



DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD

3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and mutagenic activity in Massachusetts drinking water.

The Harvard community has made this article openly available.
[Please share](#) how this access benefits you. Your story matters.

Citation	Wright, J. Michael, Joel Schwartz, Terttu Vartiainen, Jorma Mäki-Paakkanen, Larisa Altshul, Joseph J. Harrington, and Douglas W. Dockery. 2002. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and mutagenic activity in Massachusetts drinking water. <i>Environmental Health Perspectives</i> 110(2): 157-164.
Published Version	http://ehpnet1.niehs.nih.gov/docs/2002/110p157-164wright/abstract.html
Accessed	February 19, 2015 8:28:32 AM EST
Citable Link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:4889583
Terms of Use	This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

(Article begins on next page)

3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and Mutagenic Activity in Massachusetts Drinking Water

J. Michael Wright,^{1,2} Joel Schwartz,^{1,2,3} Terttu Vartiainen,^{4,5} Jorma Mäki-Paakkanen,⁴ Larisa Altshul,² Joseph J. Harrington,² and Douglas W. Dockery^{1,2,3}

¹Environmental Epidemiology Program and ²Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; ³Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA; ⁴Department of Environmental Health, National Public Health Institute, Kuopio, Finland; ⁵University of Kuopio, Department of Environmental Sciences, Kuopio, Finland

There is limited information on the prevalence of the potent mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in U.S. water supplies. We measured MX concentrations and mutagenic activity in tap water samples from 36 surface water systems throughout Massachusetts. We found MX levels much higher (up to 80 ng/L) than previously reported in the United States. We also evaluated the role of water treatment on mutagenic activity and disinfection by-product formation. After adjusting for other covariates, chloramination and filtration were the most important treatment options for reducing mutagenic activity and disinfection by-product formation. Multiple chlorine application (before and after filtration) was associated with increased mutagenicity. Chlorine dose, pH, and total organic carbon were also associated with mutagenicity, MX, and total trihalomethane (TTHM) concentration. Seasonal variation was evident for MX and mutagenic activity, with higher levels occurring in the spring compared to the fall. In contrast, TTHM concentrations were greater in the fall. **Key words:** disinfection by-products, drinking water, mutagenicity, MX, trihalomethanes. *Environ Health Perspect* 110:157–164 (2002). [Online 16 January 2002]

<http://ehpnet1.niehs.nih.gov/docs/2002/110p157-164wright/abstract.html>

Disinfection of drinking water is one of the most important public health accomplishments helping to dramatically reduce the incidence of enteric diseases such as cholera and typhoid (1). Although the effectiveness in combating infectious agents is undisputed, there has been concern over the production of disinfection by-products (DBPs) during water treatment and transport. Disinfection by-products are formed when organic and inorganic matter combines with oxidative disinfectants. Toxicologic and epidemiologic studies have reported that DBPs are associated with a variety of health effects.

The volatile DBPs (e.g., trihalomethanes) have been heavily scrutinized since chloroform (the most prevalent trihalomethane) was first reported to be a rodent carcinogen in 1976 (2). Other DBPs reported to be carcinogenic in animal studies include the haloacetic acids (3), haloacetonitriles (4), bromate (5), and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) (6). Toxicologic data have indicated that the brominated trihalomethanes (such as bromoform and bromodichloromethane) are more carcinogenic (7,8) and mutagenic (9,10) than chloroform.

Most of the genotoxicity detected in chlorinated drinking water has been attributed to by-products in the nonvolatile fraction (11). The haloacetic acids are the most prevalent nonvolatile compounds (12), but they have been shown to be weak mutagens (13–15). MX has been shown to be one of the most potent bacterial mutagens tested (16). In addition to bacterial assays, MX is a

direct-acting mutagen and genotoxin *in vivo* (17–21) and in mammalian cells *in vitro* (21–27). MX is a multisite carcinogen in male and female rats (6), with an estimated cancer potency 170 times greater than chloroform and 17 times greater than bromodichloromethane (28).

There is some inconsistency between the toxicologic and epidemiologic data with respect to organ specificity. The toxicologic evidence indicates that the liver and kidney are the primary target organs for DBPs (29). The epidemiologic data suggests that trihalomethanes may be associated with cancer of the bladder and rectum (30). Toxicologic research typically focuses on the impact of individual compounds, whereas epidemiologic studies evaluate mixtures of compounds present in drinking water. Most of the earlier epidemiologic work has focused on total trihalomethane (TTHM) as a surrogate for total DBP exposure since it is routinely monitored. Although trihalomethanes are typically the most prevalent class of DBPs, they may not be an adequate marker of exposure to individual compounds or of exposure to the mixture of compounds.

