamide	CHO cells	SCGE	146.0 µM	[159]
Chloroacetamide	CHO cells	SCGE	1.38 mM	[159]
Dichloroacetamide	CHO cells	SCGE	Negative	[159]
Trichloroacetamide	CHO cells	SCGE	6.54 mM	[159]

^a GP: the SCGE genotoxic potency for SCGE analysis which is the concentration at the midpoint of the concentration–response curve [10–13].

potent than chloroacetamide. Log *P* did not appear to play a major role in the toxicity of the monohaloacetamides.

For dihaloacetamides the cytotoxic rank order was $I_2 > IBr > ICl > Br_2 > BrCl > Cl_2$, and the genotoxicity rank order was $I_2 > IBr > ICl > BrCl > Br_2$; Cl_2 was inactive. For these compounds, the relative leaving tendencies of the halogen was correlated with toxicity; the agents containing the most iodo group(s) expressed the greatest combined cytotoxicity and genotoxicity indices, followed by bromo group(s) and chloro group(s) (Table 12). These results are difficult to explain by S_N2 reactivity alone but may be the result of metabolic activation by intracellular GSH or $-SH$ compounds, which displace one halogen and form highly reactive electrophilic intermediates. The key element of this reaction is the presence of at least one halogen with good leaving tendency. Log *P* may be more important in the activity of dihaloacetamides by affecting cellular uptake. The estimated log *P* values followed the order $I_2 > IBr > ICl > Br_2 > BrCl > Cl_2$, which is identical to their cytotoxicity and genotoxicity.

For the trihaloacetamides, the cytotoxic and genotoxic potencies ranked $Br_3 > Br_2Cl > BrCl_2 > Cl_3$ (Table 12). Cytotoxicity and genotoxicity decreased with a reduction in the number of bromo groups. The cytotoxicity of trichloroacetamide was lower than tribromoacetamide by almost three orders of magnitude [159]; this confirmed results in human leukemia P388 cells [161]. Only one bromo group was required for potent cytotoxicity. In contrast, the decrease in genotoxic potency with a decrease in the number of bromo groups was more gradual.

The CHO cell genotoxicity of the trihaloacetamides could be partially explained by electrophilic reactivity at the carbonyl carbon, as well as the possible release of electrophilic dihalocarbene intermediates. Alternatively, it is possible that reductive dehalogenation may yield genotoxic free radicals; this pathway and the metabolic competency of the CHO cells have been defined only partially [162]. GSTT1-1 catalyzed preferential activation of brominated trihalomethanes to genotoxic intermediates [72,163]; the possible role of GSTT1-1 in the activation of trihaloacetamides in CHO cells remains to be explored.

6.5.3. Carcinogenicity

No carcinogenicity studies have been reported for the haloamides.

6.6. Haloacetamides

6.6.1. Occurrence

Although they are not regulated in the United States, haloacetamides (HANs) have been measured in several occurrence studies [23,27,164,165]. Chloro-, bromochloro-, dibromo-, and trichloroacetamide (HAN4) are the most commonly measured HAN species and have been included in a survey of 35 U.S. water utilities [165], a survey of 53 Canadian water utilities [164], and the U.S. EPA's ICR effort [23]. In the ICR, HANs ranged from non-detectable ($<0.5 \mu\text{g/L}$) to $41.0 \mu\text{g/L}$ and were generally present at 12% of the levels of the four regulated trihalomethanes. These HANs were formed by treatment with chlorine, chloramine, chlorine dioxide, or ozone disinfection; plants using chloramines (with or without chlorine) had the highest levels in their finished drinking water.

Higher HAN levels were also observed in distribution-system waters treated with post-chloramination vs. free chlorine. However, the increased HAN levels with chloramination may be a result of the higher total organic carbon (TOC) levels in their source waters [23]. HANs were frequently found in drinking waters from the Canadian survey, with dichloroacetamide found in 97% of all samples [164]. The World Health Organization (WHO) has published drinking-water guidelines for two of the haloacetamides: a guideline of $70 \mu\text{g/L}$ for dibromoacetamide and a provisional guideline of $20 \mu\text{g/L}$ for dichloroacetamide [166].

Several other haloacetamides, including a number of brominated species, were also measured in a recent nationwide DBP occurrence study [9,27]. These included chloro-, bromo-, bromodichloro-, dibromochloro-, and tribromoacetamide, in addition to the four ICR haloacetamides listed above. Total HAN levels reached a maximum of $14 \mu\text{g/L}$ in this study, and they were approximately 10% of the levels of the four regulated THMs combined, although a maximum of 25% was observed. When higher bromide levels were present in the source waters, more brominated HAN species were formed. This shift in speciation was observed in another study of high-bromide waters in Israel [14], which also provided evidence that chlorine dioxide disinfection can form HANs (dibromoacetamide), as well as a new bromoacetamide species (3-bromopropanoacetamide).

6.6.2. Genotoxicity

The genotoxicities of most of the haloacetamides have been reviewed [61], and more recent studies are included in Table 13. Other than iodoacetamide, all of

Table 13
Comparative genotoxicity of haloacetonitrile DBPs

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References
Chloroacetonitrile	See review			[61]
	<i>Salmonella</i>	Fluctuation test	0.13–1.3 mM	[304]
	HeLa S3 cells	SCGE	10–1000 μ M	[304]
	CHO cells	SCGE	601 μ M (GP)	[10]
Dichloroacetonitrile	See review			[61]
	<i>S. typhimurium</i>	Fluctuation test	0.91–9.1 mM	[304]
	HeLa S3 cells	SCGE	1–10,000 μ M	[304]
	CHO cells	SCGE	2.75 mM (GP)	[10]
Trichloroacetonitrile	See review			[61]
	<i>Salmonella</i>	Fluctuation test	70 μ M	[304]
	HeLa S3 cells	SCGE	0.1–10 μ M	[304]
	CHO cells	SCGE	1.01 mM (GP)	[10]
Bromoacetonitrile	<i>Salmonella</i>	Fluctuation test	Negative	[304]
	<i>Salmonella</i>	Fluctuation test	6–10 μ g/mL	[167]
	<i>E. coli</i> PQ37	SOS chromotest	Negative	[167]
	<i>Salmonella</i>	<i>his</i> reversion	Negative	[305]
	<i>Pleurodeles</i> newt	Micronucleus test	0.125–0.25 μ g/mL	[167]
	HeLa S3 cells	SCGE	0.01–100 μ M	[304]
	CHO cells	SCGE	38.5 μ M (GP)	[10]
Dibromoacetonitrile	See review			[61]
	<i>Salmonella</i>	Fluctuation test	Negative	[304]
	HeLa S3 cells	SCGE	0.01–100 μ M	[304]
	CHO cells	SCGE	47.1 μ M (GP)	[10]
Bromochloroacetonitrile	See review			[61]
	CHO cells	SCGE	324 μ M (GP)	[10]
Iodoacetonitrile	CHO cells	SCGE	37.1 μ M (GP)	[10]

GP: the SCGE genotoxic potency for SCGE analysis which is the concentration at the midpoint of the concentration–response curve [10–13].

the haloacetonitriles in Table 13 have been tested in *Salmonella* for gene mutation, in mammalian cells for SCEs and DNA damage (SCGE assay), and for micronucleus induction in newt erythrocytes or mouse bone marrow. Brominated acetonitriles are generally not mutagenic in *Salmonella*, whereas the chlorinated analogues are positive in the presence or absence of S9. All of the compounds in Table 13 induced DNA damage in mammalian cells. Seven haloacetonitriles were recently evaluated using microplate-based CHO cell assays for chronic cytotoxicity and acute genotoxicity [10]. The cytotoxic potencies ranged from 2.8 μ M for dibromoacetonitrile to 0.16 mM for trichloroacetonitrile, with a descending rank order of dibromoacetonitrile > iodoacetonitrile \approx bromoacetonitrile > bromochloroacetonitrile > dichloroacetonitrile > chloroacetonitrile > trichloroacetonitrile. For acute genomic DNA damage, the SCGE genotoxicity potency ranged from 37 μ M iodoacetonitrile to 2.7 mM dichloroacetonitrile. The rank order of declining genotoxicity was iodoacetonitrile > bromoacetonitrile \approx dibromoacetonitrile

> bromochloroacetonitrile > chloroacetonitrile > trichloroacetonitrile > dichloroacetonitrile.

Haloacetonitriles have two potential electrophilic reactive centers: (i) displacement of a halogen atom at the α carbon by S_N2 reaction and (ii) addition at the partially positively charged carbon of the cyano group [167]. The S_N2 reactivity of the HANs is dependent on the leaving tendency of the halogen and the degree of halogenation. The leaving tendency of a halogen should decrease with increasing halogenation; therefore, the alkylating potential of the HANs should decrease with increasing halogenation. Both the alkylating potential [168] and interaction with calf thymus DNA [169] were consistent with an S_N2 mechanism. The relative order of the SCGE genotoxic potency for the haloacetonitriles was in agreement with the S_N2 SAR expectation. The higher activity of trichloroacetonitrile versus dichloroacetonitrile indicated that nucleophilic addition at the cyano carbon could also contribute to the genotoxicity.

The cytotoxic and genotoxic consequence of the interaction of haloacetonitriles with cellular macromolecules

may be affected by the presence of GSH compounds. GSH conjugation may detoxify the mono- and trihaloacetonitriles because of the elimination of reactive electrophiles. The toxicity and genotoxicity of these compounds may not be expressed until the cellular GSH pool is depleted. For dihaloacetonitriles, GSH conjugation is detoxifying only if both halogens are displaced. If only one halogen is displaced, GSH conjugation can become an activation pathway because the resulting intermediate (an α -halothioether) is a highly reactive electrophile [170]. As a class, the haloacetonitriles are highly reactive, causing DNA damage in mammalian cells *in vitro*; however, they show limited ability to induce gene mutations in bacteria and give unclear results for micronucleus induction *in vivo*.

6.6.3. Carcinogenicity

No carcinogenicity studies have been reported on the haloacetonitriles.

6.7. Tribromopyrrole

6.7.1. Occurrence

In 2003, a new halogenated pyrrole, 2,3,5-tribromopyrrole (Fig. 4), was identified in drinking water [14]. This represents the first time that a halogenated pyrrole had been observed as a drinking-water by-product for any disinfectant. This halopyrrole was found in finished drinking water (at approximately 50 ng/L) 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 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 drinking water from Israel. 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 and HAAs were formed mostly from fulvic acid.

It is interesting to note that a soil humic model [171] based on ^{13}C NMR, pyrolysis, and oxidative degradation data, included 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 compared to that in the fulvic acid. The finding of 2,3,5-tribromopyrrole at significant levels when only 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.

6.7.2. Genotoxicity

Tribromopyrrole is highly cytotoxic in CHO cells, with a cytotoxic potency of 61 μM , which is approximately 8 \times more cytotoxic than dibromoacetic acid (a regulated DBP). Tribromopyrrole was also a potent inducer of DNA damage in CHO cells, with a SCGE genotoxic potency of 299 μM , which is approximately the same genotoxic potency as MX [14].

6.7.3. Carcinogenicity

No carcinogenicity studies have been reported on tribromopyrrole.

6.8. Nitrosodimethylamine (NDMA) and other nitrosamines

6.8.1. Occurrence

U.S. EPA considers nitrosodimethylamine (NDMA) to be a probable (B2) human carcinogen (<http://www.epa.gov/iris/subst/0045.htm>) under its 1986 *Guidelines for Carcinogen Risk Assessment*. Until recently, concerns about NDMA stemmed primarily from its presence in food, beverages, consumer products, contaminated groundwater (from the use of

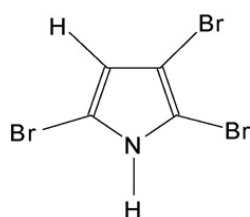


Fig. 4. Structure of 2,3,5-tribromopyrrole.

rocket fuel), and polluted air (e.g., tobacco smoke) [172]. Although it has become evident that NDMA is also a drinking-water DBP [173–177], a recent assessment indicates that oral ingestion of drinking water is likely a minor source of NDMA exposure relative to that from food [178]. NDMA in drinking water has been found primarily in chloraminated drinking water, where the nitrogen in monochloramine (NH_2Cl) is incorporated into the structure of the NDMA by-product formed [179]. Chlorination can also result in 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 [172,180].

NDMA was discovered initially in chlorinated drinking waters from Ontario, Canada [173], and it has been found recently in other locations and in laboratory studies [179,181,182]. The observation of NDMA in U.S. waters is due largely to improved analytical techniques that have allowed its determination at low nanogram per liter (parts per trillion) concentrations. Recent measurements have shown it is generally present at low ng/L levels in chloraminated/chlorinated drinking water, and it can be formed at much greater concentration in wastewater treated with chlorine. Following its discovery in California well water, the State of California issued an action level of 2 ng/L (2 parts per trillion) for NDMA, which was subsequently revised to 10 ng/L due to the analytical difficulty in measuring it at the level proposed originally. The California Department of Health Services has a website that provides further background and details about NDMA (<http://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, but it has been included (along with other nitrosamines) in proposed Unregulated Contaminants Monitoring Rule (UCMR-2; <http://www.epa.gov/ogwdw/ucmr/ucmr2/index.html>). Canada does not regulate NDMA nationally, but Ontario has established a drinking-water quality standard of 9 ng/L for NDMA (<http://www.ene.gov.on.ca/envision/gp/4449e.pdf>).

Mitch et al. published a review in 2003 that discussed 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 [172]. This review article also discussed analytical methods used for the analysis of NDMA and the sources and occurrence of NDMA.

New research is expanding beyond NDMA to other nitrosamines as potential DBPs. In fact, a new EPA method [183] 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) (http://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 as low as 1.2 ng/L.

Important new discoveries of nitrosamines beyond NDMA include ones by Charrois et al. [184] and Zhao et al. [185]. These new nitrosamine DBPs are shown in Fig. 5. Charrois et al. discovered *N*-nitrosopyrrolidine and *N*-nitrosomorpholine in finished drinking water

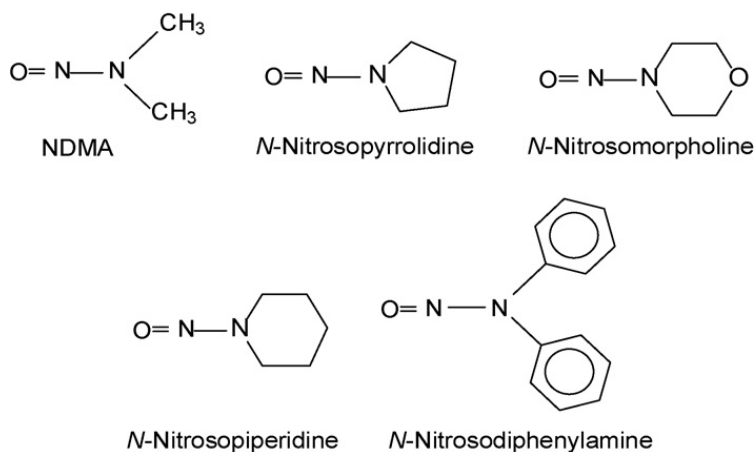


Fig. 5. Structures of NDMA and other nitrosamine DBPs.

(both at the plant and in the distribution system) from two cities in Canada that use chloramination for treatment [184]. This is the first report of nitrosamines other than NDMA in drinking water. Levels of *N*-nitrosopyrrolidine ranged from 2 to 4 ng/L, and *N*-nitrosomorpholine was found in drinking water from one city at 1 ng/L. NDMA was also found in drinking water from these cities and ranged from 2 to 180 ng/L. The 180 ng/L level found for NDMA, which was measured in the distribution system of one city, is the highest concentration that has been observed for NDMA in drinking water to date. 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 compared to the drinking-water treatment plant (e.g., from an initial 67 ng/L of NDMA at the plant to 180 ng/L in the distribution system). This study suggests that previous measurements of NDMA at the treatment plant may substantially underestimate the public's exposure to this DBP.

Zhao et al. [185] recently reported the discovery of *N*-nitrosopiperidine and *N*-nitrosodiphenylamine in finished drinking water from Canada that was treated with a combination of chloramination and UV irradiation. *N*-nitrosodiphenylamine is thermally labile (will decompose with commonly used GC/MS methods), so the researchers developed a liquid chromatography (LC)/MS/MS method to enable its measurement, along with 8 other nitrosamines. NDMA and *N*-nitrosopyrrolidine were also found in drinking-water samples collected, and as in the Charrois et al. study, the nitrosamines were found to increase in concentration in the distribution system. Maximum levels of 108, 71, 118, and 2 ng/L were observed for NDMA, *N*-nitrosopyrrolidine, *N*-nitrosopiperidine, and *N*-nitrosodiphenylamine, respectively.

In another important study, Wilczak et al. [180] 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 reaction of the diallyldimethylammonium chloride (DADMAC) polymer with chlorine and chloramines in pure water; pilot treatment-plant studies were carried out by using the DADMAC polymer in a pilot plant that utilized chlorine, chloramines, and ozone, and their combinations; 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 depended strongly 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 h 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 [186] recently investigated precursors of NDMA in natural waters. Samples from lakes, reservoirs, groundwaters, and isolated natural organic matter were reacted with monochloramine. A compound that had been suggested previously 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 by drinking-water treatment. As a result, nitrogen-containing coagulants (like DADMAC mentioned above) may also be significant precursors. Unplanned wastewater reuse was also suggested as a source of NDMA because 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 [181] measured NDMA after extended chloramination in advanced wastewater-treatment plants and in reactions of model precursors. 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.

6.8.2. Genotoxicity

Increased public health concerns were expressed after the discovery that nitrosamines were generated as DBPs [184]. Currently, five *N*-nitrosamines have been defined as DBPs: *N*-nitrosodimethylamine, *N*-nitrosopyrrolidine and *N*-nitrosomorpholine [184] and the recently discovered *N*-nitrosodiphenylamine and *N*-nitrosopiperidine [185] (Table 14).

Table 14
Comparative genotoxicity of *N*-nitrosamine DBPs

Chemical	Biosystem	Genetic endpoint	Concentration range of positive response or highest genotoxic potency	References
<i>N</i> -Nitrosodimethylamine (NDMA)	See reviews			[188,189]
	<i>Salmonella</i> YG7108-CYP2E1	<i>his</i> reversion	0.553 revertants/nmol	[190]
<i>N</i> -Nitrosopyrrolidine	<i>Salmonella</i> YG7108-CYP1A1	<i>his</i> reversion	0.12 revertants/nmol	[191]
	YG7108-CYP1A2		0.016 revertants/nmol	
	YG7108-CYP1B1		0.0085 revertants/nmol	
	YG7108-CYP2A6		4.15 revertants/nmol	
	YG7108-CYP2C8		0.0133 revertants/nmol	
	YG7108-CYP2C19		0.0761 revertants/nmol	
	YG7108-CYP2E1		1.49 revertants/nmol	
	YG7108-CYP3A4		0.0027 revertants/nmol	
	<i>Drosophila</i> <i>gpt</i> delta rats	Wing spot test <i>gpt</i> mutation	80 μ mol/vial Ten-fold increase in mutant frequency at 200 ppm in drinking water	[192] [194]
	<i>N</i> -Nitrosomorpholine	<i>Salmonella</i> YG7108-CYP1A1	<i>his</i> reversion	0.0376 revertants/nmol
YG7108-CYP1B1			0.03 revertants/nmol	
YG7108-CYP2A6			0.457 revertants/nmol	
YG7108-CYP2E1			0.0963 revertants/nmol	
YG7108-CYP3A4			0.0071 revertants/nmol	
Caco-2 cells		DNA strand breaks SCGE	0.92–5.1 mmol/L	[195]
V79/+S9 cells		6-TG resistance	15–20 mmol/L	[196]
Wistar rats		UDS	200 mg/kg	[198]
SD, F344 rats		Bone marrow and blood micronucleus	180 mg/kg	[197]
<i>Drosophila</i>		Wing spot test	7 μ mol/vial	[192]
<i>N</i> -Nitrosodiphenylamine	See review			[201]
<i>N</i> -Nitrosopiperidine	<i>Salmonella</i> YG7108-CYP2A6	<i>his</i> reversion	0.537 revertants/nmol	[191]
	YG7108-CYP2E1		0.029 revertants/nmol	
	<i>Drosophila</i>	Wing spot test	9 μ mol/vial	[192]
	Human T-lymphocytes +S9	SCGE DNA strand breaks	0.5–20 mM	[199]

As a class, *N*-nitrosamines are well-known environmental toxins that can be metabolized into potent genotoxic agents. Given the extensive number of studies of the genotoxicity of the *N*-nitrosamines, it is beyond the scope of this review to present this literature in detail. Instead, we present a summary of a model compound of this class and highlight the main features of the genotoxicity of the remaining compounds.

N-nitrosodimethylamine (NDMA) is a model compound of this class, and its genotoxicity has been reviewed extensively [187–189]. A summary of the *in vitro* and *in vivo* genotoxicity of NDMA derived from an Agency for Toxic Substances and Disease Registry in a report by the Office of Environmental Health Assess-

ment, California Environmental Protection Agency is presented in Table 15 [187]. The results show that NDMA is genotoxic in a wide array of systems *in vitro* in the presence of S9 and *in vivo*. It induces gene and chromosomal mutations as well as DNA damage *in vivo* and *in vitro*. Recent studies have shown that NDMA is activated to a mutagen by various P450 enzymes in strains of *Salmonella* containing human P450 genes [190].