Although no associations were reported for bladder or rectal cancer, epidemiologic research from Finland suggests that past exposure to mutagenic substances in drinking water may be associated with other types of cancers (31–34). These data suggest that the carcinogenic effects may be due to mutagenic nonvolatile compounds, such as MX. High MX concentrations (up to 67 ng/L) have

been reported in Finnish water systems (35). MX was highly correlated with mutagenicity in these samples, accounting for 15–57% of the mutagenic activity in Ames tester strain TA100. MX concentrations range from 3 to 9 ng/L in Japan (36) and from nondetectable to 33 ng/L in the United Kingdom (37). There are limited data on the occurrence of MX and mutagenic activity in U.S. drinking water supplies. Meier et al. (16) reported MX concentrations of 2–33 ng/L in three locations, with MX accounting for 15–34% of the mutagenic activity.

Disinfection by-product formation is dependent on a variety of water quality parameters, including total organic carbon (TOC), pH, temperature, contact time, disinfectant dose, and residual (38). TOC, ammonia, and chlorine dose have been shown to be influential in the production of mutagenic activity (39). The choice of disinfectant greatly influences the type and amount of by-products that are formed. Chloramination (40), ozonation (41), and a combination of the two (39,42) have been shown to result in reduced mutagenic activity. Alternative disinfectants result in fewer trihalomethanes (43) but produce additional by-products. For example, while ozonation is effective in limiting trihalomethane formation, it produces distinct DBPs (e.g. bromate) for which the health effects are not well understood.

Type and quality of source water are also critical to DBP formation. Due to the presence of natural organic matter, surface water typically produces higher levels of DBPs compared to groundwater. This is an important issue in the United States because surface water was the primary source for 61% of the population served by public water systems in 1997 (44). Seventy-four percent of

Address correspondence to J.M. Wright, 665 Huntington Avenue, Department of Environmental Health, Environmental Epidemiology Program, Room 1416A-Building I, Boston MA 02115 USA. Telephone: (617) 432-4404. Fax: (617) 277-2382. E-mail: mwright@hsph.harvard.edu

We thank J. Weintraub, J. Baliff, the water departments, and the Massachusetts Department of Environmental Protection for their assistance and cooperation in this survey.

This work was supported by the Kresge Center for Environmental Health (NIEHS grant ES-00002) and NIEHS training grant 5 T32 ES07069.

Received 25 May 2001; accepted 1 August 2001.

the population served by community public water systems in Massachusetts relies on surface water or a combination of surface and groundwater (45). With such a large exposed population, characterizing the presence and the health impact of DBPs is important. We initiated this survey to measure mutagenic activity and MX concentrations in Massachusetts drinking water. An additional objective was to gain a better understanding of the factors that influence the formation of MX and mutagenic compounds.

Methods

Sampling strategy. Tap water samples were collected from 36 towns in Massachusetts during 1997 and 1998. A geographically diverse sample of surface water systems was selected from towns with populations > 10,000. Fourteen of the 36 towns (11 in eastern Massachusetts and 3 in western Massachusetts) are supplied by a single source. The remaining 22 communities have independent water supplies. One town with its own surface water sources temporarily switched over to the common unified source in the summer of 1998.

Water utilities in Massachusetts employ a variety of disinfection strategies depending on the size of the system and the quality of the source water. Twenty-four of the sampled towns use chlorine as their primary disinfectant with 10 towns chlorinating their water twice (i.e., before and after filtration) prior to distribution. One town used ozone and another used chlorine dioxide as their primary disinfectants (i.e., prior to filtration) while relying on postfiltration chlorination to maintain an adequate residual in their distribution systems. The 12 remaining communities used chloramination, with two towns from the unified source beginning in September 1997. Another town that had historically rechlorinated its water (following the initial chlorination by the unified supplier) eliminated this practice in August 1997.

Tap water samples were collected from 30 towns in spring 1997. Four locations were resampled in fall 1997 along with five new sites, which were added to monitor the effect of changes in chlorination practice. Resampling occurred at 22 locations during spring 1998 and 23 towns during fall 1998. Fifteen communities had samples taken during spring 1997, spring 1998, and fall 1998. The 1997 samples were collected at regular trihalomethane sampling sites or centrally located sites in the distribution system. Most of the 1998 samples were collected simultaneously by the water utilities in conjunction with their quarterly trihalomethane monitoring.

The water departments provided data on water quality characteristics thought to influence the formation of DBPs (temperature,

pH, chlorine dose, chlorine residual, and turbidity). We requested data for the location and time closest to our samples. The water departments also provided information on their disinfection and filtration practices. The unified water system in eastern Massachusetts provided additional data on a summary measure of five haloacetic acids (HAA₅) for 15 communities during 1997 and 1998. TOC measurements from 1997–2000 were available for most communities as part of the “Information Collection Rule” (46). We categorized the TOC measurements into low, intermediate, and high levels.

Analytical protocol. Duplicate tap water samples ($n = 3$) and control blanks ($n = 5$) were collected for quality control purposes. At each location, water was allowed to run for several minutes to flush out the impurities and stabilize the temperature. A 4-L water sample was then collected in a pre-cleaned “Superfund grade” amber glass bottle with a Teflon cap. The samples were extracted at the Harvard School of Public Health Exposure Assessment Laboratories. Samples were allowed to sit for 2–5 days until they were free of chlorine. Hydrochloric acid was added to adjust the pH to 2 and stabilize the MX. Organic materials were adsorbed via a column of XAD-8 resin (Alltech Associates Inc., Deerfield, IL) at a flow rate of approximately 35 mL/min. MX is fully recovered by XAD-8 resin adsorption. The adsorbed organics were eluted with 300 mL ethyl acetate. Each extract was evaporated to 1 mL ethyl acetate.

The extracts were shipped to the Laboratory of Chemistry, National Public Health Institute, in Kuopio, Finland. The concentrated extract was then divided into two parts for the analyses of MX and mutagenicity. An internal standard mucobromic acid was added to the MX extract, corresponding to 180 ng/L of the original water.

The ethyl acetate solution was then evaporated to dryness. The resultant residues were dissolved and methylated in 300 μ L 2% (v/v) H₂SO₄. The solution was heated to 70°C to accelerate the reaction. The mixture was neutralized by addition of 600 μ g/L 2% aqueous NaHCO₃ and extracted twice with 600 μ L *n*-hexane. Upon completion of the preparation, MX concentration was measured using gas chromatography/mass spectrometry (GC/MS) (37).

Mutagenicity, measured as the number of net revertants per liter (rev/L), was assayed according to the plate incorporation method of Ames et al. (47). The Ames test was conducted on *Salmonella typhimurium* tester strain TA100, which has been shown to be the most sensitive strain for detecting mutagenicity caused by MX (22). Since MX is a direct-acting mutagen capable of inducing mutations without metabolic activation, S9 mix was not used for the Ames test. Addition of S9 mix reduces the mutagenicity of MX by 90% in TA100 assays (22). For the mutagenicity tests, the XAD extracts were dissolved in dimethyl sulfoxide. Sodium azide and dimethyl sulfoxide were used as positive and negative controls, respectively. Tests were done on five dose levels with two plates per dose. A linear dose response was used as the criterion for positive mutagenicity. The activity was calculated in the linear proportion of the dose–response curves.

The quarterly trihalomethane samples were collected by the respective towns and analyzed by Massachusetts- or U.S. Environmental Protection Agency (EPA)-certified laboratories. Trihalomethane concentrations were measured using GC or GC/MS according to U.S. EPA methods 502.2 and 524.2 (48). Standard protocol included collection of duplicate water samples in 40 mL sampling vials and addition of ascorbic acid and hydrochloric acid prior to refrigeration. The Massachusetts

Table 1. Mutagenic activity and disinfection by-product concentration in Massachusetts drinking water.

	No.	Mean	SD	Range
Mutagenicity (rev/L)				
Spring 1997	30	2,000	1,271	400–5,700
Fall 1997	10	750	273	450–1,250
Spring 1998	22	1,500	797	300–3,450
Fall 1998	26	1,100	438	350–1,950
Total	88	1,450	975	300–5,700
MX (ng/L)				
Spring 1997	30	35.5	20.8	10.1–79.9
Fall 1997	10	12.1	4.1	6.2–18.3
Spring 1998	22	25.3	15.4	4.0–61.1
Fall 1998	26	26.0	11.2	11.7–51.2
Total	88	27.5	17.0	4.0–79.9
TTHM (μ g/L)				
Spring 1997	29	31.8	22.6	5.5–82.0
Fall 1997	8	35.0	16.3	14.9–67.8
Spring 1998	22	34.5	19.2	8.0–65.5
Fall 1998	24	54.3	22.9	7.9–88.1
Total	83	39.1	23.3	5.0–88.1

Department of Environmental Protection and the individual water departments provided the trihalomethane results.

Statistical analysis. STATA, version 6, was used for the statistical analysis (Stata Corporation, College Station, TX). Pearson correlation coefficients were calculated to determine the strength of the linear relations between the DBP indicators. We used two-sided unpaired *t*-tests, assuming unequal variances to compare stratified mean DBP and mutagenicity levels. We used multiple linear regression to determine the predictors of mutagenicity, and MX and TTHM concentrations. Statistical significance was defined as $p \leq 0.05$. Contribution of MX to mutagenicity was determined by multiplying the measured concentration of MX with the tested TA100 mutagenicity of pure MX.