The genotoxicity literature is less extensive for the remaining compounds (Table 14); however, like NDMA, *N*-nitrosopyrrolidine is mutagenic in *Salmonella* in the presence of S9, and it also has been evaluated using strains of *Salmonella* containing

Table 15
Summary of the genotoxicity of *N*-nitrosodimethylamine (NDMA)^a

Endpoint	Biosystem	Result	
		–S9	+S9
<i>In vitro</i>			
DNA damage	Rat hepatocytes	+	
DNA fragmentation	Rat hepatocytes	+	
DNA repair	Rat hepatocytes	+	+
	Human lymphoblasts		+
	Mouse hepatocytes	+	
	Hamster hepatocytes	+	
	Rat pancreatic cells	–	
Gene mutation	<i>Salmonella</i>	+	+
	<i>E. coli</i>		+
	<i>S. cerevisiae</i>		+
	Chinese hamster V79 cells, Chinese hamster ovary cells	–	+
	Mouse lymphoma L5178Y cells	–	+
Chrom. aberrations	Chinese hamster lung cells	+	+
	Rat ascites hepatoma and rat esophageal tumor cells	+	
SCE	Rat esophageal tumor cells, rat ascites hepatoma	+	
	Human lymphocytes	–	+
	Human fibroblasts		+
	Chinese hamster ovary cells	–	+
	Chinese hamster V79 cells	–	+
	Chinese hamster primary lung cells	–	+
<i>In vivo</i>			
DNA methylation	Rat, mouse, hamster, gerbil liver		+
	Human liver		+
DNA fragmentation	Rat liver and kidney gel elution		+
	Mouse liver and kidney gel elution		+
DNA repair	Fetal mouse kidney and liver		+
	Mouse testes		+
	Rat liver		+
	Rat respiratory cells		+
	Rat spermatocytes		–
Germ-cell mutations	<i>Drosophila</i>		+
Chrom. aberrations	Hamster embryo fibroblasts		+
Micronucleus	Rat bone marrow		+/–
	Rat hepatocytes		+
	Mouse bone marrow		+
	Hamster embryonic fibroblasts		+
SCE	Chinese hamster bone marrow		+/–
	Rat bone marrow		+
Sperm abnormalities	Mouse		–

^a Data from ATSDR (1989) and adapted from CA EPA [187].

plasmids expressing various P450 genes. Depending on the P450 gene being expressed, mutagenic responses ranged from 0.0027 revertants/(nmol pmol) CYP in a strain expressing CYP3A4 to 4.15 revertants/(nmol pmol) in a strain expressing CYP2A6. Compared to NDMA, *N*-nitrosopyrrolidine was more responsive to

human cytochrome P450s. Although NDMA was metabolized only by CYP2E1 [190], *N*-nitrosopyrrolidine was metabolized by several cytochromes: CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2C8, CYP2C19, CYP2E1, and CYP3A4 [191]. In a direct comparison using a strain expressing CYP2E1, *N*-nitrosopyrrolidine

was $2.7\times$ more mutagenic than NDMA. However, NDMA was $160\times$ more mutagenic than *N*-nitrosopyrrolidine in the *Drosophila* wing spot somatic mutation assay [192]. An early study in *Salmonella* TA104 showed that *N*-nitrosopyrrolidine induced base substitutions primarily at AT base pairs [193]. Consistent with those findings, *N*-nitrosopyrrolidine induced primarily A to G base substitutions in the liver of *gpt* delta transgenic rats [194]. This study provides support for a genotoxic mechanism for liver cancer induced by this compound (see Section 12.1).

An early review of the genotoxicity of *N*-nitrosomorpholine [189] showed that it was a potent mutagen and clastogen, inducing micronuclei *in vivo* and chromosomal aberrations *in vitro*, as well as gene mutations in *Salmonella* and mammalian cells. Studies in *Salmonella* expressing various P450 genes showed that *N*-nitrosomorpholine exhibited mutagenic potencies ranging from 0.0071 revertants/(nmol pmol) CYP for strain YG7108-CYP3A4 to 0.457 revertants/(nmol pmol) CYP with strain YG7108-CYP2A6 [191]. Using strain YG7108-CYP2E1, it was only about 20% as mutagenic as NDMA [191]. In *in vitro* mammalian cell assays, *N*-nitrosomorpholine induced DNA strand breaks as measured by SCGE [195] and gene mutations [196]. *In vivo*, *N*-nitrosomorpholine induced somatic-cell mutations in *Drosophila* [192], micronuclei in rats [197], and unscheduled DNA synthesis [198] in rats.

N-nitrosopiperidine was mutagenic in *Salmonella* expressing various P450 genes (0.029 revertants/(nmol pmol) CYP for strain YG7108-CYP2E1 and 0.537 revertants/(nmol pmol) CYP with strain YG7108-CYP2A6) [191]. In the presence of S9, *N*-nitrosopiperidine induced DNA strand breaks in human lymphocytes [199] and was mutagenic in *Drosophila* [192]. The relationship between stereochemical features of the nitrosopiperidines and the mutagenicity of these compounds has been studied in *Salmonella* [200].

The exception to the nitrosamines described above is *N*-nitrosodiphenylamine. An extensive review of the genetic toxicology of this compound [201] showed that, unlike the other nitrosamines, *N*-nitrosodiphenylamine is generally not mutagenic in bacteria or mammalian cells and does not induce chromosomal aberrations or SCEs in mammalian cells. It is also not clastogenic *in vivo*.

An important study of the potential role of *N*-nitrosamines in drinking water on genotoxicity in humans was conducted by van Maanen et al., [202]. They found that subjects consuming well water containing 25 mg of nitrate/L had higher *HPRT* mutant frequencies in their peripheral lymphocytes than did

those consuming tap water containing 0.2 or 17.5 mg of nitrate/L. *N*-nitrosopyrrolidine was also found in the urine of 18/22 subjects. This study indicated that drinking water with high nitrate levels may pose a mutagenic risk to humans via the endogenous formation of carcinogenic *N*-nitroso compounds from nitrate-derived nitrite.

6.8.3. Carcinogenicity

Many nitrosamines have been tested extensively for carcinogenicity, and nearly all have shown carcinogenic effects in a variety of species exposed through various routes [189,203]. It is beyond the scope of this review to describe in detail the carcinogenicity studies of the *N*-nitrosoamines mentioned above; instead, we have noted the general highlights of the carcinogenicity of the DBPs in this chemical class.

The primary sites of tumor formation for the nitrosamines are the esophagus and liver; however other organs, including the urinary bladder, brain, and lungs, are also targets [189,203]. The optimal conditions for nitrosamine carcinogenicity involve low-dose exposure over long periods of time [203]. A mixture study in which low doses of *N*-nitrosodiethylamine (0.1 mg/(kg day)), *N*-nitrosopyrrolidine (0.4 mg/(kg day)), and *N*-nitrosodiphenylamine (2.0 mg/(kg day)) were given in drinking water to rats for their lifetime showed additivity for liver tumors [204].

An especially important “mega-study” evaluated liver and esophageal tumors induced by *N*-nitrosodiethylamine or *N*-nitrosodimethylamine in 4080 rats at 16 doses given in the drinking water during the lifetime of the animals [205]. The results showed that exposures to concentrations as low as 0.01 ppm in the drinking-water resulted in 25% of the animals developing liver tumors, with no indication of a threshold effect.

IRIS identifies the following nitrosoamines as probable human carcinogens under the 1986 Cancer Guidelines: *N*-nitrosopyrrolidine (<http://www.epa.gov/iris/subst/0081.htm#noncar>), *N*-nitrosodiphenylamine (<http://www.epa.gov/iris/subst/0178.htm#noncar>), *N*-nitrosodiethylamine, and NDMA (<http://www.epa.gov/iris/subst/0045.htm>). IARC [189] found sufficient evidence in animals for the carcinogenicity of NDMA, *N*-nitrosopyrrolidine, *N*-nitrosomorpholine, and *N*-nitrosopiperidine. Although no epidemiologic data were available at the time, IARC [189] noted that these compounds should be regarded as if they were carcinogenic to humans. A review [201] indicated that, unlike the other nitrosamines above, there was limited evidence for the carcinogenicity of *N*-nitrosodiphenylamine in experimental animals. The IRIS database [26]

also provides an estimate of carcinogenic potency for NDMA; the slope factor is 51 mg/(kg day). Using standard default exposure assumptions, this indicates that 7 ng/L in drinking water represents a 10^{-5} lifetime cancer risk for the average adult.

6.9. Aldehydes

6.9.1. Occurrence

Several aldehydes were measured in the ICR effort, including formaldehyde, acetaldehyde, glyoxal, methyl glyoxal, and trichloroacetaldehyde (chloral hydrate). The non-halogenated aldehydes are DBPs produced primarily by ozone treatment [2,206], although both chlorine and chlorine dioxide treatment can also form low ppb levels of formaldehyde [2,23,29]. In the ICR, these aldehydes were detected at higher concentrations in water-treatment systems using ozone (up to 30.6 $\mu\text{g/L}$) than chlorine dioxide. Among treatment systems using ozone, the 90th percentile concentration for formaldehyde was 13.7 $\mu\text{g/L}$. Formaldehyde was detected at more than 50% of the treatment plants using chlorine dioxide at a mean of 5.3 $\mu\text{g/L}$ and 90th percentile of 9.0 $\mu\text{g/L}$ [23]. Acetaldehyde, glyoxal, and methyl glyoxal were observed at maximum levels of 11,

16, and 6 $\mu\text{g/L}$, respectively, in ozonated drinking water, but were generally below the detection limit ($<5 \mu\text{g/L}$) in chlorine dioxide-treated waters. Chloral hydrate is primarily a chlorine or chloramine DBP, but the use of preozonation prior to chlorination or chloramination can increase its formation [9,27,207]. In the ICR, chloral hydrate was found to be at higher levels in the distribution system (median 2.8 $\mu\text{g/L}$; 90th percentile 11.0 $\mu\text{g/L}$) than in the finished water (median 1.7 $\mu\text{g/L}$; 90th percentile 7.4 $\mu\text{g/L}$) [23].

Other haloaldehydes have been measured in a few studies, and chloro-, dichloro-, bromochloro-, and tribromoacetaldehyde were included in the Nationwide Occurrence Study as priority DBPs [9,27]. In this study, the haloaldehydes were the third largest DBP class by weight (behind THMs and HAAs) of all the DBPs studied. Dichloroacetaldehyde was the most abundant of these haloaldehydes and was found at a maximum concentration of 16 $\mu\text{g/L}$. Ozonation followed by post-chloramination was found to increase the formation of haloaldehydes.

6.9.2. Genotoxicity

The genotoxicity of formaldehyde, which has been reported in >100 studies published during the past 77

Table 16
Summary of genotoxicity of aldehyde DBPs

Chemical [Ref.]	Endpoint	System	Result
Formaldehyde [208]	Gene mutation	Bacteria, fungi, mammalian cells, rat nasal <i>in vivo</i>	+
	SCE	Mammalian cells	+
	MN	Mammalian cells/rodents	+/w+
	CA	Mammalian cells/rodents	+/w+
	DNA damage	Bacteria, mammalian cells	+
	Germ-cell mutation	<i>Drosophila</i>	+
		Rodents	w+
Acetaldehyde [61]	Gene mutation	Bacteria	–
		Mammalian cells	+
	SCE	Mammalian cells, rodents	+
	MN	Mammalian cells	+
	CA	Mammalian cells	+
	Aneuploidy	Fungi	+
	DNA–protein links	Rat nasal tissue	+
Chloral hydrate [17]	Gene mutation	Bacteria, mammalian cells, <i>Drosophila</i>	+
	MN	Mammalian cells, rodents	+
	CA	Mammalian cells	+
	Aneuploidy	Mammalian cells, mouse, fungi	+
	DNA damage	Rodents	+
	Cell transformation	SHE cells	+
	DNA–protein links	Rodents	+
Chloroacetaldehyde	<i>his</i> reversion [308]	<i>Salmonella</i> TA100	+
	<i>HPRT</i> mutation [309]	H2E1 cells	+
	SSCE [133]	CHO cells	–
	<i>Hprt</i> mutation [310]	Mice	–

years, has been reviewed recently [208] and is summarized in Table 16. It required S9 to be mutagenic *in vitro*, and it induced gene mutation in bacteria, mammalian cells, and in rat nasal epithelia *in vivo*. It also induced SCEs in mammalian cells, as well as micronuclei and chromosomal aberrations in mammalian cells and rodents. It induced DNA damage in bacteria and mammalian cells and germ-cell mutations in *Drosophila* and possibly rodents. Formaldehyde also induced DNA–protein cross-links *in vitro* as well as in rodents and humans. In mouse lymphoma cells, formaldehyde induced gene mutations containing large deletions and recombination events [209]. Due to its highly reactive nature, the *in vivo* genotoxicity of formaldehyde is complex and difficult to assess in humans [210].

The genotoxicity of acetaldehyde has been reported in >40 studies published during the past 30 years and has been reviewed [61] and is summarized in Table 16. Acetaldehyde required S9 to be mutagenic *in vitro*; however, it was not mutagenic in bacteria. In mammalian cells, it caused gene mutations, SCEs, micronuclei, and chromosomal aberrations. In rodents, it induced SCEs and protein–DNA cross-links. Acetaldehyde also induced aneuploidy in fungi.

The genotoxicity of chloral hydrate has been reviewed recently [17,211], and a summary of the results of the more than 60 studies spanning 30 years is shown in Table 16. Chloral hydrate is a direct-acting mutagen *in vitro* and induced base-substitution mutations in bacteria; aneuploidy and micronuclei in mammals *in vivo*; and aneuploidy, micronuclei, chromosomal aberrations, gene mutations, and cell transformation in mammalian cells *in vitro*. It also caused DNA damage and protein–DNA cross links in rodents. Chloral hydrate is related to some of the other DBPs discussed here in that it is metabolized in humans and rodents to trichloroacetic acid, trichloroethanol, and dichloroacetic acid [17].

Chloroacetaldehyde is mutagenic in bacterial and mammalian cells *in vitro* [308,309] but not in mice [310]. In addition to being a DBP, this compound is also a metabolite of the well-characterized mutagen and carcinogen vinyl chloride.

Glyoxal is a related aldehyde that is mutagenic in bacteria [212–215]; in *Salmonella*, the majority of mutations were base substitutions at G:C base pairs [215]. In *E. coli*, it was suggested that glyoxal-induced mutations may be correlated to mutations induced by oxygen free-radicals [215]. Glyoxal induced DNA strand breaks and DNA–protein cross-links at a 10-fold lower frequency than did methylglyoxal. Glyoxal and

methylglyoxal also induced DNA damage in human skin cells exposed *in vitro* [216]; however, methylglyoxal produced compacted nuclei and reduced DNA migration, indicating the induction of cross-links. Using the SCGE assay with freshly isolated rat hepatocytes, glyoxal resulted in an elevated tail moment, indicating DNA damaging activity. Glyoxal also caused DNA images with small, highly condensed areas within otherwise circular DNA spots, which were probably the consequence of DNA and protein cross-links [217].

Methylglyoxal is present in many foods, drinks, and tobacco smoke; its genotoxicity has been reviewed [65]. It was mutagenic in bacteria in the absence of S9 [218,219]. In yeast, methylglyoxal induced gene mutation and gene conversion. In mammalian cells, it induced SCEs, gene mutations, chromosomal aberrations, and micronuclei [65]. Mutations induced in mammalian cells were mainly deletions and, secondarily, base substitutions [220].

6.9.3. Carcinogenicity

Chloral hydrate and chloroacetaldehyde have been shown to cause liver tumors in rodents [227] (Table 10). Formaldehyde and acetaldehyde have been shown to cause tumors in rodents when administered through inhalation, but they were not carcinogenic when administered through drinking water [221–225]. As mentioned earlier, inhalation exposures due to showering and other activities can be important sources of exposure to some DBPs. It is not currently known whether significant levels of formaldehyde or acetaldehyde would be present during showering. In water, formaldehyde should mostly form a hydrated diol, $\text{CH}_2(\text{OH})_2$ [226], which is extremely water soluble and has a low calculated Henry's constant (-7), such that, in a closed system, it would partition with the water and would not be expected to volatilize (SPARC model; <http://ibmlc2.chem.uga.edu/sparc>). The small fraction (0.4%) of the formaldehyde (HC(O)H) species present in water also has a low Henry's constant (-2) (<http://ibmlc2.chem.uga.edu/sparc>) [226]. Other aldehydes, such as acetaldehyde, would also be expected to hydrate and form equivalent diols in water, but not quite to the extent as formaldehyde. Therefore, in a closed system, these aldehydes would not be expected to volatilize from water into the air. However, a showering situation is not a closed system, and Henry's Law may not strictly apply. In addition, elevated temperatures in showers may also help to drive the equilibrium, promoting volatilization. Studies have not been conducted to address the concentrations of formaldehyde or other aldehydes volatilized into air from showering.

EPA has evaluated formaldehyde, acetaldehyde, and chloral hydrate for carcinogenicity, and summaries of these evaluations can be found on IRIS. Under the 1986 *Cancer Guidelines*, formaldehyde was considered to be B1, probable human carcinogen, based on animal data and some limited human data from occupational exposure by inhalation (<http://www.epa.gov/iris/subst/0419.htm>). Acetaldehyde was considered to be B2, probable human carcinogen based on tumors in animals exposed by inhalation (<http://www.epa.gov/iris/subst/0290.htm>). Neither of these two assessments on IRIS has been revised since 1991. In 2000, chloral hydrate carcinogenicity was summarized on IRIS as having data suggestive of carcinogenicity (under an early draft of the Revised 2005 Cancer Guidelines) and as C, possible human carcinogen using the 1986 version of the *Cancer Guidelines* (<http://www.epa.gov/iris/subst/0304.htm>). There were no quantitative risk estimates for cancer published on IRIS for any of the above aldehydes.

6.10. Chlorate

6.10.1. Occurrence

Like chlorite, chlorate is primarily a DBP from chlorine dioxide treatment, although it can also be present as a contaminant from chlorination (when solutions of sodium hypochlorite are used) [94]. Chlorate is a decomposition product of chlorine dioxide (along with chlorite), and chlorate levels can occur at approximately 20% of the original chlorine dioxide dose [107,228].

In the U.S. EPA's ICR, which represents the most extensive data for chlorate, the median level of chlorate was 0.12 mg/L at plants using chlorine dioxide for disinfection [23,111]. Recent measurements of chlorate included a study of full-scale treatment plants in Israel [14] in which chlorate was found at levels up to 0.052 mg/L; a full-scale treatment plant in Virginia [112] where chlorate was found at a median level of 0.014 mg/L; and full-scale treatment plants in Quebec [113] where chlorate was found at a maximum level of 0.19 mg/L.

6.10.2. Genotoxicity

Chlorate had limited genotoxicity data; however, a set of studies reviewed by Kurokawa [100] showed that chlorate was mutagenic in *Salmonella* and induced chromosome aberrations and micronuclei in mammalian cells.

6.10.3. Carcinogenicity

Chlorate showed no evidence of carcinogenicity in mice exposed via the drinking water; however, it

induced thyroid follicular-cell tumors in male and female rats [229].

7. Summary of the occurrence, genotoxicity, and carcinogenicity of the emerging unregulated DBPs

7.1. Summary of the occurrence of the emerging unregulated DBPs

The unregulated DBPs that occur at the highest levels include chlorate (in chlorine dioxide-treated waters); the four remaining HAAs (bromochloro-, bromodichloro-, dibromochloro-, and tribromoacetic acid); trichloronitromethane (chloropicrin); and trichloroacetaldehyde (chloral hydrate). Chlorate is generally present at high ppb levels (and sometimes ppm levels), and the others are generally present at low ppb levels. It is no surprise that these DBPs are commonly measured, along with the regulated DBPs, in occurrence studies. Halonitromethanes, iodo-THMs, iodo-acids, halo-amides, and aldehydes (including halo-aldehydes) form the next tier of DBPs, which are generally present at sub-ppb to low-ppb levels. In the Nationwide Occurrence Study of unregulated priority DBPs, the haloaldehydes were the third largest DBP class by weight (behind the THMs and HAAs) of all the DBPs studied. The lowest tier included those DBPs found at the ng/L level. Halofuranones (MX analogues) are in this group, with low-ng/L levels being common, but they can reach 1 µg/L (1 ppb) or greater if summed together as a class [9]. Nitrosamines are generally present at low-ng/L levels, but there is one report of NDMA being found at 180 ng/L in drinking water from a distribution system [184]. Finally, 2,3,5-tribromopyrrole, the first halopyrrole identified, was found to be present at approximately 50 ng/L in finished waters. So far, this DBP has been identified only in finished drinking waters from Israel that have extremely high bromide levels; it is not yet known whether this DBP will be found in other locations.

The type of disinfectant chosen can have a dramatic effect on the DBPs formed, as each disinfectant tends to produce its own suite of DBPs, e.g., THMs and HAAs from chlorine, chlorite and chlorate from chlorine dioxide, bromate and aldehydes from ozone, and nitrosamines from chloramines, with some overlap of DBPs among disinfectants. The presence of natural bromide and/or iodide in the source waters can influence the speciation of DBPs such that higher bromide/iodide levels contribute to a shift in speciation from chlorinated to brominated/iodinated DBPs. Due to

differences in kinetics, the choice of disinfectant can also play a role in speciation. For example, chloramination can result in higher levels of iodo-THMs and iodoacids than does chlorination, which preferentially forms iodate instead. The combined use of disinfectants can also influence the levels of DBPs formed. For example, the use of pre-ozonation before post-disinfection with chlorine or chloramines can dramatically increase the levels of halonitromethanes compared to chlorination or chloramination alone.

However, approximately 50% of the total organic halogen (TOX) in chlorinated drinking water and approximately 75% of the TOX in water treated with alternative disinfectants remains unidentified. Approximately 60% of the assimilable organic carbon (AOC) measured in ozonated drinking water is unaccounted for. In addition, TOX and AOC represent only a portion of the types of DBPs formed. For example, the TOX measurement for chlorinated drinking water would not include contributions from non-halogenated DBPs, such as formaldehyde or carboxylic acids. Therefore, the levels of unregulated DBPs discussed here are based on those that are currently known and have been quantified in drinking water. There is much that is still not known about the DBPs formed during disinfection.

7.2. Summary of the genotoxicity of the emerging unregulated DBPs

Studies on the genotoxicity of emerging unregulated DBPs have increased in number during the past decade; many of these compounds appear to be more genotoxic in some assays than the regulated DBPs. Chemical classes of DBPs in which there are ample genotoxicity and some carcinogenicity data include the halomethanes, haloacetic acids, halofuranones, and the nitrosamines. The dihalomethanes (dichloromethane, dibromomethane, and bromochloromethane) have been shown to be mutagenic in *Salmonella* and to have moderate cytotoxicity; however, they did not induce DNA damage in CHO cells.