Results

Distribution of mutagenic activity and DBPs. MX was not detected and mutagenicity did not exceed the limit of detection (100 rev/L) in the control blanks. The duplicate samples indicated that there was limited variability in MX and mutagenicity measurements; the standard deviation of the duplicates was 4 ng/L for MX and 200 rev/L for mutagenicity.

Eighty-eight samples were collected from 36 towns over the four sampling periods. Average mutagenicity was 1,450 rev/L with a maximum of 5,700 rev/L (Table 1). Figure 1 shows the distribution of mutagenicity and MX and TTHM concentrations. Average mutagenicity was lower in the fall (mean = 1,000 rev/L) compared to the spring (mean = 1,800 rev/L). The mean MX concentration was 28 ng/L, with a maximum of 80 ng/L (Table 1). The distribution of MX was skewed as shown in Figure 1. Twenty-six samples from 16 towns had MX concentrations > 33 ng/L and three samples were > 67 ng/L. MX levels were higher in the spring (mean = 31 ng/L) compared to the fall (mean = 22 ng/L). Matching TTHM measurements were available for 83 of the 88 samples. The mean TTHM concentration was 39 $\mu\text{g/L}$, with a maximum of 88 $\mu\text{g/L}$ (Table 1). Although the TTHM distribution was skewed toward higher values, there was a suggestion of a bimodal distribution across the sampling periods (Figure 1). In particular, the fall 1998 mean TTHM concentration was considerably higher (54 $\mu\text{g/L}$) than the earlier sampling periods (mean = 33 $\mu\text{g/L}$). This increase was most likely due to a change in chlorination practice by the major water supplier of eastern Massachusetts in the summer of 1998.

Correlation between mutagenic activity and DBP concentrations. Mutagenicity was highly correlated with MX concentrations

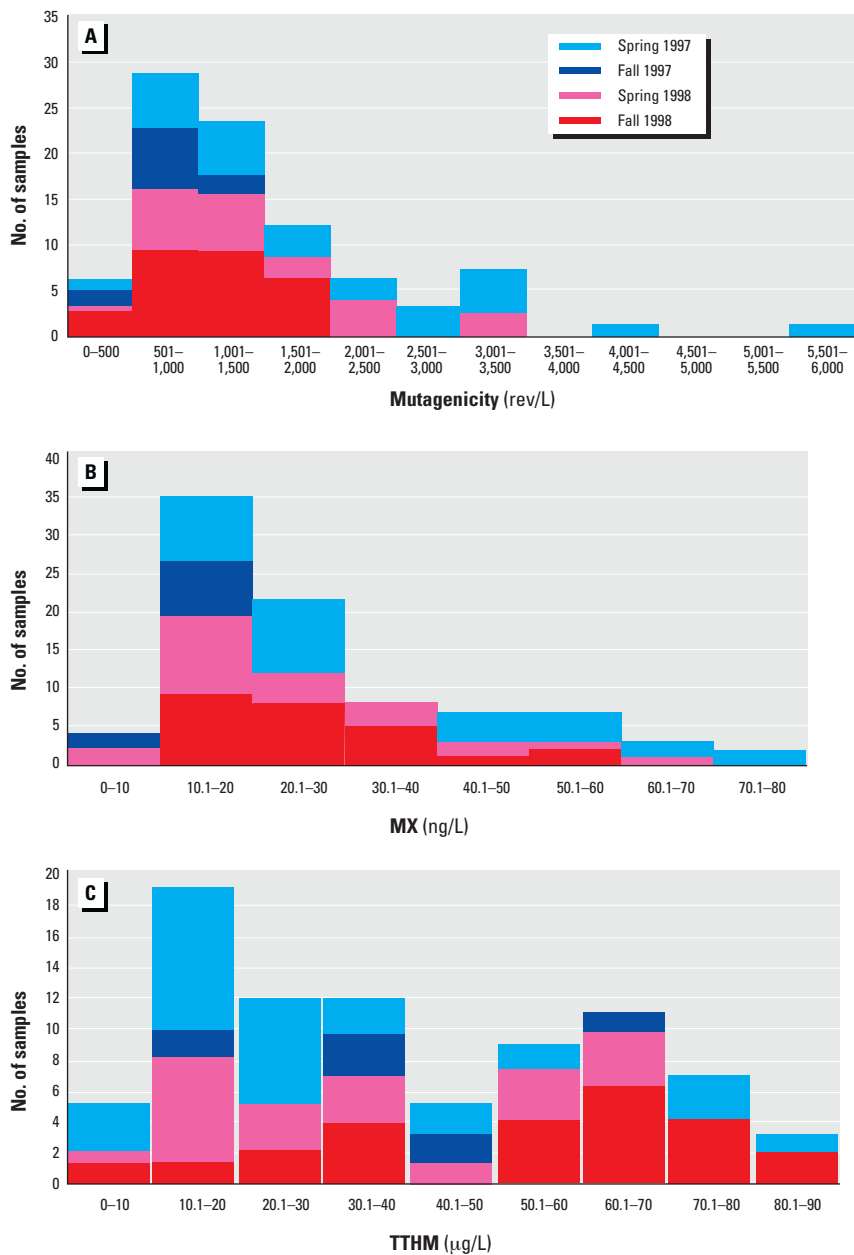


Figure 1. Mutagenicity, MX, and TTHM concentrations in Massachusetts drinking water.

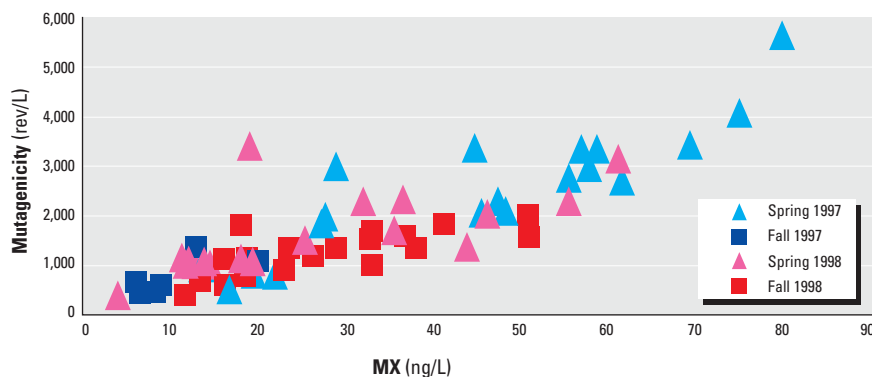


Figure 2. Mutagenicity versus MX concentration.

across all four sampling periods ($r = 0.86$; Figure 2). The period-specific correlation coefficients were 0.92 for spring 1997 ($n = 30$), 0.65 for fall 1997 ($n = 10$), 0.70 for spring 1998 ($n = 22$) and 0.81 for fall 1998 ($n = 26$; Table 2). There was considerable variation in the regression slopes of mutagenicity versus MX between sampling periods. The increase in mutagenicity associated with each 1-ng/L increase in MX concentration was most pronounced in spring 1997 and decreased over successive sampling periods. On average, MX accounted for 51% of mutagenicity across the sampling periods, 49% in spring 1997, 44% in fall 1997 and spring 1998, and 63% in fall 1998.

TTHM concentration was moderately correlated ($r = 0.35$) with HAA₅ ($n = 350$) collected for 15 towns in 1997 and 1998 (data not shown). Similar relationships were observed between TTHM and mutagenicity ($r = 0.37$; Figure 3) and TTHM and MX ($r = 0.44$; Figure 4), although there was considerable variability across the four sampling periods (Table 2). Most of the spring and fall 1998 samples were collected simultaneously with routine trihalomethane monitoring. There was a weaker correlation between the matched MX and TTHM measurements ($r = 0.36$) than the unmatched samples collected during spring and fall 1997 ($r = 0.63$). A weaker correlation was found for the matched mutagenicity and TTHM samples ($r = 0.14$) compared to the unmatched samples ($r = 0.69$). Overall, TTHM concentration was predictive of MX and mutagenic activity, but this was mainly driven by the spring 1997 and fall 1998 sampling periods (Table 2).

Predictors of mutagenic activity and DBP concentration. We stratified the mutagenicity, MX, and TTHM concentration levels by treatment plant characteristics (Table 3). Overall, there were small, but not statistically significant, increases in mean levels of mutagenicity, MX, and TTHM with the use of filtration and aluminum sulfate for coagulation. Towns using activated carbon had higher levels of mutagenic activity [600 rev/L; 95% confidence interval (CI), 50–1,150]. Chloramination was associated with decreased mutagenicity (–600 rev/L; 95% CI, –1,000 to –100) and MX concentration (–10 ng/L; 95% CI, –18 to –2). The application of chlorine before and after filtration was associated with increased mutagenicity (500 rev/L; 95% CI, 0–1,000) and MX (8 ng/L; 95% CI, –1 to 16).