Of special interest is the impact of iodinated methanes because they are produced at elevated levels by one of the alternative disinfection methods—chloramination. The iodo-THMs were more cytotoxic in CHO cells than their chlorinated and brominated analogues. Dibromo-, tribromo-, bromochloro-, dibromochloro-, and bromodichloroacetic acids are of concern because of their increased genotoxicity and cytotoxicity compared to their chlorinated analogues. Iodoacetic acid is a potent mutagen with a variety of biological responses in several bioassays. In a

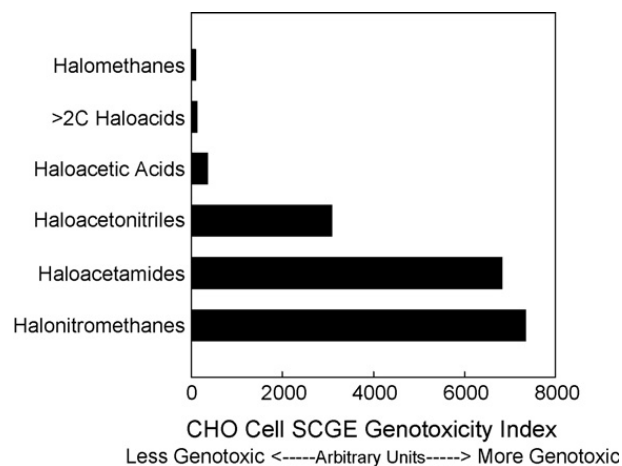


Fig. 6. Genotoxicity index for the major DBP classes evaluated in the CHO SCGE assay; derived from data from Plewa et al. [62]; see text or reference for explanation of units.

comparative analysis of over 60 DBPs using SCGE in CHO cells, iodoacetic acid was the most potent genotoxic DBP evaluated. The presence of iodinated acids and other iodo-DBPs in drinking water is an area of increased concern because of their enhanced toxicity and genotoxicity compared to their brominated and chlorinated analogues.

The unregulated DBPs in the halofuranone and nitrosamine chemical classes need further investigation as to their occurrence, toxicity, and health effects. Both of these classes have individual agents that are highly genotoxic, and some are carcinogenic. The brominated furanone DBPs should be afforded attention because they may express heightened toxicity, and a search for

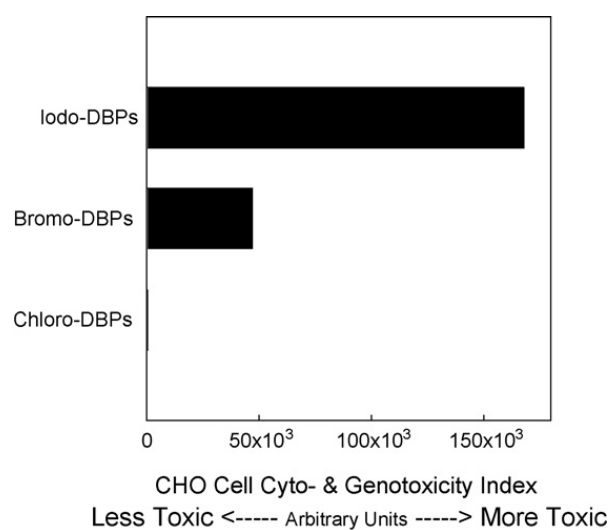


Fig. 7. Combined toxicity index values as a function of the presence of the halogen species for matched analogues of mono-, di-, and trihalogenated DBPs from the CHO cell database (a total of 18 DBPs) [62].

the presence of iodinated furanone DBPs should be undertaken. The toxicity, genotoxicity, and carcinogenicity of specific nitrosamines is well known; however, the fact that these agents can be generated by disinfecting water indicates that studies should be conducted to determine whether other novel nitrosamine DBPs occur in drinking water.

The relative genotoxicities for a number of DBP classes have been determined from a comprehensive comparative program examining DNA damage (SCGE assay) in CHO cells (Fig. 6) [62]. It is interesting that the regulated classes of DBPs appear to have lower genotoxic activities than the emerging DBP classes.

The impact of the halogen atom on a set of matched DBPs was determined from this CHO cell data published by Plewa et al. [62]. When the combined cytotoxicity and genotoxicity index values were calculated for a set of 18 DBPs with matched halogen analogues and were averaged as a function of the halogen species, the order of toxicity was $I \gg Br \gg Cl$ (Fig. 7). Finally, in a comparison of 26 carbon-based DBPs (i.e., DBPs without a nitrogen) versus 29 nitrogenous DBPs (i.e., DBPs containing nitrogen), the nitrogenous DBPs as a class were the most toxic (Fig. 8) [62]. Based on these comparisons, it is important to gather additional information on iodinated DBPs within all chemical classes, as well as further information on the halonitriles and haloamines. Additional genotoxicity data on the nitrogenous DBPs are needed to characterize the range of genotoxic activities of this class of DBPs. These emerging DBPs are important environmental compounds, and future studies on the genotoxicity of iodinated, nitrogenous, and iodinated-nitrogenous DBPs are much needed.

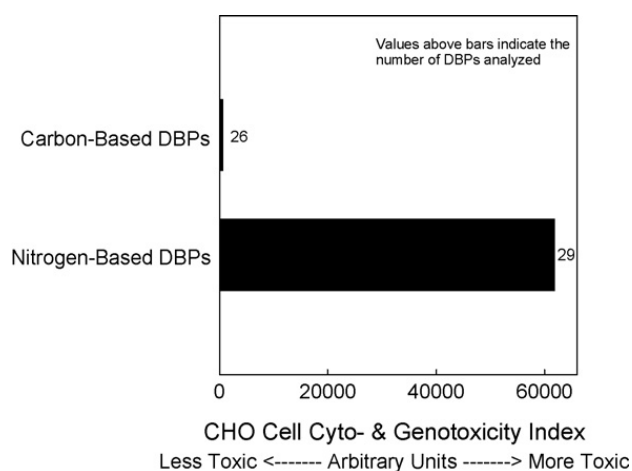


Fig. 8. Combined toxicity index values for carbonaceous DBPs (C-DBPs, i.e., those that do not contain nitrogen) versus nitrogenous DBPs (N-DBPs, i.e., those that contain nitrogen) [62].

7.3. Summary of the carcinogenicity of the emerging unregulated DBPs

Clearly the list of unregulated DBPs is quite long. Some of these chemicals are similar to those that are regulated, such as the HAAs, while others are unique. Although most of the chemicals presented here can be found in chlorinated surface waters, some are present after alternative disinfection methods, such as chlorate after chlorine dioxide disinfection. Similar to the regulated DBPs, those unregulated DBPs that result in rodent tumors induced them at doses of 75 mg/(kg day) or greater. The exception was MX (Table 10), which has been shown to result in tumors in rats at doses as low as 0.4 mg/(kg day) [154], making it the most potent animal carcinogen of all the DBPs (regulated or unregulated) and 3× more potent than the most potent regulated DBP (bromate).

Among the unregulated DBPs, there were no 2-year rodent carcinogenicity data for chloropicrin, dibromoacetonitrile, glyoxal, or methylglyoxal (Table 17). There was some evidence from 2-year studies for the carcinogenicity of chloral hydrate (Table 17), and it was genotoxic in a variety of systems (Table 16). IARC did not consider these data sufficient to classify chloral hydrate as to human carcinogenicity [17]. In 2000, the U.S. EPA published its assessment on IRIS, which indicated that chloral hydrate had data suggestive of carcinogenicity (under an early draft of the Revised 2005 Cancer Guidelines) and as C, possible human carcinogen, using the 1986 version of the *Cancer Guidelines* (<http://www.epa.gov/iris/subst/0304.htm>). Chlorate induced thyroid tumors in rats via the drinking water, but there were little genotoxicity data for this DBP, and no conclusions could be made regarding its carcinogenicity to humans. Finally, there were four unregulated DBPs (formaldehyde, acetaldehyde, MX, and the nitrosamines) for which there was some level of evidence of carcinogenicity from 2-year rodent studies, some human data (except for MX), and genotoxicity data indicating that they might have the toxicological characteristics of human carcinogens (Tables 17 and 18).

As summarized for formaldehyde [208], exposure of rats to formaldehyde by inhalation resulted in the induction of squamous-cell carcinomas of the nasal cavities in several studies; however, similar studies in hamsters and mice showed no effect. When rats were exposed to formaldehyde in the drinking water, one study found an increase in forestomach papillomas, a second found an increase in gastrointestinal leiomyosarcomas, a third found an increase in lymphomas and

Table 17
Summary of the 2-year carcinogenicity studies of the unregulated DBPs

DBP	Route of administration and target organ of tumors					
	Gavage		Drinking water		Inhalation	
	Mouse	Rat	Mouse	Rat	Mouse	Rat
Acetaldehyde						Nasal
Chloroacetaldehyde			Liver			
Bromodichloroacetic acid			Liver, lymphoma ^a	On test		
Bromochloroacetic acid			Liver ^a			
Dibromochloroacetic acid			Liver ^a			
Chloral hydrate	Liver		Liver	Negative		
	Pituitary		Liver	Negative		
Chlorate			Negative	Thyroid		
Chloropicrin	Inadequate	Inadequate				
Dibromoacetonitrile			On test	On test		
Formaldehyde				Negative	Nasal	Nasal
				Testicular interstitial cells, lymphoma + leukemia		
MX				Liver, adrenal, thyroid, lung, breast, lymphoma + leukemia		
Nitrosamines ^b						

^a A.B. DeAngelo, in preparation, personal communication.

^b Carcinogenic in a wide range of species via many routes; see text.

leukaemias and an increase in testicular interstitial-cell adenomas in males, and a fourth study gave negative results. Formaldehyde was considered a class 1 carcinogen by IARC [208], i.e., it is carcinogenic to

humans, and it was genotoxic at a wide variety of endpoints and biological systems (Table 16). U.S. EPA's assessment on IRIS has not been revised since 1991. However, at that time, formaldehyde was considered to

Table 18
DBPs having some or all of the toxicological features of human carcinogens based on occurrence, genotoxicity, and rodent carcinogenicity data^a

DBP	Occurrence ^b	Genotoxicity						Carcinogenicity			
		Gene mutation		Chrom. mutation		DNA damage		Rodent		Human ^c	
		Bacteria	MC ^d	<i>In vitro</i>	<i>In vivo</i>	<i>In vitro</i>	<i>In vivo</i>	Mouse	Rat	IARC	EPA
Regulated											
Bromodichloromethane ^{e,f}	*****	+				–		+, –, –	+, +, –	2B	B2
Bromate	***	+	+	+	+	+	+	+, –	+, +, –	2B	B2
Dichloroacetic acid	*****	+	+	+	+	–	–	+	+	2B	B2
Dibromoacetic acid	*****	+				+		+	+		
Unregulated											
Formaldehyde ^f	***	+	+	+	+	+		+	+, –	1	
Acetaldehyde	***	–	+	+			+		+	2B	
MX ^g	**	+	+	+		+	+		+	2B	
NDMA	**	+	+	+	+	+	+	+	+	Yes	B2

^a Toxicologic properties of human carcinogens are those carcinogens that are trans-species and mutagenic in a variety of mutagenicity assays as described [277,278].

^b *Low-ng/L levels; **ng/L to sub-μg/L levels; ***sub- to low-μg/L levels; ****low-μg/L levels; ***** low- to mid-μg/L levels.

^c IARC: 1, human carcinogen; 2B, possibly carcinogenic to humans; Yes, should be regarded as a human carcinogen. EPA: B2, probably carcinogenic to humans as evaluated using the U.S. EPA's 1986 *Guidelines for Carcinogen Risk Assessment*.

^d MC, mammalian cells.

^e Although only two reports have studied bromodichloromethane in cells expressing the GSTT1-1 enzyme, both were highly positive.

^f Bromodichloromethane and formaldehyde are genotoxic in humans.

^g MX is the most potent *in vitro* mutagen in *Salmonella* and most potent rodent carcinogen of all DBPs; it also induces tumors at more organ sites (7) than does any other DBP.

be B1, probable human carcinogen (under the 1986 *Cancer Guidelines*) based on animal data and some limited human data from occupational exposure by inhalation (<http://www.epa.gov/iris/subst/0419.htm>).

Acetaldehyde [61] induced adenocarcinomas and squamous-cell carcinomas of the nasal mucosa in rats and laryngeal carcinomas in hamsters exposed by inhalation. Intratracheal inhalation of acetaldehyde did not cause an increase in any types of tumors in hamsters [61]. It was genotoxic in many systems and endpoints (Table 16), and it was considered a class 2B, possible human carcinogen, by IARC [61]. U.S. EPA's assessment in 1991 was that acetaldehyde was considered to be B2, probable human carcinogen, based on tumors in animals exposed by inhalation (<http://www.epa.gov/iris/subst/0290.htm>).

Chloroacetaldehyde induced liver tumors in mice [227] and was genotoxic *in vitro*, although not *in vivo*. Beyond being a DBP, this compound is also a metabolite of vinyl chloride.

As discussed in detail above (Section 6.4), MX was a multi-site carcinogen in rats via the drinking water, and its carcinogenic potency was the greatest of any DBP (regulated or unregulated). It was genotoxic in a wide array of systems (Table 11), and although it has not been tested in any species other than rats, it was considered a possible human carcinogen (2B) by IARC [17].

As noted previously, the nitrosamines, which as a class are generally carcinogenic, can be present in drinking-water disinfected with chloramines. Compounds of this class have been shown to cause cancer in rodents by a variety of routes, including through the drinking water [203,204,230]. The nitrosamines were genotoxic in a wide array of systems and endpoints (Table 14), and they were considered human carcinogens by IARC [230]; several (NDMA, *N*-nitrosodiethylamine, *N*-nitrosopyrrolidine, and *N*-nitrosodiphenylamine) were considered B2, probable human carcinogens, by the U.S. EPA IRIS (<http://www.epa.gov/iris/subst/0045.htm>).

One study found that chlorate induced thyroid tumors in rat when administered via the drinking water; however, chlorate was negative in mice via this exposure route (Table 10).

8. DBPs formed from anthropogenic contaminants

All of the DBP studies discussed above involve primarily the DBPs formed from natural organic matter found in drinking-water source waters. However, source waters are also impacted by municipal and industrial emissions [231,232], and recent investigations have

shown that some of these water contaminants can also react with disinfectants used in drinking-water treatment to form their own by-products. Chlorination in swimming pools has also been shown to transform active compounds used in sunscreens to halogenated by-products [73,233]. To date, most of these disinfectant reactions have been carried out in controlled laboratory studies and have not been identified in finished drinking water, but the potential is there for their formation in drinking-water treatment.

Most of this research has been conducted in order to find ways to degrade and remove these contaminants from wastewater effluents and drinking-water sources. It is not surprising that DBPs can form from these contaminants because many of them have activated aromatic rings that can be reacted readily with oxidants like chlorine and ozone. However, until recently, the occurrence and toxicology of DBPs formed from anthropogenic contaminants have not been investigated. Because this research is so new, there is not much known about the genotoxicity or carcinogenicity of the contaminant by-products formed.

As discussed below, recent reports have shown the formation of DBPs by chlorine or ozone treatment of pesticides, pharmaceuticals, antibacterial agents, textile dyes, bisphenol A, alkylphenol ethoxylate surfactants, and cyanobacterial toxins. Pharmaceutical DBPs have included chlorinated [234] and ozonated [235] by-products of ethinylestradiol. One of these chlorinated by-products had an estrogenic activity similar to the parent pharmaceutical, but the ozonation by-products had reduced estrogenic activity. The pharmaceutical, carbamazepine, forms oxidation products when treated with ozone [236], and acetaminophen can produce chlorinated and non-chlorinated products when treated with chlorine [237]. The antibacterial agent triclosan, which is used in many hand soaps, can form chloroform and other chlorinated DBPs when reacted with chlorine or monochloramine under drinking-water treatment conditions [238,239]. The antibacterial agent sulfamethoxazole produces chlorinated and non-chlorinated by-products when reacted with chlorine [240], and the veterinary antibacterial agent carbadox can form oxidation products when reacted with chlorine [241]. These oxidation products of carbadox retained their biologically active *N*-oxide group, which suggests that the by-products may still be active antibacterial agents.

Pesticide DBPs have included oxidation products of S-triazine herbicides (prometryne, terbutryne, ametryne, and desmetryne) when reacted with chlorine or chlorine dioxide [242]; chlorinated and non-chlorinated by-products of the herbicide isoproturon when reacted

with chlorine or chlorine dioxide [243]; chlorinated by-products of the herbicide chlortoluron when reacted with chlorine [244]; an oxidation product of isoxaflutole (under chlorination conditions) [245]; an oxidation product of chlorpyrifos (chloropyrifos oxon, which is more toxic than the parent pesticide) when treated with chlorine [246]; oxidation products of clethodim when reacted with chlorine [247]; by-products of chloroacetamide herbicides (acetochlor, alachlor, metolachlor, and dimetheamide) when reacted with either ozone or chlorine under simulated drinking-water conditions [248]; by-products of diazinon during UV and UV/hydrogen peroxide treatment [249]; and an oxidation product (methanediol) from glyphosate [250].

Alkylphenol ethoxylate surfactants, which are used in many laundry detergents, produce brominated nonylphenolic by-products when they are reacted with chlorine [251]. The industrial contaminant bisphenol A can form monochloro-, dichloro-, trichloro-, and tetrachloro-derivatives when chlorinated [252]. The cyanobacterial toxin microcystin-LR was found to react with chlorine dioxide to form dihydroxy isomers, which were nontoxic in a protein phosphatase-inhibition assay [253].

Chlorination of disperse azo dyes was found to produce a chlorinated product that was highly mutagenic [254–257]. These studies were carried out because a local drinking-water treatment plant in Brazil had repeatedly detected mutagenic activity that could not be explained by traditional DBPs, and the source waters had been contaminated by a dye-processing plant. These and other studies suggest the potential value of biomonitoring various source waters for mutagenicity using the *Salmonella* assay. A series of studies from Japan has identified various mutagens derived from azo dyes in source waters, with some being chlorinated via water-treatment plants [258–262]. Mechanistic studies can also aid in decision-making regarding ecological and public health risks due to source-water contaminants [263,264].

9. Mutagenicity of organic extracts or concentrates of drinking water

As discussed above, alternatives to chlorination, such as ozonation and chloramination, have generally accomplished the intended reduction in the levels of regulated THMs and HAAs compared to the levels produced by chlorination. However, these alternative methods have also produced higher levels of other DBPs and even new classes of DBPs, some of which appear to be more toxic or genotoxic than those

currently regulated. Despite the presence of these newly identified DBPs in water prepared by alternative methods, studies done more than a decade ago showed that all of the organic extracts (XAD/ethyl acetate) of water prepared by alternative disinfection methods were less mutagenic in the *Salmonella* mutagenicity assay than were those from chlorinated water [37–39]. A recent study has confirmed this for concentrates prepared by reverse osmosis [40]. Although the levels of bromide in these waters were not determined at the time, some may have had at least moderate levels because they were coastal waters. However, a more systematic analysis should be performed in which bromide levels are measured and other assays in addition to the *Salmonella* mutagenicity assay are used to evaluate the genotoxicity of extracts of drinking water prepared by different disinfection methods.

In general, extracts or concentrates of ozonated water were only slightly mutagenic. However, introduction of the Cl atom by post-treatment of ozonated water with either chloramine or chlorine greatly increased the mutagenic activity. Nonetheless, extracts of water prepared by ozonation, chloramination, or post-treatment of ozonated water by chloramine or chlorine were all less mutagenic than chlorinated water, which was generally 2–40× as mutagenic as any of the other waters. Analysis of the mutations induced by these extracts showed that they all produced a similar spectrum of mutations, predominantly GC to TA base substitutions [37,265]. Collectively, these studies indicated that alternative disinfection methods have reduced the levels of THMs and HAAs and also produced water that was less mutagenic (as assessed in the *Salmonella* assay) than that produced by chlorination. These results give some indication that alternative disinfection may be producing safer water than that produced by chlorination, despite the formation of new, highly genotoxic DBPs in waters prepared by alternative disinfection methods. Nonetheless, further studies are needed in which (a) various source waters are used, (b) the waters are chemically characterized before and after treatment, and (c) additional bioassays along with the *Salmonella* mutagenicity assay are used to characterize the genotoxicity of the extracts of drinking waters produced by different disinfection methods.

10. Carcinogenicity of raw waters or organic extracts of drinking water or mixtures of DBPs

Unlike for mutagenicity, only a few carcinogenicity studies of drinking water or concentrates/extracts of

drinking water have been performed, and all were negative. All exposures were done as drinking water. However, inhalation and dermal exposure to DBPs must also be considered major exposure routes because showering or bathing typically entails larger volumes of water than drinking. It must also be noted that higher concentrations of some volatile DBPs are found in the blood or breath after dermal exposure compared to oral exposure (e.g., THMs) [71]. Some of the DBPs reviewed here are clearly (sometimes only) carcinogenic by inhalation, and none has been examined for carcinogenicity by the dermal route of exposure. Thus, the lack of carcinogenic effect noted for drinking water or drinking-water concentrates/extracts may reflect both inherent limitations of such studies and the absence of dermal/inhalation exposure.

Two carcinogenicity studies have been performed using organic extracts of drinking water, and neither found carcinogenic effects. Kool et al. [266] evaluated drinking water from a city in the Netherlands whose water yielded organic extracts shown to be mutagenic in the *Salmonella* assay. The water was concentrated on XAD-4/8 resin, the organics were eluted with dimethylsulfoxide (DMSO), and ~10,000× concentrates were prepared. No other procedures were used to prepare the extract, and no chemical analyses were performed. Elution by DMSO may have failed to recover the volatile or semi-volatile DBPs and may not have provided a very extensive organic extraction. Male and female Wistar SSP TOX rats were given the water concentrate in their drinking water at 40 or 68 times, respectively, the levels typically consumed by humans. After 106 weeks of exposure, no carcinogenic or other adverse effects were found.