We trichotomized the water quality indicators to determine their effect on mutagenic activity and DBP formation (Table 4). Chlorine dose was associated with mutagenicity, MX, and TTHM, whereas residual chlorine in the distribution system was not associated with the DBP indicators. Higher

pH ($p < 0.01$) and turbidity ($p = 0.01$) were associated with TTHM concentration but not with MX or mutagenic activity. A linear trend in TTHM concentration was detected for increasing temperature ($p < 0.01$). Inverse relationships were observed between temperature and both mutagenicity and MX, although the tests for trend were not statistically significant. Linear trends with increasing TOC levels were detected for mutagenic activity and MX concentration.

We identified predictors of mutagenicity, MX, and TTHM via univariate linear regression models. The crude and adjusted

results are shown in Table 5. Chloramination, chlorine dose, and a marker for season were consistent predictors of mutagenicity, MX, and TTHM and were included in the core multivariate models. The other covariates were included in the regression models to determine if they had an independent effect on mutagenic activity and DBP formation.

After adjusting for the other covariates, seasonality had the largest impact on mutagenic activity. A difference of 800 rev/L (95% CI, –1,300 to –300) in mutagenic activity was observed in fall samples compared to spring samples. A similar effect was

Table 2. Univariate linear regression of mutagenic activity and disinfection by-product concentration.

	No.	Slope (SE)	Intercept (SE)	r^a
Mutagenicity versus MX				
Spring 1997	30	56.1 (4.6)	–5 (188)	0.92
Fall 1997	10	43.4 (17.8)	206 (226)	0.65
Spring 1998	22	36.2 (8.3)	587 (243)	0.70
Fall 1998	26	31.9 (4.7)	272 (132)	0.81
Total	88	49.2 (3.2)	111 (102)	0.86
Mutagenicity versus TTHM				
Spring 1997	29	44.8 (6.1)	547 (236)	0.77
Fall 1997	8	9.3 (6.2)	448 (237)	0.28
Spring 1998	22	13.0 (8.8)	1,053 (345)	0.40
Fall 1998	24	11.5 (3.4)	450 (197)	0.52
Total	83	15.5 (4.3)	857 (195)	0.44
MX versus TTHM				
Spring 1997	29	0.7 (0.1)	13.4 (4.1)	0.82
Fall 1997	8	0.1 (0.1)	9.3 (3.5)	0.48
Spring 1998	22	0.3 (0.2)	14.2 (6.5)	0.31
Fall 1998	24	0.3 (0.1)	11.5 (5.3)	0.59
Total	83	0.3 (0.7)	14.9 (3.2)	0.37

^aPearson correlation coefficient.

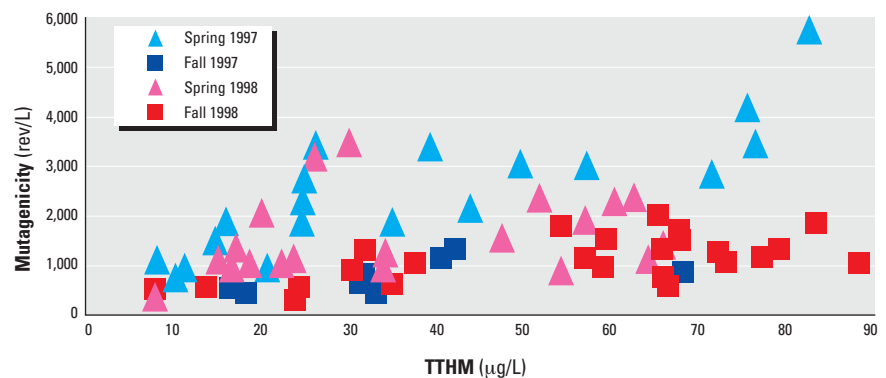


Figure 3. Mutagenicity versus TTHM concentration.

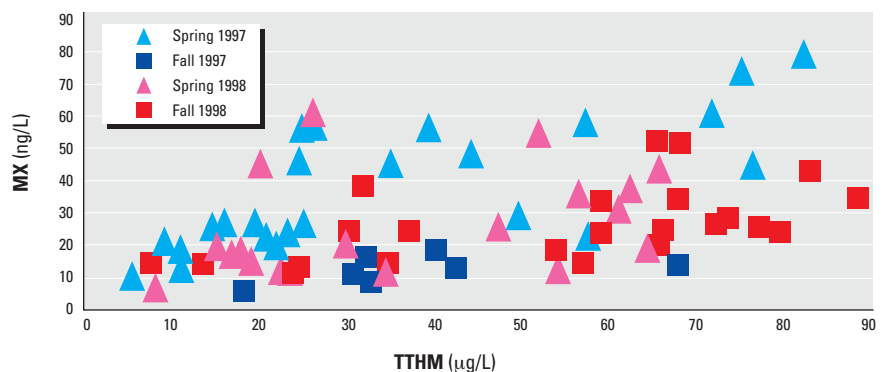


Figure 4. MX concentration versus TTHM concentration.

observed in samples collected from chloraminated systems (–800 rev/L; 95% CI, –1,450 to –200). Filtration was also influential, with filtered supplies resulting in less mutagenicity than unfiltered water (–650; 95% CI, –1,250 to –50). Communities with multiple chlorine application (before and after filtration) had

higher levels of mutagenic activity (550; 95% CI, –50 to 1,100). Chlorine dose, TOC, and pH were also associated with increased mutagenicity.