A similar study was performed by Condie et al. [267] in male and female Fisher 344 rats exposed to concentrates of three different waters in their drinking water: Denver, Colorado finished drinking water; reverse-osmosis reclaimed water; or ultrafiltered reclaimed water. The concentrates were prepared by passing the waters through XAD-2/4/8 resin, eluting the organics with acetone, and preparing 150× and 500× concentrates in Emulphor. Three volatile organics (chloroform, bromodichloromethane, and 1,1-dichloropropane) were added to the concentrates to restore the concentrations of these compounds to the levels found in the water prior to concentration/extraction. No neoplasms or other pathologies were found after 104 weeks of exposure to any of the three types of water.

Several reports [268–270] evaluated the carcinogenicity of a defined mixture of DBPs consisting of potassium bromate, MX, chloroform, and bromodi-

chloromethane. Eker rats, which develop renal neoplasia and are especially susceptible to renal carcinogens, were exposed via drinking water to a low and a high concentration of this defined mixture for 4 or 10 months. Although this defined mixture produced a dose response for pre-neoplastic and neoplastic renal lesions, the authors reported generally subadditive results for the pre-neoplastic lesions and carcinogenicity. The exception was that additive responses were obtained for pre-neoplastic lesions in males at the high dose. The high dose of MX alone caused transitional epithelial hyperplasia and cell proliferation in the urinary bladder, but this effect was reduced in animals exposed to the high dose of the mixture. The four DBPs individually as well as the mixture induced aberrant crypt foci, which is the putative preneoplastic lesion of colon cancer.

As noted previously, drinking water is a complex mixture, containing more than 600 identified DBPs, with much of the organic halogen fraction unidentified. Thus, consideration of the carcinogenicity or genotoxicity of each individual DBP in isolation is inadequate for understanding the biological consequences of the whole mixture. It is beyond the scope of this review to discuss models of additivity or results with other complex mixtures or defined mixtures not related to drinking water. However, when assessing risk of a mixture from tests of the individual compounds, the U.S. EPA may assume additivity of low-dose response for independent events [271]. Efforts have been made to consider not only additivity but other models appropriate for assessments of a complex mixture such as drinking water [272–274], and new studies using reverse-osmosis concentrates of drinking water should provide additional data to improve risk assessment procedures for drinking water [275].

11. Risk assessment of DBPs

It has been 30 years since research on DBPs began in earnest, and not surprisingly, there are as many scientific questions as there are accepted answers. Some of these questions are on the types of data and evaluations needed to demonstrate that DBPs are controlled to an acceptable level while maintaining the needed degree of protection against microbial disease that water disinfection provides. The U.S. EPA and other groups have used the tools of risk assessment in their analysis of potential health effects of DBPs (for a recent discussion of EPA risk assessment practices, see *An Examination of Risk Assessment Principles and Practices*, EPA/1-/B-04/001, available at <http://epa.gov/osa/ratf.htm>). In general, assessment of human health risk from chemicals includes four steps.

- *Hazard Identification*, which evaluates the likelihood (or weight of the evidence) that a chemical can cause adverse effects in humans. This step also characterizes the type of health effects mostly likely to result from environmental exposure.
- *Dose–response assessment*, which determines the potency of the contaminant in producing health effects.
- *Exposure assessment*, wherein the risk assessor determines how people are exposed or come in contact with the contaminant.
- *Risk characterization*, the final step that combines all the preceding information and judgments.

The regulated DBPs have all been evaluated multiple times using these tools, most recently in preparation for the proposal and promulgation of the Stage 2 D/DBP Rule. That set of risk assessments reflected the growing concern for reproductive and developmental effects that may be associated with DBP exposure. These assessments also incorporated new approaches to judging cancer risk that were being developed in the U.S. EPA's revised *Guidelines for Cancer Risk Assessment* (<http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283>). These newer approaches emphasize making maximal use of appropriate data rather than relying on default procedures. The *Cancer Guidelines* also identify the mode of action (MOA) as the critical information needed to determine if data are relevant to humans and how to approach dose–response assessment. A side effect (or by-product) of the Guidelines is an increased reliance on genetic toxicity data in several steps of risk assessment.

There are many uses of genetic toxicity assessment, but several are particularly germane to the evaluation of DBPs. Data on mutation and other DNA interactions can contribute to the weight of the evidence that a chemical may be identified as a likely or probable human carcinogen (<http://epa.gov/osa/ratf.htm>). Most importantly, genetic toxicity data can be used to establish the mode of action whereby a chemical caused cancer in animals or humans. The U.S. EPA and the International Programme on Chemical Safety (IPCS) both use a framework for evaluating all data contributing to determination of a mode of action (<http://epa.gov/osa/ratf.htm>). This framework was used, for example, in the U.S. EPA's judgment that chloroform causes cancer by virtue of its metabolism to phosgene, causing toxicity in the kidney and liver and subsequent compensatory hyperplasia. For a summary of chloroform's mode of action, see IRIS at <http://www.epa.gov/iris/subst/0025.htm>. The genetic toxicity

testing data were critical to the determination that chloroform was not likely to cause cancer through mutation; data from some *in vivo* assays were particularly useful in this evaluation.

EPA and other risk assessors have begun full-scale application of the MOA framework only recently; thus, there are few published examples. It has become clear, however, that genetic toxicity data are most useful when considered as a whole and in conjunction with other toxicity data, rather than in isolation. Data on many different genetic toxicity endpoints can be arrayed, as we attempted in this review, and evaluated in a comprehensive fashion [276]. Then a mode of action can best be constructed with data that may include genetic toxicity, pharmacokinetics, tumor types, structural alerts, cytotoxicity, systemic toxicity, and carcinogenicity studies.

The current U.S. regulations to control levels of DBPs rely on a type of indicator approach. Enforceable MCLs were set for the four THMs, five HAAs, bromate, and chlorite. U.S. EPA regulators believe that practices that reduce these indicator DBPs at or below the MCL will result in an associated reduction of the entire suite of DBPs and, ultimately, in a decrease in the likelihood of adverse health effects. Although these regulations represent the best current approach to protecting public health, questions remain that need to be addressed. For example, levels of many DBPs in the U.S. Nationwide Occurrence Study did not always change proportionately with the regulated DBPs; in fact, some DBPs increased in concentration when the regulated DBPs were decreased [9,27]. These DBPs included iodo-THMs and iodo-acids, which showed the highest levels with chloramination; halonitromethanes and haloaldehydes, which were enhanced with preozonation; and MX compounds, which showed an unexpectedly high occurrence with chlorine dioxide disinfection. In addition, NDMA and other nitrosamines tend to form at greater levels with chloramination.

12. Conclusions and research needs from current analysis

12.1. Categories of DBPs to prioritize testing and aid in decision-making

Our analysis identifies three categories of DBPs for priority testing and decision-making based on the combination of occurrence, genotoxicity, and carcinogenicity data reviewed here. These categories contain those DBPs that (1) have some or all of the toxicologic characteristic of human carcinogens, (2) occur at

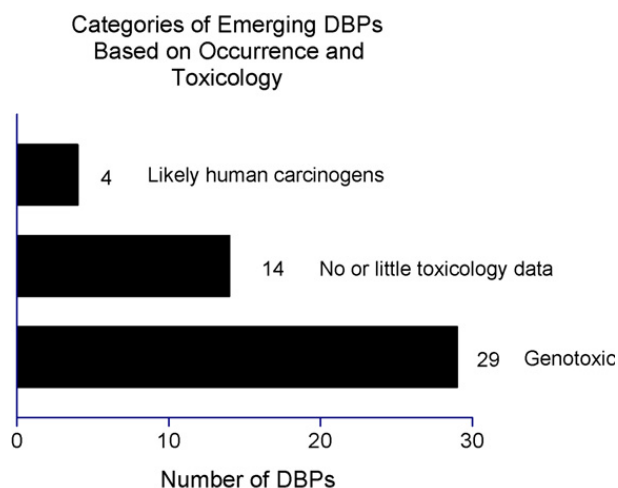


Fig. 9. Categories of emerging DBPs based on occurrence and toxicology data. Four have some or all of the toxicologic characteristics of human carcinogens; 14 occur at moderate levels and are genotoxic; 29 occur at moderate levels but have not been studied toxicologically. See Section 12.1 for details.

moderate concentrations and are genotoxic, and (3) occur at moderate concentrations but for which little or no toxicology data are available. The DBPs in these categories are described below, and the possible guidance such categories may provide for future research and decision-making are discussed (Fig. 9).

12.1.1. DBPs that have some or all of the toxicologic characteristics of human carcinogens

The majority of the chemicals categorized as carcinogenic to humans by IARC (group 1 or sufficient evidence) are carcinogenic in more than one species (i.e., are trans-species carcinogens) [277], and they are mutagenic, frequently inducing both gene and chromosomal mutation [278]. Among the 11 regulated DBPs, five are trans-species carcinogens (bromodichloromethane, chloroform, dibromoacetic acid, dichloroacetic acid, and bromate). Among these, four are genotoxic: bromodichloromethane, bromate, dichloroacetic acid, and dibromoacetic acid. Table 18 summarizes the results for these four DBPs, along with their occurrence levels, which range from low to high.

Bromodichloromethane is carcinogenic in mice and rats, producing tumors at three organ sites. One of these sites is the large intestine, which is analogous to the human cancer site (colon) that has been associated with cancer in drinking-water epidemiology studies. Bromodichloromethane is also mutagenic via metabolism through GSTT1-1. Bromodichloromethane has recently been shown to produce systemic genotoxicity (mutagenic urine) in humans exposed either dermally or orally to this brominated, regulated THM [71].

Together, the occurrence, genotoxicity, and carcinogenicity data available for bromodichloromethane suggest that it has most of the toxicologic characteristics of a human carcinogen as defined in the publications of Tennant and Shelby [277,278].

Bromate is a trans-species carcinogen, and it induces tumors at multiple sites: kidney, thyroid, and mesothelioma. As summarized in Table 18, it induces both gene and chromosomal mutation both *in vivo* and *in vitro*. A role for oxidative damage has been indicated for the induction of renal tumors by bromate (see Section 4.3.3). Collectively, these occurrence, genotoxicity, and carcinogenicity data suggest that bromate has essentially all of the toxicologic characteristics of a human carcinogen discussed by Tennant and Shelby [277,278].

Dichloroacetic acid is a high-occurrence, trans-species carcinogen that induces liver tumors. It is genotoxic at a variety of endpoints and has been classified by various organizations as a possible or probable human carcinogen. The high doses required for its genotoxicity, along with other mechanistic information, have suggested that the genotoxicity of dichloroacetic acid plays a minor role in its carcinogenicity.

Dibromoacetic acid is a high-occurrence, trans-species carcinogen, producing liver and lung tumors in mice and leukemia and mesothelioma in rats. It has had limited genotoxicity testing, but it is mutagenic in bacteria and induces DNA damage in mammalian cells.

Among the unregulated DBPs, four display some or all of the toxicologic characteristics of human carcinogens: formaldehyde, acetaldehyde, MX, and NDMA (Table 18). Formaldehyde is the only DBP that has been identified as clearly carcinogenic to humans by IARC (albeit not by the U.S. EPA). It is a trans-species carcinogen, causing nasal tumors in both mice and rats exposed by inhalation, and it is genotoxic in multiple systems. It occurs at moderate levels in drinking water. However, as mentioned earlier, it is unclear whether formaldehyde volatilizes from water while showering or bathing. Thus, it is uncertain whether inhalation is a relevant exposure route for this chemical in water or for other aldehydes in water. Acetaldehyde has been tested in only one species; however, it is widely genotoxic and is possibly carcinogenic to humans by IARC's criteria; it is present at moderate levels in drinking water. Although MX is present at low to moderate levels, it is the most potent direct-acting *in vitro* mutagen of all DBPs tested. Importantly, it is the most potent rodent carcinogen of all DBPs tested, being 3× more potent than the regulated bromate. MX induces tumors at more sites (seven) than any DBP, and it is genotoxic in many

assay types. It is classified as a possible human carcinogen by IARC. Like MX, NDMA occurs at low to moderate levels (low-ng/L to sub- μ g/L levels). It is a trans-species carcinogen and induces tumors in a variety of organs via various routes of exposure. It is genotoxic in every category of test shown in Table 18.

Among this list of DBPs that have some or all of the toxicologic characteristics of human carcinogens (Table 18), four are regulated already, and four are not. The analysis presented here provides combined occurrence, genotoxicity, and carcinogenicity data that highlight the potential importance of these four unregulated DBPs with regard to human health effects. Although these DBPs have many of the toxicologic characteristics of human carcinogens, there are still significant data gaps for these compounds, e.g., MX and acetaldehyde have been tested for carcinogenicity in only one species (Table 18). Research could be directed to providing data to either confirm or deny the importance of these four unregulated DBPs and to provide additional support for the regulation of the four regulated DBPs as to their carcinogenic potential in humans exposed via drinking water.

An additional area of study involves the potential carcinogenicity of these DBPs as a function of lifestage. Oxidative metabolism by CYP2E1, for example, detoxifies some DBPs in this category, such as bromodichloromethane and MX. However, many such enzymes are not fully expressed in children until the age of one year [146]. In addition, some DBPs have been found to induce germ-cell mutations in *Drosophila*; however, no such studies have been performed in rodents. The ability of some DBPs to induce germ-cell mutations should also be investigated and evaluated for potential human hazard.

12.1.2. Emerging DBPs with moderate occurrence that are genotoxic

A second category of concern includes those emerging DBPs with moderate occurrence levels that are genotoxic; our analysis has identified 29 DBPs on this list (Table 19). One of these, chloral hydrate, is also carcinogenic via drinking water and gavage, producing pituitary and liver tumors in the mouse. Another, chloroacetaldehyde, induced liver tumors in mice. Thus, these are unregulated DBPs for which there is some evidence for genotoxicity (and even carcinogenicity) and that are in drinking water at concentrations similar to those of many of the regulated DBPs, including those that have the features of human carcinogens (Table 18). Such compounds seem deserving of further study with regard to occurrence,

Table 19
Unregulated genotoxic DBPs with moderate occurrence

Occurrence ^a	DBP ^b
****	Trichloronitromethane (chloropicrin) Tribromoacetic acid Chloral hydrate
***	Dibromonitromethane Bromodichloronitromethane Dibromochloronitromethane Tribromochloronitromethane Iodoacetic acid Iodoform Bromoiodoacetic acid 2-Iodo-3-methylbutenedioic acid Chloroacetaldehyde Dichloromethane Ten haloamides Six haloacetoneitriles

^a ****Sub- to low- μ g/L levels; ****low- μ g/L levels.

^b Formaldehyde and acetaldehyde also are in this category of DBPs; however, they are listed in Table 17 as likely human carcinogens.

genotoxicity, and mechanisms of action. Depending on the results, some of these DBPs may be justified for evaluation for rodent carcinogenicity.

12.1.3. Emerging DBPs with moderate occurrence and no toxicology data

A third list that derives from our analysis is composed of those emerging DBPs that are present at moderate concentrations in drinking water but for which there are little or no toxicology data. Among the 14 compounds on this list (Table 20), almost none have

Table 20
Unregulated DBPs with moderate occurrence but with little or no toxicology data

Occurrence ^a	DBP
****	Bromochloroacetic acid ^b Bromodichloroacetic acid ^b Dibromochloroacetic acid ^b
***	Dichloriodomethane Bromodichloriodomethane Dibromiodomethane Chlorodiodomethane Bromodiodomethane Bromodichloroacetoneitrile Dibromochloroacetoneitrile Tribromoacetoneitrile Dichloroacetaldehyde Bromochloroacetaldehyde Tribromoacetaldehyde

^a ****Sub- to low- μ g/L levels; ****low- μ g/L levels.

^b These are rodent carcinogens; however, they have not been tested for genotoxicity.

genotoxicity data or any other types of toxicology data. Nonetheless, three of them are present at concentrations similar to those of some regulated DBPs. Despite a lack of genotoxicity data, two DBPs on this list, bromochloroacetic acid and bromodichloroacetic acid, have recently been shown to induce tumors in the mouse via drinking-water exposure (A.B. DeAngelo, personal communication). Table 19 provides guidance for prioritizing any further testing of unregulated DBPs beyond those DBPs in Table 19, for which there is already some evidence of genotoxic effect. Below, we discuss additional research needs and some approaches to further testing.

12.2. Systematic generation of quantitative genotoxicity data for classes of DBPs

Although the genotoxicity of DBPs has been studied for more than 70 years, until recently there has been little systematic effort to evaluate all the members of various chemical classes in the same manner in a set of genotoxicity assays. In fact, such systematic data were first published for the regulated haloacetic acids in *Salmonella* in 2002 [77] with a comparison of their chronic cytotoxicity and genotoxicity in mammalian cells [15]. A systematic analysis (i.e., a study in the same assay under the same conditions) of the eight chlorinated and brominated halomethanes and halonitromethanes (in *Salmonella*) was not published until 2004 [121,122]. The lack of such a systematically generated database has resulted not only in gaps in the literature, but it has prevented any comparison of the genotoxic potencies of compounds either within a chemical class or among classes within a single assay. Despite years of study, the genotoxicity profile of some of the most important regulated DBPs is incomplete (Table 18), and the genotoxicity data available for those DBPs that are genotoxic and occur at moderate levels (Table 19) consist largely of just two endpoints: mutagenicity in *Salmonella* and DNA damage in mammalian cells.

The most complete data base of DBPs evaluated systematically for genotoxicity is that generated in the laboratory of Plewa et al. for >60 DBPs for chronic cytotoxicity and DNA damage (SCGE assay) in CHO cells [62]. The analysis encompasses the brominated, chlorinated, and many of the iodinated analogues of important DBP classes, including the haloacetic acids [11,13,15,123], halonitromethanes [12], pyrroles [14], halonitriles [10], halomethanes [62,129], MX, and bromate [15], and the haloacetamides [159]. These studies also include an analysis of the structure–activity

relationships of the DBPs within each chemical class. In these studies on mammalian cells, the most genotoxic of the regulated DBPs was bromoacetic acid [11], whereas the most genotoxic of the unregulated DBPs was iodoacetic acid [11,62]. Although both DBPs are haloacetic acids, the overall cytotoxicity/genotoxicity of the entire chemical class is dependent on the combined toxicity of its members. Using a combined toxicity index [10] that integrates the chronic cytotoxicity and genotoxicity of agents of each chemical class, the rank order of combined cytotoxicity and genotoxicity index of the DBP classes was halonitromethanes > haloacetamides > haloacetanitriles > haloacetic acids > halomethanes [62] (Genotoxicity presented in Fig. 6).

From the CHO cell DBP database, the impact of the halogen atom on a set of matched compounds was also determined. The genotoxicity ranking as a function of the halogen for the monohalo-DBPs was $I > Br \gg Cl$. When the combined cytotoxicity and genotoxicity index values were calculated for a set of 16 DBPs with matched halogen analogues and averaged as a function of the halogen species, the order of cytotoxicity was $I \gg Br \gg Cl$ (Fig. 7). Finally, in a comparison of 26 carbon-based DBPs versus 29 nitrogenous DBPs, the nitrogenous DBPs as a class were the most toxic (Fig. 8) [62].

Likewise, studies in the laboratory of DeMarini et al. characterized the mutagenicity of the chlorinated and brominated halomethanes and halonitromethanes for mutagenicity in a variety of strains of *Salmonella* [121,122]. In addition to mutagenic potency, these studies also provided mechanistic information in the form of mutation spectra, the role of GSTT1-1 activation (which activates many of the halomethanes but none of the halonitromethanes), and structure–activity analyses. The types of systematic, analytical, and biological approaches described above, combined with occurrence data, will produce cytotoxicity and genotoxicity data bases that will help identify the most toxicologically important DBPs for more detailed and expensive *in vivo* studies. Application of this approach to the DBPs and related DBPs in Tables 19 and 20 would provide useful guidance on managing the health risks of drinking-water containing these DBPs.

12.3. Studies on the route of exposure and the role of genotype

As mentioned previously, there are concerns that, with the exceptions of bromodichloromethane and bromoform, the regulated DBPs produce primarily liver cancer

in rodents (Table 7) rather than the bladder and colon cancers observed in human epidemiology studies. The route of exposure (e.g., inhalation and dermal absorption) may be an important determining factor on blood levels and tissue target dose [8,71,73,279] (<http://cfpub.epa.gov/ncea/cfm/recorddisplay.cfm?deid=153303>).

The first and only epidemiologic study to stratify route of exposure with regard to drinking water and cancer risk was published in 2007 [32]. In a case-control bladder cancer study of the effects of route of exposure to THMs from chlorinated drinking water, Villanueva et al. [32,41] found a 2-fold increased risk for bladder cancer among men who showered or bathed with chlorinated water irrespective of whether the men drank chlorinated or bottled water. Genotyping showed that most of the risk was among people who had the glutathione-S-transferase-theta (*GSTT1-1*) gene [35]. This is consistent with studies done 10 years ago showing that some THMs other than chloroform were activated to mutagens by the GSTT1-1 enzyme in a transgenic strain of *Salmonella* bacteria containing the rat *GSTT1-1* gene [60,63]. These results implicate the brominated THMs (and possibly other DBPs that are activated by GSTT1-1) in the etiology of drinking-water-associated bladder cancer in humans.

The same study by Villanueva et al. [32] also included the first epidemiologic study of swimmers and cancer; they found that life-long swimmers had a 1.6-fold increased risk for bladder cancer, providing further support for the important role of inhalation and dermal exposure—most likely to some of the THMs. This study should be replicated and expanded to confirm these important observations regarding route of exposure and genotype. As reviewed recently [73], studies of swimmers might also contribute to an understanding of the health effects associated with chlorinated water via dermal/inhalation exposure.

The mechanistic basis for the role of dermal/inhalation exposure has been explored to a limited extent. In rats, the levels of GSTT1-1 enzyme available to activate one of these THMs, bromodichloromethane (BDCM), relative to the levels of the cytochrome enzyme CYP2E1 to inactivate BDCM, were greater in the kidney and large intestine than in the liver [64]. Studies in humans have found higher levels of THMs in the blood when people are exposed to chlorinated water via showering (dermal/inhalation) than when exposed orally [280]. A recent pharmacokinetic study in humans found that bromodichloromethane produced mutagenic urine by either oral or dermal exposure, but dermal exposure resulted in blood levels 25–130× higher than those from oral exposure [71].

These studies have led to the proposal [64] that oral exposure to the THMs results in the inactivation and elimination of the THMs by first-pass metabolism in the liver before the THMs can reach the systemic circulation. In contrast, dermal or inhalation exposure would result in the THMs entering the blood stream directly (by-passing the liver) and being distributed at high concentrations to various organs throughout the body. In those organs in which cancer has been observed, such as the bladder, the THMs other than chloroform might then be activated to mutagens by the GSTT1-1 enzyme, initiating the production of cancer. Such a mechanism provides support for the recent observations of Villanueva et al. [32] and Cantor et al. [35] described above indicating that the increased risk for bladder cancer associated with chlorinated water may be due to dermal/inhalation exposure by showering, bathing, and swimming [73].

A recent case-control study found that water intake (oral consumption) was inversely associated with bladder cancer risk and was unrelated to the estimated levels of THM exposure [311]. This provides support for the notion that fluid intake “flushes” the bladder of mutagens and carcinogens, and it suggests that the bladder cancer risk associated with drinking water from other studies may reflect dermal/inhalation exposure rather than oral exposure.

These results from newly published epidemiologic and other human studies set the stage for a research agenda exploring the role of dermal (and inhalation) exposure of at least the brominated THMs, especially bromodichloromethane. Currently, no dermal studies of any DBP have been performed in rodents. Nonetheless, a DBP such as bromodichloromethane might provide an excellent model to investigate the health effects of dermal exposure. Among all DBPs, formaldehyde and acetaldehyde were carcinogenic via inhalation exposure (Table 17) and exhibited most or all of the toxicologic features of human carcinogens (Table 18). Chloroform, a regulated DBP, was carcinogenic via combined inhalation and drinking-water exposure (Table 7). As noted earlier, showering and bathing (and swimming) result in larger volume exposures than does drinking, and higher concentrations of some of the volatile DBPs were observed in blood from dermal/inhalation exposure (e.g., THMs) compared to oral ingestion.

The new human studies [32,71] also support earlier findings in *Salmonella* of a role of genotype (at least *GSTT1-1*) in modifying the health effects from exposure to some THMs. Approximately 75% of the U.S. population has the *GSTT1-1* gene, and if further studies confirm that having this genotype enhances the risk for

bladder cancer from exposure to some of the THMs in drinking water, then such information will help inform the management of the health effects associated with drinking water. Little systematic effort has been made to explore the role of genotype and health risks associated with drinking water, and these early studies suggest the need for further investigations in this area. Thus, genotype and route-of-exposure studies might help to resolve the current lack of concordance between DBP-induced tumors in rodents and epidemiologic findings in humans exposed to drinking water.

12.4. Chemical identification of the unknown fraction of drinking water

Although 600 DBPs have been identified already, >50% of the total organic halide (TOX) formed during the chlorination of drinking water [19], and >50% of the assimilable organic carbon (AOC) formed during ozonation of drinking water have not been identified as specific compounds [20]. Obviously, there is also nothing known about the potential toxicity of the many unidentified DBPs in drinking water. Additional analytical studies are needed to guide toxicological studies and to provide additional information about the role of various disinfection methods on the production of various types and classes of DBPs [281].

12.5. Evaluate DBPs from alternative disinfection methods

It is especially important to investigate DBPs formed by alternative disinfectants because more water-treatment plants in the U.S. are changing from chlorine to alternative disinfectants to meet requirements of the new regulations. Beyond the three most popular alternative disinfectants (chloramine, ozone, and chlorine dioxide), there is also a move to non-chemical disinfection, such as UV irradiation and membrane technology. UV irradiation is sometimes presented as a DBP-free disinfectant, but it has the potential to form hydroxyl radicals in water (as ozone does), which can produce oxygen-containing DBPs.

Membrane filtration may not be the panacea either. Although membrane filtration appears to be a promising non-chemical means of disinfection, there will need to be a post-filtration disinfectant added to the water to maintain disinfection in the distribution system. Furthermore, the use of membranes (particularly reverse-osmosis membranes used in desalination plants with seawater) can increase the bromide levels and cause unexpected shifts to brominated DBPs when the

post-disinfectant is added. As we have described in this review, the brominated DBPs are generally more cytotoxic, genotoxic, and carcinogenic than the chlorinated DBPs.

12.6. Evaluate source-water contamination

As reviewed in Section 8, recent studies have used mutagenicity assays (primarily *Salmonella*) and chemical analyses to identify DBPs that result from the reaction of chlorine or other disinfectants with industrial contaminants in the source water. The studies reviewed here have illustrated the power of bioassay-directed fractionation and chemical analysis to identify and characterize contaminants in source waters that may account for much of the mutagenic activity of the resulting drinking water. Application of these methodologies may reveal that the problem of anthropogenic contamination of source water, resulting in mutagenic drinking water, may not be confined to the specialized cases reviewed here.

12.7. Complex mixture studies

Finally, we emphasize again the importance of viewing drinking water as a complex mixture. Despite the relatively weak carcinogenic potency of most of the DBPs, people are exposed to drinking/shower/bathing water as a mixture of at least 600 identified DBPs (and countless unidentified ones) via dermal, inhalation, and ingestion routes. This complexity is not reflected in any of the toxicology studies of individual DBPs. Although concentrates or extracts of drinking water have been shown to induce mammalian cell transformation *in vitro* [282], they have not shown evidence of carcinogenic effects in rodents. These *in vivo* studies involved exposure via the drinking water and did not consider inhalation or dermal routes of exposure. The tumor site differences between rodent and human studies may also reflect this lack of dermal exposure. Also, as noted previously, it is unlikely that these extracts contained volatile DBPs due to the extraction procedure used.

The full toxicological effects of the complex mixtures of DBPs present in drinking water are largely unknown except through epidemiologic studies [17]. Thus, further consideration of toxicological studies involving various routes of exposure to concentrates or extracts of drinking waters prepared by different disinfection methods from various source waters (high- vs. low-bromide content, for example), should be considered to better understand the toxicological effects of the mixture of DBPs in drinking water.

Although not reviewed here, a large number of studies over the past 30 years examined the mutagenicity of drinking-water extracts or concentrates—largely in *Salmonella*, but also in mammalian-cell assays. Only a few studies (most published a decade ago) involved drinking waters prepared by alternative disinfection methods. All showed that drinking water prepared by alternative disinfection methods was considerably less mutagenic than chlorinated drinking water. However, none of these studies systematically examined waters prepared from various types of source waters (including high-bromide/iodide source waters), and only one incorporated chemical analysis [40,275]. Thus, additional genotoxicity studies of drinking-water extracts or concentrates that explored these outstanding issues would provide additional insight into the toxicological effects associated with drinking water prepared by alternative disinfection methods.

The primary goal of water disinfection remains the same as it was in the last century: protection of human health from microbial disease. The goal of decreasing human exposure to DBPs, and thus reducing health risk from these compounds, has certainly advanced through 30 years of research, evaluation, and control. What also has emerged is a set of complicated questions that will be addressed only by carefully planned, systematic research of the type suggested by the analysis presented here.

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References

- [1] E.V. Ohanian, C.S. Mullin, J. Orme, Health effects of disinfectants and disinfection by-products: a regulatory perspective, *Water Chlorination: Chem. Environ. Impact Health Effects* 6 (1989).
- [2] S.D. Richardson, Drinking water disinfection by-products, *Encyclopedia Environ. Anal. Remed.* 3 (1998) 1398–1421.
- [3] J.J. Rook, Formation of haloforms during chlorination of natural waters, *Water Treat. Exam.* 23 (1974) 234–243.
- [4] T.A. Bellar, J.J. Lichtenbert, R.C. Kroner, The occurrence of organohalides in chlorinated drinking waters, *J. Am. Water Works Assoc.* 66 (1974) 703–706.
- [5] F.C. Kopfler, R.G. Melton, R.D. Lingg, W.E. Coleman, GC/MS determination of volatiles for the national organics reconnaissance survey (NORS) of drinking water, in: L.H. Keith (Ed.), *Identification and Analysis of Organic Pollutants in Water*, Ann Arbor Science, Ann Arbor, MI, 1976, pp. 87–104.
- [6] National Cancer Institute, Report on the carcinogenesis bioassay of chloroform (CAS no. 67-66-3), National Cancer Institute, TR-000 NTIS Rpt No. PB264018, Bethesda, MD, 1976.
- [7] J.C. Loper, Mutagenic effects of organic compounds in drinking water, *Mutat. Res.* 76 (1980) 241–268.
- [8] S.D. Richardson, J.E. Simmons, G. Rice, Disinfection byproducts: the next generation, *Environ. Sci. Technol.* 36 (2002) 198A–205A.
- [9] S.W. Krasner, H.S. Weinberg, S.D. Richardson, S.J. Pastor, R. Chinn, M.J. Scrimanti, G.D. Onstad, A.D. Thruston Jr., The occurrence of a new generation of disinfection by-products, *Environ. Sci. Technol.* 40 (2006) 7175–7185.
- [10] M.G. Muellner, E.D. Wagner, K. McCalla, S.D. Richardson, Y.T. Woo, M.J. Plewa, Haloacetonitriles vs. regulated haloacetic acids: are nitrogen containing DBPs more toxic? *Environ. Sci. Technol.* 41 (2007) 645–651.
- [11] E. Cemeli, E.D. Wagner, D. Anderson, S.D. Richardson, M.J. Plewa, Modulation of the cytotoxicity and genotoxicity of the drinking water disinfection byproduct iodoacetic acid by suppressors of oxidative stress, *Environ. Sci. Technol.* 40 (2006) 1878–1883.
- [12] M.J. Plewa, E.D. Wagner, P. Jazwierska, S.D. Richardson, P.H. Chen, A.B. McKague, Halonitromethane drinking water disinfection byproducts: chemical characterization and mammalian cell cytotoxicity and genotoxicity, *Environ. Sci. Technol.* 38 (2004) 62–68.
- [13] M.J. Plewa, E.D. Wagner, S.D. Richardson, A.D. Thruston Jr., Y.T. Woo, A.B. McKague, Chemical and biological characterization of newly discovered iodoacid drinking water disinfection byproducts, *Environ. Sci. Technol.* 38 (2004) 4713–4722.
- [14] S.D. Richardson, A.D. Thruston Jr., C. Rav-Acha, L. Groisman, I. Popilevsky, O. Juraev, V. Glezer, A.B. McKague, M.J. Plewa, E.D. Wagner, Tribromopyrrole, brominated acids, and other disinfection byproducts produced by disinfection of drinking water rich in bromide, *Environ. Sci. Technol.* 37 (2003) 3782–3793.
- [15] M.J. Plewa, Y. Kargalioglu, D. Vanker, R.A. Minear, E.D. Wagner, Mammalian cell cytotoxicity and genotoxicity analysis of drinking water disinfection by-products, *Environ. Mol. Mutagen.* 40 (2002) 134–142.
- [16] R.J. Bull, S.W. Krasner, P.A. Daniel, R.D. Bull, Health Effects and Occurrence of Disinfection By-products, AWWA Research Foundation, Denver, CO, 2001.
- [17] IARC, Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Drinking-water Disinfectants and Contaminants,

- Including Arsenic, vol. 84, International Agency for Research on Cancer, Lyon, France, 2004
- [18] G.A. Boorman, V. Dellarco, J.K. Dunnick, R.E. Chapin, S. Hunter, F. Hauchman, H. Gardner, M. Cox, R.C. Sills, Drinking water disinfection byproducts: review and approach to toxicity evaluation, *Environ. Health Perspect.* 107 (1999) 207–217.
- [19] S.W. Krasner, B.M. Coffey, P.A. Hacker, C.J. Hwang, C. Kuo, A.A. Mofidi, M.J. Scimmenti, International working group on biodegradable organic matter in drinking water, in: Proceedings of the Fourth International BOM Conference, Waterloo, Canada, 1996.
- [20] A.A. Stevens, L.A. Moore, C.J. Slocum, B.L. Smith, D.R. Seeger, J.C. Ireland, Disinfection By-products: Current Perspectives, American Water Works Association, Denver, CO, 1989.
- [21] U.S. Environmental Protection Agency, National interim primary drinking water regulations: control of trihalomethanes in drinking water: final rules, Fed. Reg. 44 (1979) 68624–68705.
- [22] U.S. Environmental Protection Agency, National primary drinking water regulations: disinfectants and disinfection byproducts; final rule, Fed. Reg. 63 (1998) 69390–69476.
- [23] M.J. McGuire, J.L. McLain, A. Obolensky, Information Collection Rule Data Analysis, AWWA Foundation and AWWA, Denver, CO, 2002.
- [24] U.S. Environmental Protection Agency, National primary drinking water regulations: stage 2 disinfectants and disinfection byproducts rule, Fed. Reg. 71 (2006) 387–493.
- [25] Y. Kurokawa, S. Aoki, Y. Matsushima, N. Takamura, T. Imazawa, Y. Hayashi, Dose–response studies on the carcinogenicity of potassium bromate in F344 rats after long-term oral administration, *J. Natl. Cancer Inst.* 77 (1986) 977–982.
- [26] U.S. Environmental Protection Agency, IRIS—Integrated Risk Information System, <http://www.epa.gov/iriswebp/iris/index/html>, 2003.
- [27] H.S. Weinberg, S.W. Krasner, S.D. Richardson, A.D. Thruston Jr., The occurrence of disinfection by-products (DBPs) of health concern in drinking water: results of a nationwide DBP occurrence study, EPA/600/R02/068 (2002).
- [28] S.D. Richardson, A.D. Thruston Jr., T.V. Caughran, P.H. Chen, T.W. Collette, T.L. Floyd, K.M. Schenck, B.W. Lykins Jr., G. Sun, G. Majetich, Identification of new drinking water disinfection byproducts formed in the presence of bromide, *Environ. Sci. Technol.* 33 (1999) 3378–3383.
- [29] S.D. Richardson, Disinfection by-products and other emerging contaminants in drinking water, *Trends Anal. Chem.* 22 (2003) 666–684.
- [30] IARC, Monographs on the Evaluation of Carcinogenic Risks to Humans. Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals, vol. 63, International Agency for Research on Cancer, Lyon, France, 1995.
- [31] K.P. Cantor, Drinking water and cancer, *Cancer Causes Control* 8 (1997) 292–308.
- [32] C.M. Villanueva, K.P. Cantor, J.O. Grimalt, N. Malats, D. Silverman, A. Tardon, R. Garcia-Closas, C. Serra, A. Carrato, G. Castano-Vinyals, R. Marcos, N. Rothman, F.X. Real, M. Dosemeci, M. Kogevinas, Bladder cancer and exposure to water disinfection by-products through ingestion, bathing, showering and swimming in pools, *Am. J. Epidemiol.* 165 (2007) 148–156.
- [33] M. Koivusalo, J.J. Jaakkola, T. Vartiainen, T. Hakulinen, S. Karjalainen, E. Pukkala, J. Tuomisto, Drinking water mutagenicity and gastrointestinal and urinary tract cancers: an ecological study in Finland, *Am. J. Public Health* 84 (1994) 1223–1228.
- [34] G.E. Bove Jr., P.A. Rogerson, J.E. Vena, Case control study of the geographic variability of exposure to disinfectant byproducts and risk for rectal cancer, *Int. J. Health Geogr.* 6 (2007) 18.
- [35] K.P. Cantor, C. Villanueva, M. Garcia-Closas, D. Silverman, F.X. Real, M. Dosemeci, N. Malats, M. Yeager, R. Welch, S. Chanock, Bladder cancer, disinfection byproducts, and markers of genetic susceptibility in a case–control study from Spain, *Epidemiology* 17 (2006) S150.
- [36] C. Chevrier, B. Junod, S. Cordier, Does ozonation of drinking water reduce the risk of bladder cancer? *Epidemiology* 15 (2004) 605–614.
- [37] D.M. DeMarini, A. Abu-Shakra, C.F. Felton, K.S. Patterson, M.L. Shelton, Mutation spectra in *Salmonella* of chlorinated, chloraminated, or ozonated drinking water extracts: comparison to MX, *Environ. Mol. Mutagen.* 26 (1995) 270–285.
- [38] D.K. Noot, W.B. Anderson, S.A. Daignault, D.T. Williams, P.M. Huck, Evaluating treatment processes with the Ames mutagenicity assay, *J. Am. Water Works Assoc.* 81 (1989) 87–102.
- [39] K.S. Patterson, B.W. Lykins Jr., S.D. Richardson, Mutagenicity of drinking water following disinfection, *Aqua-J. Water Supply: Res. Technol.* 44 (1995) 1–9.
- [40] L.D. Claxton, R. Pegram, K.M. Schenck, J.E. Simmons, S.H. Warren, Integrated disinfection byproducts research: *Salmonella* mutagenicity of water concentrates disinfected by chlorination and ozonation/postchlorination, *J. Toxicol. Environ. Health*, in press.
- [41] C.M. Villanueva, K.P. Cantor, J.O. Grimalt, G. Castaño-Vinyals, N. Malats, D. Silverman, A. Tardon, R. Garcia-Closas, C. Serra, A. Carrato, Assessment of lifetime exposure to trihalomethanes through different routes, *Occup. Environ. Med.* 63 (2006) 273–277.
- [42] K. Waller, S.H. Swan, G. DeLorenze, B. Hopkins, Trihalomethanes in drinking water and spontaneous abortion, *Epidemiology* 9 (1998) 134–140.
- [43] S.H. Swan, K. Waller, B. Hopkins, G. Windham, L. Fenster, C. Schaefer, R.R. Neutra, A prospective study of spontaneous abortion: relation to amount and source of drinking water consumed in early pregnancy, *Epidemiology* 9 (1998) 126–133.
- [44] K. Waller, S.H. Swan, G.C. Windham, L. Fenster, Influence of exposure assessment methods on risk estimates in an epidemiologic study of total trihalomethane exposure and spontaneous abortion, *J. Exp. Anal. Environ. Epidemiol.* 11 (2001) 522–531.
- [45] M.J. Nieuwenhuijsen, M.B. Toledano, N.E. Eaton, J. Fawell, P. Elliott, Chlorination disinfection byproducts in water and their association with adverse reproductive outcomes: a review, *Occup. Environ. Med.* 57 (2000) 73–85.
- [46] M.D. Kramer, C.F. Lynch, P. Isacson, J.W. Hanson, The association of waterborne chloroform with intrauterine growth retardation, *Epidemiology* 3 (1992) 407–413.
- [47] A. Aschengrau, S. Zierler, A. Cohen, Quality of community drinking water and the occurrence of late adverse pregnancy outcomes, *Arch. Environ. Health* 48 (1993) 105–113.
- [48] F.J. Bove, M.C. Fulcomer, J.B. Klotz, J. Esmart, E.M. Dufficy, J.E. Savrin, Public drinking water contamination and birth outcomes, *Am. J. Epidemiol.* 141 (1995) 850–862.
- [49] D.A. Savitz, K.W. Andrews, L.M. Pastore, Drinking water and pregnancy outcome in central North Carolina: source, amount, and trihalomethane levels, *Environ. Health Perspect.* 103 (1995) 592–596.