Seasonal differences were observed in the MX samples—higher concentrations were found in spring (10 ng/L; 95% CI, 2–19)

compared to fall (Table 5). Communities that used filtration (–12 ng/L; 95% CI, –23 to –2) and chloramination (–17 ng/L; 95% CI, –27 to –6) had significantly lower MX levels compared to other methods of treatment. Chlorine dose, TOC, and pH were also associated with increased MX concentrations.

Table 3. Influence of water treatment practices on mutagenic activity and disinfection by-product concentration.

	Mutagenicity (rev/L)			MX (ng/L)			TTHM (µg/L)		
	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
Total	84	1,500	987	84	27.7	17.3	82	39.3	23.4
Plant characteristics									
Filtration									
Yes	37	1,600	875	37	29.6	15.6	37	39.5	21.7
No	47	1,400	1,070	47	26.3	18.5	45	39.1	24.9
Difference		200			3.3			0.4	
Aluminum sulfate									
Yes	30	1,650	826	30	29.7	15.0	30	41.7	21.2
No	54	1,400	1,064	54	26.6	18.5	52	37.9	24.6
Difference		250			3.1			3.8	
Activated carbon									
Yes	18	1,950	1,005	18	33.4	17.1	18	40.9	18.5
No	66	1,350	953	66	26.2	17.1	64	38.8	24.7
Difference		600 ^a			7.2			2.1	
Chloramination									
Yes	29	1,100	414	29	21.2	10.7	28	37.5	25.0
No	55	1,700	1,140	55	31.2	19.1	54	40.2	22.7
Difference		–600 ^a			–10.0 ^a			–2.7	
Multiple chlorine application ^b									
Yes	19	1,900	1,010	19	33.5	16.4	19	38.0	20.1
No	65	1,400	955	65	26.0	17.3	63	39.6	24.4
Difference		500			7.5			–1.6	

^aStatistically significant difference ($p < 0.05$). ^bChlorine applied before and after filtration.

Table 4. Influence of water quality indicators on mutagenic activity and disinfection by-product concentration.

	Mutagenicity (rev/L)			MX (ng/L)			TTHM (µg/L)		
	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
Total	84	1,500	987	84	27.7	17.3	82	39.3	23.4
Water quality indicators									
Cl ₂ dose									
0.55–1.52 mg/L	27	1,250	660	27	24.8	13.7	27	28.0	20.2
1.53–1.95 mg/L	28	1,500	913	28	26.2	14.1	28	50.4	21.6
1.96–3.07 mg/L	26	1,850	1,260	26	34.4	21.9	25	40.4	23.8
Trend test		$p = 0.02$			$p = 0.02$			$p = 0.01$	
Cl ₂ residual									
0.01–0.40 mg/L	25	1,250	822	25	24.5	15.2	24	34.9	25.1
0.41–0.87 mg/L	27	1,800	1,266	27	32.9	20.4	26	41.7	22.8
0.88–2.00 mg/L	27	1,500	804	27	27.8	16.0	27	39.2	23.8
Trend Test		$p = 0.68$			$p = 0.69$			$p = 0.47$	
pH									
5.66–7.39	23	2,100	1,311	23	35.9	21.3	23	36.3	24.4
7.40–8.16	27	1,150	718	27	21.9	13.8	26	29.2	19.1
8.17–9.90	28	1,450	689	28	28.9	14.0	28	50.5	22.2
Trend test		$p = 0.09$			$p = 0.61$			$p = 0.002$	
Temperature									
1.1–6.7 (°C)	28	1,950	1,276	28	34.6	21.4	27	38.7	25.2
6.8–11.0 (°C)	26	1,400	749	26	25.8	14.0	26	30.2	22.2
11.1–22.6 (°C)	26	1,250	690	26	25.1	13.6	26	49.0	19.5
Trend test		$p = 0.05$			$p = 0.12$			$p = 0.002$	
Turbidity									
0.01–0.18 NTU	28	1,600	911	28	30.8	16.9	28	37.7	23.0
0.19–0.34 NTU	28	1,350	840	28	24.9	14.7	27	39.3	24.4
0.35–1.50 NTU	28	1,500	1,192	28	27.5	20.0	27	40.9	23.5
Trend test		$p = 0.66$			$p = 0.29$			$p = 0.01$	
TOC									
2.0–2.8 mg/L	36	1,150	628	36	21.2	11.7	35	39.4	22.5
2.9–3.9 mg/L	19	1,350	761	19	27.2	15.6	19	29.1	19.4
4.0–7.3 mg/L	20	2,000	1,271	20	36.3	19.5	20	50.0	23.2
Trend test		$p = 0.01$			$p = 0.01$			$p = 0.19$	

NTU, nephelometric turbidity unit.