- [50] M.D. Gallagher, J.R. Nuckols, L. Stallones, D.A. Savitz, Exposure to trihalomethanes and adverse pregnancy outcomes, *Epidemiology* 9 (1998) 484–489.
- [51] P. Magnus, J.J. Jaakkola, A. Skrondal, J. Alexander, G. Becher, T. Krogh, E. Dybing, Water chlorination and birth defects, *Epidemiology* 10 (1999) 513–517.
- [52] J.B. Klotz, L.A. Pynch, Neural tube defects and drinking water disinfection by-products, *Epidemiology* 10 (1999) 383–390.
- [53] L. Dodds, W. King, C. Woolcott, J. Pole, Trihalomethanes in public water supplies and adverse birth outcomes, *Epidemiology* 10 (1999) 233–237.
- [54] W.D. King, L. Dodds, A.C. Allen, Relation between stillbirth and specific chlorination by-products in public water supplies, *Environ. Health Perspect.* 108 (2000) 883–886.
- [55] L. Dodds, W.D. King, Relation between trihalomethane compounds and birth defects, *Occup. Environ. Med.* 58 (2001) 443–446.
- [56] D.A. Savitz, P.C. Singer, K.E. Hartmann, A.H. Herring, H.S. Weinberg, C. Makarushka, C. Hoffman, R. Chan, R. Macle-hose, *Drinking Water Disinfection By-products and Pregnancy Outcome*, AWWA Research Foundation, Denver, CO, 2005.
- [57] W. Glaze, H. Weinberg, *Identification and Occurrence of Ozonation By-products in Drinking Water*, AWWA Research Foundation, Denver, CO, 1993.
- [58] D.J. Munch, M.A. Feige, H.J. Brass, The analysis of purgeable compounds in the national organics monitoring survey by gas chromatography/mass spectrometry, in: *Proceedings of the AWWA Water Quality Technology Conference*, Denver, CO, 1977.
- [59] IARC, *Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances*, vol. 73, International Agency for Research on Cancer, Lyon, France, 1999.
- [60] R.A. Pegram, M.E. Andersen, S.H. Warren, T.M. Ross, L.D. Claxton, Glutathione S-transferase-mediated mutagenicity of trihalomethanes in *Salmonella typhimurium*: contrasting results with bromodichloromethane or chloroform, *Toxicol. Appl. Pharmacol.* 144 (1997) 183–188.
- [61] IARC, *Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide*, vol. 71, International Agency for Research on Cancer, Lyon, France, 1999.
- [62] M.J. Plewa, E.D. Wagner, M.G. Muellner, K.M. Hsu, S.D. Richardson, Comparative mammalian cell toxicity of N-DBPs and C-DBPs, in: T. Karanfil, S.W. Krasner, P. Westerhoff, Y. Xie (Eds.), *Occurrence, Formation, Health Effects and Control of Disinfection By-products in Drinking Water*, American Chemical Society, Washington, DC, in press.
- [63] D.M. DeMarini, M.L. Shelton, S.H. Warren, T.M. Ross, J.Y. Shim, A.M. Richard, R.A. Pegram, Glutathione S-transferase-mediated induction of GC → AT transitions by halo-methanes in *Salmonella*, *Environ. Mol. Mutagen.* 30 (1997) 440–447.
- [64] M.K. Ross, R.A. Pegram, In vitro biotransformation and genotoxicity of the drinking water disinfection byproduct bromodichloromethane: DNA binding mediated by glutathione transferase theta 1-1, *Toxicol. Appl. Pharmacol.* 195 (2004) 166–181.
- [65] IARC, *Monographs on the Evaluation of Carcinogenic Risks to Humans. Coffee, Tea, Mate, Methylxanthines and Methyl-glyoxal*, vol. 51, International Agency for Research on Cancer, Lyon, France, 1991.
- [66] K. Nagano, H. Kano, H. Arito, S. Yamamoto, T. Matsushima, Enhancement of renal carcinogenicity by combined inhalation and oral exposures to chloroform in male rats, *J. Toxicol. Environ. Health Part A* 69 (2006) 1827–1842;
- (a) A.B. DeAngelo, D.R. Geter, D.W. Rosenberg, C.K. Crary, M.H. George, The induction of aberrant crypt foci (CF) in the colons of rats by trihalomethanes administered in the drinking water, *Cancer Lett.* 187 (2002) 25–31;
- (b) D.R. Geter, M.H. George, T.M. Moore, S. Kilburn, G. Huggins-Clark, A.B. DeAngelo, Vehicle and mode of administration effects on the induction of aberrant crypt foci in the colons of male F344/N rats exposed to bromodichloromethane, *J. Toxicol. Environ. Health A* 67 (2004) 23–29;
- (c) D.R. Geter, M.H. George, T.M. Moore, S.R. Kilburn, G. Huggins-Clark, A.B. DeAngelo, The effects of a high animal fat diet on the induction of aberrant crypt foci in the colons of male F344/N rats exposed to trihalomethanes in the drinking water, *Toxicol. Lett.* 147 (2004) 245–252;
- (d) D.R. Geter, T.M. Moore, M.H. George, S.R. Kilburn, J.W. Allen, G.M. Nelson, E. Winkfield, A.B. DeAngelo, Tribromo-methane exposure and dietary folate deficiency in the formation of aberrant crypt foci in the colons of F344/N rats, *Food Chem. Toxicol.* 43 (2005) 1405–1412.
- [67] D.C. Wolf, B.E. Butterworth, Risk assessment of inhaled chloroform based on its mode of action, *Toxicol. Pathol.* 25 (1997) 49–52.
- [68] G.C. Hard, G.A. Boorman, D.C. Wolf, Re-evaluation of the 2-year chloroform drinking water carcinogenicity bioassay in Osborne–Mendel rats supports chronic renal tubule injury as the mode of action underlying the renal tumor response, *Toxicol. Sci.* 53 (2000) 237–244.
- [69] M.V. Templin, J.L. Larson, B.E. Butterworth, K.C. Jamison, J.R. Leininger, S. Mery, K.T. Morgan, B.A. Wong, D.C. Wolf, A 90-day chloroform inhalation study in F-344 rats: profile of toxicity and relevance to cancer studies, *Toxicol. Sci.* 32 (1996) 109–125.
- [70] M.V. Templin, J.L. Larson, B.E. Butterworth, K.C. Jamison, J.R. Leininger, S. Mery, K.T. Morgan, B.A. Wong, D.C. Wolf, A 90-day chloroform inhalation study in F344 rats: profile of toxicity and relevance to cancer studies, *Fundam. Appl. Toxicol.* 32 (1996) 109–125.
- [71] T.L. Leavens, B.C. Blount, D.M. DeMarini, M.C. Madden, J.L. Valentine, M.W. Case, L.K. Silva, S.H. Warren, N.M. Hanley, R.A. Pegram, Disposition of bromodichloromethane in humans following oral and dermal exposure, *Toxicol. Sci.* 99 (2007) 432–445.
- [72] M.K. Ross, R.A. Pegram, Glutathione transferase theta 1-1-dependent metabolism of the water disinfection byproduct bromodichloromethane, *Chem. Res. Toxicol.* 16 (2003) 216–226.
- [73] C. Zwiener, S.D. Richardson, D.M. DeMarini, T. Grummt, T. Glauner, F.H. Frimmel, Drowning in disinfection byproducts? Assessing swimming pool water, *Environ. Sci. Technol.* 41 (2007) 363–372.
- [74] X. Zhang, S. Echigo, R.A. Minear, M.J. Plewa, Characterization and comparison of disinfection by-products of four major disinfectants, in: S.E. Barrett, S.W. Krasner, G.L. Amy (Eds.), *Natural Organic Matter and Disinfection By-products: Characterization and Control in Drinking Water*, American Chemical Society, Washington, DC, 2000, pp. 299–314.
- [75] C.A. Colclough, J.D. Johnson, R.F. Christman, D.S. Millington, Organic reaction products of chlorine dioxide and natural aquatic fulvic acids, in: R.L. Jolley (Ed.), *Water Chlorination:*

- Environmental Impact and Health Effects, Ann Arbor Science, Ann Arbor, MI, 1983, pp. 219–229.
- [76] S. Monarca, M. Rizzoni, B. Gustavino, C. Zani, A. Alberti, D. Feretti, I. Zerbini, Genotoxicity of surface water treated with different disinfectants using in situ plant tests, *Environ. Mol. Mutagen.* 41 (2003) 353–359.
- [77] Y. Kargalioglu, B.J. McMillan, R.A. Minear, M.J. Plewa, Analysis of the cytotoxicity and mutagenicity of drinking water disinfection by-products in *Salmonella typhimurium*, *Teratogen. Carcinogen. Mutagen.* 22 (2002) 113–128.
- [78] C.A. Hilliard, M.J. Armstrong, C.I. Bradt, R.B. Hill, S.K. Greenwood, S.M. Galloway, Chromosome aberrations in vitro related to cytotoxicity of nonmutagenic chemicals and metabolic poisons, *Environ. Mol. Mutagen.* 31 (1998) 316–326.
- [79] D. DeMarini, E. Perry, M. Shelton, Dichloroacetic acid and related compounds: induction of prophage in *E. coli* and mutagenicity and mutation spectra in *Salmonella* TA100, *Mutagenesis* 9 (1994) 429–437.
- [80] S. Giller, F. Le Curieux, F. Erb, D. Marzin, Comparative genotoxicity of halogenated acetic acids found in drinking water, *Mutagenesis* 12 (1997) 321–328.
- [81] K. Harrington-Brock, Mutagenicity of three disinfection by-products: di- and trichloroacetic acid and chloral hydrate in L5178Y/TK-3.7. 2C mouse lymphoma cells, *Mutat. Res.* 413 (1998) 265–276.
- [82] A.W. Fox, X. Yang, H. Murli, T.E. Lawlor, M.A. Cifone, F.E. Reno, Absence of mutagenic effects of sodium dichloroacetate, *Fundam. Appl. Toxicol.* 32 (1996) 87–95.
- [83] J.C. Fuscoe, A.J. Afshari, M.H. George, A.B. DeAngelo, R.R. Tice, T. Salman, J.W. Allen, In vivo genotoxicity of dichloroacetic acid: evaluation with the mouse peripheral blood micronucleus assay and the single cell gel assay, *Environ. Mol. Mutagen.* 27 (1996) 1–9.
- [84] L.W. Chang, F.B. Daniel, A.B. DeAngelo, Analysis of DNA strand breaks induced in rodent liver in vivo, hepatocytes in primary culture, and a human cell line by chlorinated acetic acids and chlorinated acetaldehydes, *Environ. Mol. Mutagen.* 20 (1992) 277–288.
- [85] S.A. Leavitt, A.B. DeAngelo, M.H. George, J.A. Ross, Assessment of the mutagenicity of dichloroacetic acid, *Carcinogenesis* 18 (1997) 2101–2106.
- [86] A.B. DeAngelo, F.B. Daniel, B.M. Most, G.R. Olson, Failure of monochloroacetic acid and trichloroacetic acid administered in the drinking water to produce liver cancer in male F344/N rats, *J. Toxicol. Environ. Health.* 52 (1997) 425–445.
- [87] National Toxicology Program, Toxicology and Carcinogenesis Studies of Monochloroacetic Acid (CAS no. 79-11-8) in F344/N rats and B6C3F1 Mice (Gavage Studies), vol. 396, 1992, pp. 1–245.
- [88] R.L. Melnick, A. Nyska, P.M. Foster, J.H. Roycroft, G.E. Kissling, Toxicity and carcinogenicity of the water disinfection byproduct, dibromoacetic acid, in rats and mice, *Toxicology* 230 (2007) 126–136.
- [89] J.H. Carter, H.W. Carter, J.A. Deddens, B.M. Hurst, M.H. George, A.B. DeAngelo, A 2-year dose–response study of lesion sequences during hepatocellular carcinogenesis in the male B6C3F(1) mouse given the drinking water chemical dichloroacetic acid, *Environ. Health Perspect.* 111 (2003) 53–64.
- [90] J. Kato-Weinstein, M.K. Lingohr, G.A. Orner, B.D. Thrall, R.J. Bull, Effects of dichloroacetate on glycogen metabolism in B6C3F1 mice, *Toxicology* 130 (1998) 141–154.
- [91] M.K. Lingohr, R.J. Bull, J. Kato-Weinstein, B.D. Thrall, Dichloroacetate stimulates glycogen accumulation in primary hepatocytes through an insulin-independent mechanism, *Toxicol. Sci.* 68 (2002) 508–515.
- [92] L. Tao, W. Wang, L. Li, P.M. Kramer, M.A. Pereira, Effect of dibromoacetic acid on DNA methylation, glycogen accumulation, and peroxisome proliferation in mouse and rat liver, *Toxicol. Sci.* 82 (2004) 62–69.
- [93] G. Gordon, B. Sloodmaekers, S. Tachiyashiki, D.W. Wood, Minimizing chlorite ion and chlorate ion in water treated with chlorine dioxide, *J. Am. Water Works Assoc.* 82 (1990) 160–165.
- [94] M. Bolyard, P.S. Fair, D.P. Hautman, Occurrence of chlorate in hypochlorite solutions used for drinking water disinfection, *Environ. Sci. Technol.* 26 (1992) 1663–1665.
- [95] M.M. Moore, T. Chen, Mutagenicity of bromate: Implications for cancer risk assessment, *Toxicology* 221 (2006) 190–196.
- [96] D.E. Levin, M. Hollstein, M.F. Christman, E.A. Schwiers, B.N. Ames, A new *Salmonella* tester strain (TA102) with AT base pairs at the site of mutation detects oxidative mutagens, *Proc. Natl. Acad. Sci. U.S.A.* 79 (1982) 7445–7449.
- [97] J.M. Poul, S. Huet, T. Godard, P. Sanders, Lack of genotoxicity of potassium iodate in the alkaline comet assay and in the cytokinesis-block micronucleus test. Comparison to potassium bromate, *Food Chem. Toxicol.* 42 (2004) 203–209.
- [98] G. Speit, S. Haupter, P. Schutz, P. Kreis, Comparative evaluation of the genotoxic properties of potassium bromate and potassium superoxide in V79 Chinese hamster cells, *Mutat. Res.* 439 (1999) 213–221.
- [99] T. Umemura, Y. Kurokawa, Etiology of bromate-induced cancer and possible modes of action—studies in Japan, *Toxicology* 221 (2006) 154–157.
- [100] Y. Kurokawa, S. Takayama, Y. Konishi, Y. Hiasa, S. Asahina, M. Takahashi, A. Maekawa, Y. Hayashi, Long-term in vivo carcinogenicity tests of potassium bromate, sodium hypochlorite, and sodium chlorite conducted in Japan, *Environ. Health Perspect.* 69 (1986) 221–235.
- [101] A.B. DeAngelo, M.H. George, S.R. Kilburn, T.M. Moore, D.C. Wolf, Carcinogenicity of potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N rats, *Toxicol. Pathol.* 26 (1998) 587–594.
- [102] K. Sai, A. Takagi, T. Umemura, R. Hasegawa, Y. Kurokawa, Relation of 8-hydroxydeoxyguanosine formation in rat kidney to lipid peroxidation, glutathione level and relative organ weight after a single administration of potassium bromate, *Jpn. J. Cancer Res.* 82 (1991) 165–169.
- [103] K. Sai, S. Uchiyama, Y. Ohno, R. Hasegawa, Y. Kurokawa, Generation of active oxygen species in vitro by the interaction of potassium bromate with rat kidney cell, *Carcinogenesis* 13 (1992) 333–339.
- [104] K. Sai, C.A. Tyson, D.W. Thomas, J.E. Dabbs, R. Hasegawa, Y. Kurokawa, Oxidative DNA damage induced by potassium bromate in isolated rat renal proximal tubules and renal nuclei, *Cancer Lett.* 87 (1994) 1–7.
- [105] T. Umemura, A. Takagi, K. Sai, R. Hasegawa, Y. Kurokawa, Oxidative DNA damage and cell proliferation in kidneys of male and female rats during 13-weeks exposure to potassium bromate (KBrO₃), *Arch. Toxicol.* 72 (1998) 264–269.
- [106] D. Delker, G. Hatch, J. Allen, B. Crissman, M. George, D. Geter, S. Kilburn, T. Moore, G. Nelson, B. Roop, R. Slade, A. Swank, W. Ward, A. DeAngelo, Molecular biomarkers of oxidative stress associated with bromate carcinogenicity, *Toxicology* 221 (2006) 158–165.

- [107] R. Miltner, The effect of chlorine dioxide on trihalomethanes in drinking water, Ph.D. Dissertation, University of Cincinnati, 1976.
- [108] K.S. Werdehoff, P.C. Singer, Chlorine dioxide effects on THMFP, TOXFP, and the formation of inorganic by-products, *J. Am. Water Works Assoc.* 79 (1987) 107–113.
- [109] M.G. Noack, R.L. Doerr, Reactions of chlorine, chlorine dioxide and mixtures thereof with humic acid, in: R.L. Jolley, H. Gorchev, D.H. Hamilton, Jr. (Eds.), *Water Chlorination: Chemistry, Environmental Impact and Health Effects*, vol. 2, Ann Arbor Science, Ann Arbor, MI, 1981, pp. 49–58.
- [110] D.J. Gates, *The Chlorine Dioxide Handbook; Water Disinfection Series*, American Water Works Association, Denver, CO, 1998.
- [111] U.S. Environmental Protection Agency, ICR Auxiliary 1 Database: Version 5.0, Query Tool—Version 2.0, Office of Water, Washington, DC, 2000.
- [112] R.C. Hoehn, C.S. Ellenberger, D.L. Gallagher, E.V. Wiseman, R.W. Benninger, A. Rosenblatt, ClO_2 and by-product persistence in a drinking water system, *J. Am. Water Works Assoc.* 95 (2003) 141–150.
- [113] H. Baribeau, M. Prevost, R. Desjardins, P. Lafrance, D.J. Gates, Chlorite and chlorate ion variability in distribution systems, *J. Am. Water Works Assoc.* 94 (2002) 96–105.
- [114] C. Korn, R.C. Andrew, M.D. Escobar, Development of chlorine dioxide-related by-product models for drinking water treatment, *Water Res.* 36 (2002) 330–342.
- [115] H. Ueno, K. Oishi, Y. Sayato, K. Nakamuro, Oxidative cell damage in kat-sod assay of oxyhalides as inorganic disinfection by-products and their occurrence by ozonation, *Arch. Environ. Contam. Toxicol.* 38 (2000) 1–6.
- [116] S.D. Richardson, A.D. Thruston Jr., T.V. Caughran, P.H. Chen, T.W. Collette, T.L. Floyd, K.M. Schenck, B.W. Lykins Jr., G. Sun, G. Majetich, Identification of new ozone disinfection byproducts in drinking water, *Environ. Sci. Technol.* 33 (1999) 3368–3377.
- [117] S.W. Krasner, R. Chinn, C.J. Hwang, S.E. Barrett, in: *Proceedings of the AWWA Water Quality Technology Conference*, AWWA, Denver, CO, 1991.
- [118] J. Hoigné, H. Bader, Formation of trichloronitromethane (chloropicrin) and chloroform in a combined ozonation/chlorination treatment of drinking water, *Water Res.* 22 (1988) 313–319.
- [119] J. Choi, S.D. Richardson, Formation of halonitromethanes in drinking water, in: *Proceedings of the International Workshop on Optimizing the Design and Interpretation of Epidemiologic Studies to Consider Alternative Disinfectants of Drinking Water*, U.S. EPA, Raleigh, NC, 2005.
- [120] P.H. Chen, S.D. Richardson, S.W. Krasner, G. Majetich, G.L. Glish, Hydrogen abstraction and decomposition of bromopicrin and other trihalogenated disinfection byproducts by GC/MS, *Environ. Sci. Technol.* 36 (2002) 3362–3371.
- [121] B. Kundu, S.D. Richardson, C.A. Granville, D.T. Shaughnessy, N.M. Hanley, P.D. Swartz, A.M. Richard, D.M. DeMarini, Comparative mutagenicity of halomethanes and halonitromethanes in *Salmonella* TA100: structure–activity analysis and mutation spectra, *Mutat. Res.* 554 (2004) 335–350.
- [122] B. Kundu, S.D. Richardson, P.D. Swartz, P.P. Matthews, A.M. Richard, D.M. DeMarini, Mutagenicity in *Salmonella* of halonitromethanes: a recently recognized class of disinfection by-products in drinking water, *Mutat. Res.* 562 (2004) 39–65.
- [123] M.J. Plewa, Y. Kargalioglu, D. Vanker, R.A. Minear, E.D. Wagner, Development of quantitative comparative cytotoxicity and genotoxicity assays for environmental hazardous chemicals, *Water Sci. Technol.* 42 (2000) 109–116.
- [124] M. Schneider, G.B. Quistad, J.E. Casida, Glutathione activation of chloropicrin in the *Salmonella* mutagenicity test, *Mutat. Res.* 439 (1999) 233–238.
- [125] S.C. Sparks, G.B. Quistad, J.E. Casida, Metabolism of [^{14}C] chloropicrin in mice, *Chem. Res. Toxicol.* 10 (1997) 1001–1007.
- [126] S.E. Sparks, G.B. Quistad, W. Li, J.E. Casida, Chloropicrin dechlorination in relation to toxic action, *J. Biochem. Mol. Toxicol.* 14 (2000) 26–32.
- [127] S. Giller, F. Le Curieux, L. Gauthier, F. Erb, D. Marzin, Genotoxicity assay of chloral hydrate and chloropicrine, *Mutat. Res.* 348 (1995) 147–152.
- [128] National Toxicology Program, NTP/NCI Bioassay of Chloropicrin for Possible Carcinogenicity, vol. 65, 1978, pp. 1–91.
- [129] S.D. Richardson, F.G. Crumley, J.J. Ellington, J.J. Evans, B.C. Blount, L.K. Silva, F.L. Cardinali, M.J. Plewa, E.D. Wagner, Occurrence and toxicity of iodo-acid and iodo-THM DBPs in chloraminated drinking water, in: *Proceedings of Symposium on Safe Drinking Water: Where Science Meets Policy*, Carolina Environmental Program, 2006.
- [130] Y. Bichsel, U. Von Gunten, Formation of iodo-trihalomethanes during disinfection and oxidation of iodide-containing waters, *Environ. Sci. Technol.* 34 (2000) 2784–2791.
- [131] Y. Bichsel, U. Von Gunten, Oxidation of iodide and hypiodous acid in the disinfection of natural waters, *Environ. Sci. Technol.* 33 (1999) 4040–4045.
- [132] P.C. Singer, Regulation of only five haloacetic acids is neither sound science nor good policy, in: *Proceedings of the Symposium on Safe Drinking Water: Where Science Meets Policy*, Carolina Environmental Program, 2006.
- [133] M.J. Plewa, E.D. Wagner, M.G. Muellner, K.M. Hsu, S.D. Richardson, Comparative mammalian cell toxicity of N-DBPs and C-DBPs, in: *Proc. Am. Chem. Soc. Meeting*, Chicago, IL, 2007.
- [134] W.E. Glaze, J.E. Henderson, G. Smith, Analysis of new chlorinated organic compounds formed by chlorination of municipal wastewater, in: R.J. Jolley (Ed.), *Water Chlorination: Environmental Impact and Health Effects*, Ann Arbor Science, Ann Arbor, MI, 1975, pp. 139–159.
- [135] H.J. Brass, M.A. Feige, T. Halloran, J.W. Mello, D. Munch, R.F. Thomas, The national organic monitoring survey: sampling and analyses for purgeable organic compounds, in: R.B. Pojasek (Ed.), *Drinking Water Quality Enhancement through Source Protection*, Ann Arbor Science, Ann Arbor, MI, 1977, pp. 393–416.
- [136] B. Cancho, F. Ventura, M. Galceran, A. Diaz, S. Ricart, Determination, synthesis and survey of iodinated trihalomethanes in water treatment processes, *Water Res.* 34 (2000) 3380–3390.
- [137] Y.T. Woo, D. Lai, J.L. McLain, M.K. Manibusan, V. Dellarco, Use of mechanism-based structure–activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products, *Environ. Health Perspect.* 110 (Suppl. 1) (2002) 75–87.
- [138] S. Landi, A. Naccarati, M.K. Ross, N.M. Hanley, L. Dailey, R.B. Devlin, M. Vasquez, R.A. Pegram, D.M. DeMarini, Induction of DNA strand breaks by trihalomethanes in primary human lung epithelial cells, *Mutat. Res.* 538 (2003) 41–50.

- [139] R. Thier, S.E. Pemble, J.B. Taylor, W.G. Humphreys, M. Persmark, B. Ketterer, F.P. Guengerich, Expression of rat glutathione S-transferase 5-5 in *Salmonella typhimurium* TA1535 leads to base pair mutations upon exposure to dihalomethanes, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 8576–8580.
- [140] L.P. Liu, K.M. Williams, F.P. Guengerich, A.E. Pegg, O-6-Alkylguanine–DNA alkyltransferase has opposing effects in modulating the genotoxicity of dibromomethane and bromomethyl acetate, Chem. Res. Toxicol. 17 (2004) 742–752.
- [141] B. Holmbom, R.H. Voss, R.D. Mortimer, A. Wong, Fractionation, isolation and characterization of Ames-mutagenic compounds in kraft chlorination effluents, Environ. Sci. Technol. 18 (1984) 333–337.
- [142] L. Kronberg, T. Vartiainen, 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-butenic acid in chlorine-treated tap waters, Mutat. Res. 206 (1988) 177–182.
- [143] L. Kronberg, B. Holmbom, M. Reunanen, L. Tikkanen, 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-oxo-butenic acid in chlorine-treated humic water and drinking water extracts, Environ. Sci. Technol. 22 (1988) 1097–1103.
- [144] N. Suzuki, J. Nakanishi, Brominated analogues of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone) in chlorinated drinking water, Chemosphere 30 (1995) 1557–1564.
- [145] J.M. Wright, J. Schwartz, T. Vartiainen, J. Maki-Paakkanen, L. Altshul, J.J. Harrington, D.W. Dockery, 3-Chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone (MX) and mutagenic activity in Massachusetts drinking water, Environ. Health Perspect. 110 (2002) 157–164.
- [146] T.A. McDonald, H. Komulainen, Carcinogenicity of the chlorination disinfection by-product MX, J. Environ. Sci. Health C: Environ. Carcinogen. Ecotoxicol. Rev. 23 (2005) 163–214.
- [147] R.T. LaLonde, L. Bu, A. Henwood, J. Fiumano, L. Zhang, Bromine-, chlorine-, and mixed halogen-substituted 4-methyl-2(5H)-furanones: synthesis and mutagenic effects of halogen and hydroxyl group replacements, Chem. Res. Toxicol. 10 (1997) 1427–1436.
- [148] A. Nishikawa, K. Sai, K. Okazaki, H.Y. Son, K. Kanki, M. Nakajima, N. Kinae, T. Nohmi, J.E. Trosko, T. Inoue, M. Hirose, MX, a by-product of water chlorination, lacks in vivo genotoxicity in gpt delta mice but inhibits gap junctional intercellular communication in rat WB cells, Environ. Mol. Mutagen. 47 (2006) 48–55.
- [149] L. Verschaeve, P. Heikkinen, G. Verheyen, U. Van Gorp, F. Boonen, F. Vander Plaetse, A. Maes, T. Kumlin, J. Maki-Paakkanen, L. Puranen, J. Juutilainen, Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo, Radiat. Res. 165 (2006) 598–607.
- [150] R.T. LaLonde, H.R. Leo, Interactive chlorine-by-bromine and hydrogen-by-hydroxyl group replacement effects in 2(5H)-furanone mutagenicity, Chem. Res. Toxicol. 7 (1994) 779–783.
- [151] J.M. Hyttinen, M. Niittykoski, K. Jansson, Lack of uniformity in the mutational spectra of chlorohydroxyfuranones in *Salmonella typhimurium* strain TA100, Mutagenesis 10 (1995) 321–323.
- [152] K. Jansson, J.M. Hyttinen, M. Niittykoski, J. Maki-Paakkanen, Mutagenicity in vitro of 3,4-dichloro-5-hydroxy-2(5H)-furanone (mucochloric acid), a chlorine disinfection by-product in drinking water, Environ. Mol. Mutagen. 25 (1995) 284–287.
- [153] K. Fekadu, W. Parzefall, L. Kronberg, R. Franzen, R. Schulte-Hermann, S. Knasmuller, Induction of genotoxic effects by chlorohydroxyfuranones, byproducts of water disinfection, in *E. coli* K-12 cells recovered from various organs of mice, Environ. Mol. Mutagen. 24 (1994) 317–324.
- [154] H. Komulainen, V.M. Kosma, S.L. Vaittinen, Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone in the rat, J. Natl. Cancer Inst. 89 (1997) 848–856.
- [155] P. Heikkinen, H. Ernst, H. Huuskonen, H. Komulainen, T. Kumlin, J. Maki-Paakkanen, L. Puranen, J. Juutilainen, No effects of radiofrequency radiation on 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone-induced tumorigenesis in female Wistar rats, Radiat. Res. 166 (2006) 397–408.
- [156] P. Hakulinen, E. Rintala, J. Maki-Paakkanen, H. Komulainen, Altered expression of connexin43 in the inhibition of gap junctional intercellular communication by chlorohydroxyfuranones in WB-F344 rat liver epithelial cells, Toxicol. Appl. Pharmacol. 212 (2006) 146–155.
- [157] J. Yuan, H. Liu, L.H. Zhou, Y.L. Zou, W.Q. Lu, Oxidative stress and DNA damage induced by a drinking-water chlorination disinfection byproduct 3-chloro-4-(dichloromethyl)-5-hydroxy-2 (5H)-furanone (MX) in mice, Mutat. Res. 609 (2006) 129–136.
- [158] S. Itoh, A. Nakano, T. Araki, Reevaluation of the toxicity of chlorinated water and the usefulness of MX as an index, J. Water Health 4 (2006) 523–531.
- [159] M.J. Plewa, M.G. Muellner, S.D. Richardson, F. Fasano, K.M. Buettner, Y.T. Woo, A.B. McKague, E.D. Wagner, Occurrence, synthesis and mammalian cell cytotoxicity and genotoxicity of haloacetamides: an emerging class of nitrogenous drinking water disinfection by-products, Environ. Sci. Technol., in press.
- [160] B. van De Water, Y. Wang, S. Asmellash, H. Liu, Y. Zhan, E. Miller, J.L. Stevens, Distinct endoplasmic reticulum signaling pathways regulate apoptotic and necrotic cell death following iodoacetamide treatment, Chem. Res. Toxicol. 12 (1999) 943–951.
- [161] H. Kigoshi, T. Ichino, N. Takada, K. Suenaga, A. Yamada, K. Yamada, D. Uemura, Isolation of tribromoacetamide from an Okinawan alga and biological activities of its analogs, Chem. Lett. 33 (2004) 98–99.
- [162] D.B. McGregor, I. Edwards, C.R. Wolf, L.M. Forrester, W.J. Caspary, Endogenous xenobiotic enzyme levels in mammalian cells, Mutat. Res. 261 (1991) 29–39.
- [163] D. Geter, L. Chang, N. Hanley, M. Ross, R. Pegram, A. DeAngelo, Analysis of in vivo and in vitro DNA strand breaks from trihalomethane exposure, J. Carcinogen. 3 (2004) 2.
- [164] D.T. Williams, G.L. LeBel, F.M. Benoit, Disinfection by-products in Canadian drinking water, Chemosphere 34 (1997) 299–316.
- [165] S.W. Krasner, M.J. McGuire, J.G. Jacangelo, N.L. Patania, K.M. Reagan, E.M. Aieta, The occurrence of disinfection by-products in United States drinking water, J. Am. Water Works Assoc. 81 (1989) 41–53.
- [166] World Health Organization, Guidelines for Drinking Water Quality, 3rd ed., 2004 http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en.
- [167] F. Le Curieux, S. Giller, L. Gauthier, F. Erb, D. Marzin, Study of the genotoxic activity of six halogenated acetonitriles, using the SOS chromotest, the Ames-fluctuation test and the newt micro-nucleus test, Mutat. Res. 341 (1995) 289–302.

- [168] F.B. Daniel, K.M. Schenck, J.K. Mattox, E.L. Lin, D.L. Haas, M.A. Pereira, Genotoxic properties of haloacetonitriles: drinking water by-products of chlorine disinfection, *Fundam. Appl. Toxicol.* 6 (1986) 447–453.
- [169] A.M. Nouraldeen, A.E. Ahmed, Studies on the mechanisms of haloacetonitrile-induced genotoxicity, *Toxicol. In Vitro* 10 (1996) 17–26.
- [170] A.A.H. Abdelaziz, S.Z. Abdelrahman, A.M. Nouraldeen, S.A. Shouman, J.P. Loh, A.E. Ahmed, Effect of glutathione modulation on molecular interaction of [¹⁴C]-chloroacetonitrile with maternal and fetal DNA, *Reprod. Toxicol.* 7 (1993) 263–272.
- [171] H.R. Schulten, M. Schnitzer, A state of the art structural concept for humic substances, *Naturwissenschaften* 80 (1993) 29–30.
- [172] W.A. Mitch, J.O. Sharp, R.R. Trussell, R.L. Valentine, L. Alvarez-Cohen, D.L. Sedlak, *N*-Nitrosodimethylamine (NDMA) as a drinking water contaminant: a review, *Environ. Eng. Sci.* 20 (2003) 389–404.
- [173] D.B. Jobb, R. Hunsinger, O. Meresz, V.Y. Taguchi, A study of the occurrence and inhibition of formation of *N*-nitrosodimethylamine (NDMA) in the Ohsweken water supply, in: *Proceedings of the Fifth National Conference on Drinking Water*, Winnipeg, Manitoba, Canada, 1992.
- [174] J.E. Graham, S.A. Andrews, G.J. Farquhar, O. Meresz, Factors affecting NDMA formation during drinking water treatment, in: *Proceedings of the AWWA Water Quality Technology Conference*, New Orleans, LA, 1995.
- [175] K.D. Kohut, S.A. Andrews, *N*-Nitrosodimethylamine formation in drinking water due to amine-based polyelectrolytes, in: *Proceedings of the AWWA Quality Technology Conference*, 2002.
- [176] S.A. Andrews, V.Y. Taguchi, Canadian issues, in: *Proceedings of the AWWA Quality Technology Conference*, 2000.
- [177] I. Najm, R.R. Trussell, NDMA formation in water and wastewater, *J. Am. Water Works Assoc.* 93 (2001) 92–98.
- [178] A. Fristachi, G. Rice, Estimation of the total daily oral intake of NDMA attributable to drinking water, *J. Water Health* 5 (2007) 341–355.
- [179] J. Choi, R.L. Valentine, Formation of *N*-nitrosodimethylamine (NDMA) from reaction of monochloramine: a new disinfection by-product, *Water Res.* 36 (2002) 817–824.
- [180] A. Wilczak, A. Assadi-Rad, H.H. Lai, L.L. Hoover, J.F. Smith, R. Berger, F. Rodigari, J.W. Beland, L.J. Lazzelle, E.G. Kinicannon, H. Baker, C.T. Heaney, Formation of NDMA in chloraminated water coagulated with DADMAC cationic polymer, *J. Am. Water Works Assoc.* 95 (2003) 94–106.
- [181] W.A. Mitch, D.L. Sedlak, Formation of *N*-nitrosodimethylamine (NDMA) from dimethylamine during chlorination, *Environ. Sci. Technol.* 36 (2002) 588–595.
- [182] I.M. Schreiber, W.A. Mitch, Occurrence and fate of nitrosamines and nitrosamine precursors in wastewater-impacted surface waters using boron as a conservative tracer, *Environ. Sci. Technol.* 40 (2006) 3203–3210.
- [183] J.W. Munch, M.V. Bassett, Method development for the analysis of *N*-nitrosodimethylamine and other *N*-nitrosamines in drinking water at low nanogram/liter concentrations using solid-phase extraction and gas chromatography with chemical ionization tandem mass spectrometry, *J. AOAC Int.* 89 (2006) 486–497.
- [184] J.W. Charrois, M.W. Arend, K.L. Froese, S.E. Hrudey, Detecting *N*-nitrosamines in drinking water at nanogram per liter levels using ammonia positive chemical ionization, *Environ. Sci. Technol.* 38 (2004) 4835–4841.
- [185] Y.Y. Zhao, J. Boyd, S.E. Hrudey, X.F. Li, Characterization of new nitrosamines in drinking water using liquid chromatography tandem mass spectrometry, *Environ. Sci. Technol.* 40 (2006) 7636–7641.
- [186] A.C. Gerecke, D.L. Sedlak, Precursors of *N*-nitrosodimethylamine in natural waters, *Environ. Sci. Technol.* 37 (2003) 1331–1336.
- [187] California Environmental Protection Agency, Public Health Goal for *N*-nitrosodimethylamine in Drinking Water, California Environmental Protection Agency, 2006.
- [188] R.G. Liteplo, M.E. Meek, *N*-Nitrosodimethylamine: hazard characterization and exposure—response analysis, *J. Environ. Sci. Health C: Environ. Carcinogen. Ecotoxicol. Rev.* 19 (2001) 281–304.
- [189] IARC, Monographs on the Evaluation of the Carcinogenic Risks to Humans, Some *N*-nitroso Compounds, vol. 17, International Agency for Research on Cancer, Lyon, France, 1978.
- [190] K. Fujita, T. Kamataki, Role of human cytochrome P450 (CYP) in the metabolic activation of *N*-alkylnitrosamines: application of genetically engineered *Salmonella typhimurium* YG7108 expressing each form of CYP together with human NADPH-cytochrome P450 reductase, *Mutat. Res.* 483 (2001) 35–41.
- [191] K. Fujita, T. Kamataki, Predicting the mutagenicity of tobacco-related *N*-nitrosamines in humans using 11 strains of *Salmonella typhimurium* YG7108, each coexpressing a form of human cytochrome P450 along with NADPH-cytochrome P450 reductase, *Environ. Mol. Mutagen.* 38 (2001) 339–346.
- [192] T. Negishi, T. Shiotani, K. Fujikawa, H. Hayatsu, The genotoxicities of *N*-nitrosamines in *Drosophila melanogaster* in vivo: the correlation of mutagenicity in the wing spot test with the DNA damages detected by the DNA-repair test, *Mutat. Res.* 252 (1991) 119–128.
- [193] J.B. Guttenplan, Y.C. Hu, Mutagenesis by *N*-nitroso compounds in *Salmonella typhimurium* TA102 and TA104: evidence for premutagenic adenine or thymine DNA adducts, *Mutat. Res.* 141 (1984) 153–159.
- [194] K. Kanki, A. Nishikawa, K. Masumura, T. Umemura, T. Imazawa, Y. Kitamura, T. Nohmi, M. Hirose, In vivo mutational analysis of liver DNA in gpt delta transgenic rats treated with the hepatocarcinogens *N*-nitrosopyrrolidine, 2-amino-3-methylimidazo[4,5-*f*]quinoline, and di(2-ethylhexyl)phthalate, *Mol. Carcinogen.* 42 (2005) 9–17.
- [195] S. Robichova, D. Slamenova, Study of *N*-nitrosomorpholine-induced DNA strand breaks in Caco-2 cells by the classical and modified comet assay: influence of vitamins E and C, *Nutr. Cancer.* 39 (2001) 267–272.
- [196] S. Robichova, D. Slamenova, A. Gabelova, J. Sedlak, J. Jakubikova, An investigation of the genotoxic effects of *N*-nitrosomorpholine in mammalian cells, *Chem. Biol. Interact.* 148 (2004) 163–171.
- [197] A. Wakata, Y. Miyamae, S. Sato, T. Suzuki, T. Morita, N. Asano, T. Awogi, K. Kondo, M. Hayashi, Evaluation of the rat micronucleus test with bone marrow and peripheral blood: summary of the 9th collaborative study by CSGMT/JEMS, MMS, *Environ. Mol. Mutagen.* 32 (1998) 84–100.
- [198] H. Korr, B. Botzem, C. Schmitz, H. Enzmann, *N*-Nitrosomorpholine induced alterations of unscheduled DNA synthesis, mitochondrial DNA synthesis and cell proliferation in different cell types of liver, kidney, and urogenital organs in the rat, *Chem. Biol. Interact.* 134 (2001) 217–233.

- [199] M. Andersson, E. Agurell, H. Vaghef, G. Bolcsfoldi, B. Hellman, Extended-term cultures of human T-lymphocytes and the comet assay: a useful combination when testing for genotoxicity in vitro? *Mutat. Res.* 540 (2003) 43–55.
- [200] S. Gonzalez-Mancebo, J. Gaspar, E. Calle, S. Pereira, A. Mariano, J. Rueff, J. Casado, Stereochemical effects in the metabolic activation of nitrosopiperidines: correlations with genotoxicity, *Mutat. Res.* 558 (2004) 45–51.
- [201] D. McGregor, The genetic toxicology of *N*-nitrosodiphenylamine, *Mutat. Res.* 317 (1994) 195–211.
- [202] J.M.S. van Maanen, I.J. Welle, G. Hageman, J.W. Dallinga, P.L.J.M. Mertens, J.C.S. Kleinjans, Nitrate contamination of drinking water: relationship with HPRT variant frequency in lymphocyte DNA and urinary excretion of *N*-nitrosamines, *Environ. Health Perspect.* 104 (1996) 522–528.
- [203] J.L. Brown, *N*-Nitrosamines, *Occup. Med.* 14 (1999) 839–848.
- [204] M.R. Berger, D. Schmahl, H. Zerban, Combination experiments with very low doses of three genotoxic *N*-nitrosamines with similar organotropic carcinogenicity in rats, *Carcinogenesis* 8 (1987) 1635–1643.
- [205] R. Peto, R. Gray, P. Brantom, P. Grasso, Effects on 4080 rats of chronic administration of *N*-nitrosodiethylamine or *N*-nitrosodimethylamine. A detailed dose response study, *Cancer Res.* 51 (1991) 6451–6515.
- [206] W.H. Glaze, H.S. Weinberg, S.W. Krasner, M.J. Scimenti, Trends in aldehyde formation and removal through plants using ozonation and biological active filters, in: *Proceedings of the AWWA*, 1991, pp. 913–943.
- [207] A. McKnight, D.A. Reckhow, Reactions of ozonation byproducts with chlorine and chloramines, in: *Proceedings of the AWWA*, Vancouver, British Columbia, (1992), pp. 399–409.
- [208] IARC, Monographs on the Evaluation of Carcinogenic Risks to Humans. Formaldehyde, 2-Butoxyethanol and 1-*tert*-Butoxypropan-2-ol, vol. 88, International Agency for Research on Cancer, Lyon, France, 2006.
- [209] G. Speit, O. Merk, Evaluation of mutagenic effects of formaldehyde in vitro: detection of crosslinks and mutations in mouse lymphoma cells, *Mutagenesis* 17 (2002) 183–187.
- [210] G. Speit, O. Schmid, Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells, *Mutat. Res.* 613 (2006) 1–9.
- [211] M.M. Moore, K. Harrington-Brock, Mutagenicity of trichloroethylene and its metabolites: implications for the risk assessment of trichloroethylene, *Environ. Health Perspect.* 108 (Suppl. 2) (2000) 215–223.
- [212] R. Jung, G. Engelhart, B. Herbolt, R. Jackh, W. Muller, Collaborative study of mutagenicity with *Salmonella typhimurium* TA102, *Mutat. Res.* 278 (1992) 265–270.
- [213] W. Muller, G. Engelhart, B. Herbold, R. Jackh, R. Jung, Evaluation of mutagenicity testing with *Salmonella typhimurium* TA102 in three different laboratories, *Environ. Health Perspect.* 101 (Suppl. 3) (1993) 33–36.
- [214] N. Murata-Kamiya, H. Kaji, H. Kasai, Types of mutations induced by glyoxal, a major oxidative DNA-damage product, in *Salmonella typhimurium*, *Mutat. Res.* 377 (1997) 13–16.
- [215] N. Murata-Kamiya, H. Kamiya, H. Kaji, H. Kasai, Mutational specificity of glyoxal, a product of DNA oxidation, in the *lacI* gene of wild-type *Escherichia coli* W3110, *Mutat. Res.* 377 (1997) 255–262.
- [216] M.J. Roberts, G.T. Wondrak, D.C. Laurean, M.K. Jacobson, E.L. Jacobson, DNA damage by carbonyl stress in human skin cells, *Mutat. Res.* 522 (2003) 45–56.
- [217] F. Kuchenmeister, P. Schmezer, G. Engelhardt, Genotoxic bifunctional aldehydes produce specific images in the comet assay, *Mutat. Res.* 419 (1998) 69–78.
- [218] H. Kasai, K. Kumeno, Z. Yamaizumi, S. Nishimura, M. Nagao, Y. Fujita, T. Sugimura, H. Nukaya, T. Kosuge, Mutagenicity of methylglyoxal in coffee, *Gann* 73 (1982) 681–683.
- [219] M. Nagao, Y. Fujita, T. Sugimura, T. Kosuge, Methylglyoxal in beverages and foods: its mutagenicity and carcinogenicity, *IARC Sci. Publ.* 70 (1986) 283–291.
- [220] N. Murata-Kamiya, H. Kamiya, Methylglyoxal, an endogenous aldehyde, crosslinks DNA polymerase and the substrate DNA, *Nucleic Acids Res.* 29 (2001) 3433–3438.
- [221] W.D. Kerns, K.L. Pavkov, D.J. Donofrio, E.J. Gralla, J.A. Swenberg, Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure, *Cancer Res.* 43 (1983) 4382–4392.
- [222] M. Soffritti, F. Belpoggi, L. Lambertin, M. Lauriola, M. Padovani, C. Maltoni, Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats, *Ann. N.Y. Acad. Sci.* 982 (2002) 87–105.
- [223] J. Swenberg, W. Kerns, K. Pavkov, R. Mitchell, E.J. Gralla, Carcinogenicity of formaldehyde vapor: interim findings in a long-term bioassay of rats and mice, *Dev. Toxicol. Environ. Sci.* 8 (1980) 283–286.
- [224] J.A. Swenberg, W.D. Kerns, R.I. Mitchell, E.J. Gralla, K.L. Pavkov, Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor, *Cancer Res.* 40 (1980) 3398–3402.
- [225] R.A. Woutersen, L.M. Appelman, A. Van Garderen-Hoetmer, V.J. Feron, Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study, *Toxicology* 41 (1986) 213–231.
- [226] S.H. Hilal, L.L. Bornander, L.A. Carreira, Hydration equilibrium constants of aldehydes, ketones, and quinazolines, *QSAR Combinatorial Sci.* 24 (2005) 631–638.
- [227] F.B. Daniel, A.B. DeAngelo, J.A. Stober, G.R. Olson, N.P. Page, Hepatocarcinogenicity of chloral hydrate, 2-chlororoacetaldehyde, and dichloroacetic acid in the male B6C3F1 mouse, *Fundam. Appl. Toxicol.* 19 (1992) 159–168.
- [228] A.L. Thompson, Chlorine dioxide process treatment data and cost, in: *Proceedings of the AWWA Second International Symposium on Chlorine Dioxide and Drinking Water Issues*, AWWA, Denver, CO, 1992.
- [229] National Toxicology Program, Toxicology and carcinogenesis studies of sodium chlorate (CAS no. 7775-09-9) in F344/N rats and B6C3F1 mice (drinking water studies), *Natl. Toxicol. Program Tech. Rep. Ser.* 517 (2005) 1–255.
- [230] IARC, Monographs on the Evaluation of the Carcinogenic Risks to Humans, Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, vols. 1–42, International Agency for Research on Cancer, Lyon, France, 1987.
- [231] P.A. White, J.B. Rasmussen, The genotoxic hazards of domestic wastes in surface waters, *Mutat. Res.* 410 (1998) 223–236.
- [232] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: a national reconnaissance, *Environ. Sci. Technol.* 36 (2002) 1202–1211.
- [233] C. Zwiener, T. Glauner, F.H. Frimmel, Abstracts of the Workshop on Pool Water Chemistry and Health, University of Karlsruhe, Germany, 2003 <http://www.wasserchemie.uni-karlsruhe.de>.

- [234] K. Moriyama, H. Matsufuji, M. Chino, M. Takeda, Identification and behavior of reaction products formed by chlorination of ethynylestradiol, *Chemosphere* 55 (2004) 839–847.
- [235] M.M. Huber, T.A. Ternes, U. von Gunten, Removal of estrogenic activity and formation of oxidation products during ozonation of 17-ethynylestradiol, *Environ. Sci. Technol.* 38 (2004) 5177–5186.
- [236] D.C. McDowell, M.M. Huber, M. Wagner, U. von Gunten, T.A. Ternes, Ozonation of carbamazepine in drinking water: identification and kinetic study of major oxidation products, *Environ. Sci. Technol.* 39 (2005) 8014–8022.
- [237] M. Bedner, W.A. MacCrehan, Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and *N*-acetyl-*p*-benzoquinone imine, *Environ. Sci. Technol.* 40 (2006) 516–522.
- [238] K.L. Rule, V.R. Ebbett, P.J. Vikesland, Formation of chloroform and chlorinated organics by free-chlorine-mediated oxidation of triclosan, *Environ. Sci. Technol.* 39 (2005) 3176–3185.
- [239] A.E. Greynshock, P.J. Vikesland, Triclosan reactivity in chloraminated waters, *Environ. Sci. Technol.* 40 (2006) 2615–2622.
- [240] M.C. Dodd, C.H. Huang, Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics, mechanisms, and pathways, *Environ. Sci. Technol.* 38 (2004) 5607–5615.
- [241] A.D. Shah, J.H. Kim, C.H. Huang, Reaction kinetics and transformation of carbadox and structurally related compounds with aqueous chlorine, *Environ. Sci. Technol.* 40 (2006) 7228–7235.
- [242] G. Mascolo, A. López, R. Passino, G. Ricco, G. Tiravanti, Degradation of sulphur containing s-triazines during water chlorination, *Water Res.* 28 (1994) 2499–2506.
- [243] A. Lopez, G. Mascolo, G. Tiravanti, R. Passino, Formation of herbicide degradation by-products during groundwater disinfection: an LC–MS investigation, *J. Anal. Chem.* 53 (1998) 856–860.
- [244] C.G. Zambonin, I.I. Losito, F. Palmisano, Liquid chromatography/electrospray ionisation sequential mass spectrometric identification of the main chlortoluron by-products during water disinfection using chlorine, *Rapid Commun. Mass Spectrom.* 14 (2000) 824–828.
- [245] C.H. Lin, R.N. Lerch, H.E. Garrett, M.F. George, Degradation of isoxaflutole (balance) herbicide by hypochlorite in tap water, *J. Agric. Food Chem.* 51 (2003) 8011–8014.
- [246] S.E. Duijk, T.W. Collette, Degradation of chlorpyrifos in aqueous chlorine solutions: pathways, kinetics, and modeling, *Environ. Sci. Technol.* 40 (2006) 546–551.
- [247] P. Sandín-España, J.O. Magrans, J.M. García-Baudín, Study of clethodim degradation and by-product formation in chlorinated water by HPLC, *Chromatographia* 62 (2005) 133–137.
- [248] M.L. Hladik, A.L. Roberts, E.J. Bouwer, Removal of neutral chloroacetamide herbicide degradates during simulated unit processes for drinking water treatment, *Water Res.* 39 (2005) 5033–5044.
- [249] H. Shemer, K.G. Linden, Degradation and by-product formation of diazinon in water during UV and UV/H₂O₂ treatment, *J. Hazard. Mater.* 136 (2006) 553–559.
- [250] A. Mehrsheikh, M. Bleeke, S. Brosillon, A. Laplanche, P. Roche, Investigation of the mechanism of chlorination of glyphosate and glycine in water, *Water Res.* 40 (2006) 3003–3014.
- [251] M. Petrovic, A. Diaz, F. Ventura, D. Barcelo, Occurrence and removal of estrogenic short-chain ethoxy nonylphenolic compounds and their halogenated derivatives during drinking water production, *Environ. Sci. Technol.* 37 (2003) 4442–4448.
- [252] J.Y. Hu, T. Aizawa, S. Ookubo, Products of aqueous chlorination of bisphenol A and their estrogenic activity, *Environ. Sci. Technol.* 36 (2002) 1980–1987.
- [253] T.P. Kull, P.H. Backlund, K.M. Karlsson, J.A. Meriluoto, Oxidation of the cyanobacterial hepatotoxin microcystin-LR by chlorine dioxide: reaction kinetics, characterization, and toxicity of reaction products, *Environ. Sci. Technol.* 38 (2004) 6025–6031.
- [254] D.P. Oliveira, P.A. Carneiro, C.M. Rech, M.V. Zaroni, L.D. Claxton, G.A. Umbuzeiro, Mutagenic compounds generated from the chlorination of disperse azo-dyes and their presence in drinking water, *Environ. Sci. Technol.* 40 (2006) 6682–6689.
- [255] R.O.A. de Lima, A.P. Bazo, D.M.F. Salvadori, C.M. Rech, D. de Palma Oliveira, G. de Aragão Umbuzeiro, Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source, *Mutat. Res.* 626 (2007) 53–60.
- [256] G. de Aragao Umbuzeiro, H.S. Freeman, S.H. Warren, D.P. de Oliveira, Y. Terao, T. Watanabe, L.D. Claxton, The contribution of azo dyes to the mutagenic activity of the Cristais river, *Chemosphere* 60 (2005) 55–64.
- [257] G. De Aragao Umbuzeiro, D.A. Roubicek, C.M. Rech, M.I. Sato, L.D. Claxton, Investigating the sources of the mutagenic activity found in a river using the *Salmonella* assay and different water extraction procedures, *Chemosphere* 54 (2004) 1589–1597.
- [258] T. Watanabe, H. Ohba, M. Asanoma, T. Hasei, T. Takamura, Y. Terao, T. Shiozawa, T. Hirayama, K. Wakabayashi, H. Nukaya, Isolation and identification of non-chlorinated phenylbenzotriazole (non-CIPBTA)-type mutagens in the Ho River in Shizuoka Prefecture, Japan, *Mutat. Res.* 609 (2006) 137–145.
- [259] T. Watanabe, Y. Takahashi, T. Takahashi, H. Nukaya, Y. Terao, T. Hirayama, K. Wakabayashi, Seasonal fluctuation of the mutagenicity of river water in Fukui, Japan, and the contribution of 2-phenylbenzotriazole-type mutagens, *Mutat. Res.* 519 (2002) 187–197.
- [260] T. Watanabe, T. Shiozawa, Y. Takahashi, T. Takahashi, Y. Terao, H. Nukaya, T. Takamura, H. Sawanishi, T. Ohe, T. Hirayama, K. Wakabayashi, Mutagenicity of two 2-phenylbenzotriazole derivatives, 2-[2-acetylamino]-4-(diethylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole and 2-[2-(acetylamino)-4-(diallylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole and their detection in river water in Japan, *Mutagenesis* 17 (2002) 293–299.
- [261] T. Watanabe, H. Nukaya, Y. Terao, Y. Takahashi, A. Tada, T. Takamura, H. Sawanishi, T. Ohe, T. Hirayama, T. Sugimura, K. Wakabayashi, Synthesis of 2-phenylbenzotriazole-type mutagens, PBTA-5 and PBTA-6, and their detection in river water from Japan, *Mutat. Res.* 498 (2001) 107–115.
- [262] H. Nukaya, T. Shiozawa, A. Tada, Y. Terao, T. Ohe, T. Watanabe, M. Asanoma, H. Sawanishi, T. Katsuhara, T. Sugimura, K. Wakabayashi, Identification of 2-[2-(acetylamino)-4-amino-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-4) as a potent mutagen in river water in Kyoto and Aichi Prefectures, Japan, *Mutat. Res.* 492 (2001) 73–80.
- [263] R. Tornero-Velez, M.K. Ross, C. Granville, J. Laskey, J.P. Jones, D.M. DeMarini, M.V. Evans, Metabolism and mutagenicity of source water contaminants 1,3-dichloropropane and 2,2-dichloropropane, *Drug Metab. Dispos.* 32 (2004) 123–131.

- [264] C.A. Granville, M.K. Ross, R. Tornero-Velez, N.M. Hanley, R.D. Grindstaff, A. Gold, A.M. Richard, K. Funasaka, A.H. Tennant, A.D. Kligerman, M.V. Evans, D.M. DeMarini, Genotoxicity and metabolism of the source-water contaminant 1,1-dichloropropene: activation by GSTT1-1 and structure-activity considerations, *Mutat. Res.* 572 (2005) 98–112.
- [265] G. de Aragão Umbuzeiro, S.H. Warren, L.D. Claxton, The mutation spectra of chlorinated drinking water samples using the base-specific TA7000 strains of *Salmonella* in the micro-suspension assay, *Mutat. Res.* 609 (2006) 26–33.
- [266] H.J. Kool, F. Kuper, H. van Haeringen, J.H. Koeman, A carcinogenicity study with mutagenic organic concentrates of drinking-water in the Netherlands, *Food Chem. Toxicol.* 23 (1985) 79–85.
- [267] L.W. Condie, W.C. Lauer, G.W. Wolfe, E.T. Czeh, J.M. Burns, Denver potable water reuse demonstration project: comprehensive chronic rat study, *Food Chem. Toxicol.* 32 (1994) 1021–1030.
- [268] M.J. Hooth, K.S. McDorman, S.D. Hester, M.H. George, L.R. Brooks, A.E. Swank, D.C. Wolf, The carcinogenic response to a mixture of drinking water disinfection by-products in Tsc2 mutant Long-Evans (Eker) rats was less than additive, *Toxicol. Sci.* 69 (2002) 322–331.
- [269] K.S. McDorman, M.J. Hooth, T.B. Starr, D.C. Wolf, Analysis of preneoplastic and neoplastic renal lesions in Tsc2 mutant Long-Evans (Eker) rats following exposure to a mixture of drinking water disinfection by-products, *Toxicology* 187 (2003) 1–12.
- [270] K.S. McDorman, S. Chandra, M.J. Hooth, S.D. Hester, R. Schoonhoven, D.C. Wolf, Induction of transitional cell hyperplasia in the urinary bladder and aberrant crypt foci in the colon of rats treated with individual and a mixture of drinking water disinfection by-products, *Toxicol. Pathol.* 31 (2003) 235–242.
- [271] U.S. Environmental Protection Agency, Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures, EPA/630/R-00/002, Washington, DC, <http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=20533>, 2002.
- [272] L.K. Teuschler, G.E. Rice, C.R. Wilkes, J.C. Lipscomb, F.W. Power, A feasibility study of cumulative risk assessment methods for drinking water disinfection by-product mixtures, *J. Toxicol. Environ. Health A* 67 (2004) 755–777.
- [273] C. Gennings, W.H. Carter Jr., R.A. Carchman, L.K. Teuschler, J.E. Simmons, E.W. Carney, A unifying concept for assessing toxicological interactions: changes in slope, *Toxicol. Sci.* 88 (2005) 287–297.
- [274] L.K. Teuschler, Deciding which chemical mixtures risk assessment methods work best for what mixtures, *Toxicol. Appl. Pharmacol.*, in press.
- [275] J.E. Simmons, S.D. Richardson, T.F. Speth, R.J. Miltner, G. Rice, K.M. Schenck, E.S. Hunter III, L.K. Teuschler, Integrated disinfection byproducts mixtures research: issues underlying the integrated chemical and toxicological assessment of complex mixtures, *J. Toxicol. Environ. Health*, in press.
- [276] K.L. Dearfield, M.M. Moore, Use of genetic toxicology information for risk assessment, *Environ. Mol. Mutagen.* 46 (2005) 236–245.
- [277] R.W. Tennant, Stratification of rodent carcinogenicity bioassay results to reflect relative human hazard, *Mutat. Res.* 286 (1993) 111–118.
- [278] M.D. Shelby, E. Zeiger, The genetic toxicity of human carcinogens and its implications, *Mutat. Res.* 234 (1990) 257–261.
- [279] C.R. Wilkes, G.L. Kedderis, A.D. Mason, L.L. Niang, J.C. Lipscomb, Exposures and internal doses of trihalomethanes in humans: multi-route contributions from drinking water, Office of Research and Development, US EPA, National Center for Environmental Assessment, Cincinnati, OH, EPA 600/R-06/087 (2006).
- [280] L.C. Backer, D.L. Ashley, M.A. Bonin, F.L. Cardinali, S.M. Kieszak, J.V. Wooten, Household exposures to drinking water disinfection by-products: whole blood trihalomethane levels, *J. Exp. Anal. Environ. Epidemiol.* 10 (2000) 321–326.
- [281] S.D. Richardson, F.G. Crumley, F. Fasano, M.J. Plewa, E.D. Wagner, L. Williamson, M. Bartlett, P. Angel, R. Orlando, Mass Spectrometry and Toxicity Characterization of Drinking Water Fractions: Are High Molecular Weight DBPs Toxic? 19th Annual Tandem Mass Spectrometry Workshop, Lake Louise, Canada, 2006.
- [282] J.C. Loper, D.R. Lang, R.S. Schoeny, B.B. Richmond, P.M. Gallagher, C.C. Smith, Residue organic mixtures from drinking water show in vitro mutagenic and transforming activity, *J. Toxicol. Environ. Health* 4 (1978) 919–938.
- [283] S. Landi, N.M. Hanley, S.H. Warren, R.A. Pegram, D.M. DeMarini, Induction of genetic damage in human lymphocytes and mutations in *Salmonella* by trihalomethanes: role of red blood cells and GSTT1-1 polymorphism, *Mutagenesis* 14 (1999) 479–482.
- [284] R.J. Brennan, R.H. Schiestl, Chloroform and carbon tetrachloride induce intrachromosomal recombination and oxidative free radicals, *Mutat. Res.* 397 (1998) 271–278.
- [285] B.E. Butterworth, M.V. Templin, A.A. Constan, C.S. Sprinkle, B.A. Wong, L.J. Pluta, J.I. Everitt, L. Recio, Long-term mutagenicity studies with chloroform and dimethylnitrosamine, *Environ. Mol. Mutagen.* 31 (1998) 248–256.
- [286] A. Araki, N. Kamigaito, T. Sasaki, T. Matsushima, Mutagenicity of carbon tetrachloride and chloroform in *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, and *Escherichia coli* WP2uvrA/pKM101 and WP2/pKM101, using a gas exposure method, *Environ. Mol. Mutagen.* 43 (2004) 128–133.
- [287] K. Yasunaga, A. Kiyonari, T. Oikawa, N. Abe, K. Yoshikawa, Evaluation of the *Salmonella* umu test with 83 NTP chemicals, *Environ. Mol. Mutagen.* 44 (2004) 329–345.
- [288] National Toxicology Program, Toxicology and carcinogenesis studies of bromodichloromethane (CAS no. 75-27-4) in male F344/N rats and female B6C3F1 mice (drinking water studies), *Natl. Toxicol. Program Tech. Rep. Ser.* (2006) 1–248.
- [289] Y. Aida, K. Yasunaga, K. Takada, Y. Kurokawa, M. Tobe, Chronic toxicity of microencapsulated bromodichloromethane administered in the diet to Wistar rats, *J. Toxicol. Sci.* 17 (1992) 51–68.
- [290] M.H. George, G.R. Olson, D. Doerfler, T. Moore, S. Kilburn, A.B. DeAngelo, Carcinogenicity of bromodichloromethane administered in drinking water to male F344/N rats and B6C3F1 mice, *Int. J. Toxicol.* 21 (2002) 219–230.
- [291] National Toxicology Program, Toxicology and carcinogenesis studies of tribromomethane (bromoform) (CAS no. 75-25-2) in F344/N rats and B6C3F1 mice (gavage studies), *Natl. Toxicol. Program Tech. Rep. Ser.* 350 (1989) 1–194.
- [292] National Toxicology Program, Toxicology and carcinogenesis studies of chlorodibromomethane (CAS no. 124-48-1) in F344/N rats and B6C3F1 mice (gavage studies), *Natl. Toxicol. Program Tech. Rep. Ser.* 282 (1985) 1–174.
- [293] T.A. Jorgenson, E.F. Meierhenry, C.J. Rushbrook, R.J. Bull, M. Robinson, Carcinogenicity of chloroform in drinking water to male Osborne-Mendel rats and female B6C3F1 mice, *Fundam. Appl. Toxicol.* 5 (1985) 760–769.

- [294] R.J. Bull, I.M. Sanchez, M.A. Nelson, J.L. Larson, A.J. Lansing, Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate, *Toxicology* 63 (1990) 341–359.
- [295] A.B. DeAngelo, M.H. George, D.E. House, Hepatocarcinogenicity in the male B6C3F1 mouse following a lifetime exposure to dichloroacetic acid in the drinking water: dose-response determination and modes of action, *J. Toxicol. Environ. Health A* 58 (1999) 485–507.
- [296] A.B. DeAngelo, F.B. Daniel, B.M. Most, G.R. Olson, The carcinogenicity of dichloroacetic acid in the male Fischer 344 rat, *Toxicology* 114 (1996) 207–221.
- [297] Y. Kurokawa, Y. Hayashi, A. Maekawa, M. Takahashi, T. Kokubo, S. Odashima, Carcinogenicity of potassium bromate administered orally to F344 rats, *J. Natl. Cancer Inst.* 71 (1983) 965–972.
- [298] Y. Yokose, K. Uchida, D. Nakae, K. Shiraiwa, K. Yamamoto, Y. Konishi, Studies of carcinogenicity of sodium chlorite in B6C3F1 mice, *Environ. Health Perspect.* 76 (1987) 205–210.
- [299] D.B. McGregor, R. Martin, P. Cattanaach, I. Edwards, D. McBride, W.J. Caspary, Responses of the L5178Y tk+/tk– mouse lymphoma cell forward mutation assay to coded chemicals. I. Results for nine compounds, *Environ. Mutagen.* 9 (1987) 143–160.
- [300] M.H. George, T. Moore, S. Kilburn, G.R. Olson, A.B. DeAngelo, Carcinogenicity of chloral hydrate administered in drinking water to the male F344/N rat and male B6C3F1 mouse, *Toxicol. Pathol.* 28 (2000) 610–618.
- [301] National Toxicology Program, Toxicology and carcinogenesis studies of chloral hydrate, NTP Technical Report 503, Research Triangle Park, NC, 2002.
- [302] J. Leuschner, N. Beuscher, Studies on the mutagenic and carcinogenic potential of chloral hydrate, *Arzneimittelforschung* 48 (1998) 961–968.
- [303] H.P. Til, R.A. Woutersen, V.J. Feron, V.H. Hollanders, H.E. Falke, J.J. Clary, Two-year drinking-water study of formaldehyde in rats, *Food Chem. Toxicol.* 27 (1989) 77–87.
- [304] V. Muller-Pillet, M. Joyeux, D. Ambroise, P. Hartemann, Genotoxic activity of five haloacetonitriles: comparative investigations in the single cell gel electrophoresis (comet) assay and the Ames-fluctuation test, *Environ. Mol. Mutagen.* 36 (2000) 52–58.
- [305] K. Mortelmans, S. Haworth, T. Lawlor, W. Speck, B. Tainer, E. Zeiger, *Salmonella* mutagenicity tests. II. Results from the testing of 270 chemicals, *Environ. Mutagen.* 8 (Suppl. 7) (1986) 1–119.
- [306] T. Roldán-Arjona, C. Pueyo, Mutagenic and lethal effects of halogenated methanes in the Ara test of *Salmonella typhimurium*: quantitative relationship with chemical reactivity, *Mutat. Res.* 8 (1993) 127–131.
- [307] H. Hikiba, E. Watanabe, J.C. Barrett, T. Tsutsui, Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells, *J. Pharmacol. Sci.* 97 (2005) 146–152.
- [308] H. Bartsch, C. Malaveille, A. Barbin, H. Bresil, L. Tomatis, R. Montesano, Mutagenicity and metabolism of vinyl chloride and related compounds, *Environ. Health Perspect.* 17 (1976) 193–198.
- [309] S.Y. Chiang, J.A. Swenberg, W.H. Weisman, T.R. Skopek, Mutagenicity of vinyl chloride and its reactive metabolites, chloroethylene oxide and chloroacetaldehyde, in a metabolically competent human B-lymphoblastoid line, *Carcinogenesis* 18 (1997) 31–36.
- [310] R.J. Hansen, R. Nagasubramanian, S.M. Delaney, L.D. Samson, M.E. Dolan, Role of *O*⁶-methylguanine-DNA methyltransferase in protecting from alkylating agent-induced toxicity and mutations in mice, *Carcinogenesis* 28 (2007) 1111–1116.
- [311] D.S. Michaud, M. Kogevinas, K.P. Cantor, C.M. Villanueva, M. Garcia-Closas, N. Rothman, N. Malats, F.X. Real, C. Serra, R. Garcia-Closas, A. Tardon, A. Carrato, M. Dosemeci, D.T. Silverman, Total fluid and water consumption and the joint effect of exposure to disinfection by-products on risk of bladder cancer, *Environ. Health Perspect.*, in press.