In contrast to MX and mutagenicity, season was inversely associated with TTHM levels, with higher values occurring in the fall ($-15 \mu\text{g/L}$; 95% CI, -26 to -4). Chloramination was associated with lower TTHM concentrations ($-15 \mu\text{g/L}$; 95% CI, -28 to -1). Other predictors of TTHM concentration included TOC and pH. Chlorine dose was marginally significant ($p = 0.06$) with every 1 mg/L increase in chlorine resulting in an 8 $\mu\text{g/L}$ (95% CI, 0–17) increase in TTHM.

Temporal changes in DBPs and mutagenic activity. Water samples were collected for 15 communities in spring 1997, spring 1998, and fall 1998 to evaluate the role of changes in treatment practices over time. This analysis was restricted to 14 communities because one town changed its water source during summer 1998. MX concentrations were higher in fall 1998 samples than in spring samples in 1997 and 1998 (Table 6). TTHM levels were also higher during fall 1998 compared to spring 1998. Mutagenic activity was highest in spring 1997 but decreased during the next two sampling periods.

MX and TTHM concentrations were relatively stable over time when stratified by water source, whereas mutagenicity decreased in the towns with independent water supplies. Mutagenicity, MX, and TTHM were similar in spring 1997 and spring 1998 for the seven eastern Massachusetts communities. This may have been due to the elimination of a rechlorination step in one of the towns in January 1998. When the analysis was restricted to the other six towns, TTHM, MX, and mutagenicity all increased over the three sampling periods. The temporal differences observed from spring to fall of 1998 within the unified eastern Massachusetts system coincided with changes in disinfection. The chlorine dose was increased as part of the chloramination disinfection before spring 1998. Free chlorine was also added at an earlier stage in the treatment process at one of the main holding reservoirs, and the chlorine

contact time was increased before the addition of ammonia.

Discussion

MX concentrations in Massachusetts drinking water were higher than previously reported in the United States (maximum = 33 ng/L) (16) and in Finland (maximum = 67 ng/L) (35,49). Nearly one-third of the Massachusetts drinking water samples were $> 33 \text{ ng/L}$ and three samples were $> 67 \text{ ng/L}$. This suggests that high MX concentrations are more prevalent in U.S. surface water supplies than previously indicated.

Due to the multitude of mutagenic compounds present in drinking water, it was useful to obtain a direct measurement of mutagenicity. We were interested in determining whether MX measurements provide any additional information on potential mutagenic activity and DBP levels beyond that given by routine trihalomethane sampling. In accordance with other findings, our data indicated that MX concentrations were highly correlated with mutagenic activity ($r = 0.86$). TTHM concentrations were not highly correlated with mutagenic activity ($r = 0.44$) for our samples collected during the fall and spring months. Vartiainen et al. (39) reported higher correlations in samples collected in April ($r = 0.63$). This is in agreement with our data, since we found the highest correlations in samples collected exclusively in March

(spring 1997; Table 2). Nestmann et al. (50) previously described seasonal variability in the relationship between mutagenicity and chloroform (higher correlation in summer vs. winter). We also detected weaker correlations between TTHM and HAA₅ ($r = 0.35$) and for TTHM and MX concentrations ($r = 0.37$). These findings have important epidemiologic implications because they suggest that TTHM may not be a good surrogate for nonvolatile DBPs or mutagenic activity.

Our findings indicate that mutagenic activity is influenced by physical and chemical treatment of drinking water. Filtration was associated with lower levels of MX after adjusting for other water quality characteristics and time of collection (Table 5). Similar to previous reports, chloramination appeared to limit the formation of TTHM, MX, and mutagenic compounds. Chlorine dose and total organic carbon were strong predictors of mutagenicity and MX concentration. These findings are in accordance with mutagenic models developed by Vartiainen et al. (39), who found that TOC, chlorine dose, and to a lesser extent ammonia were important predictors of mutagenic activity.

Trihalomethane formation has been shown to be dependent on pH, with greater concentrations occurring at higher pH (51). In contrast, higher pH results in lower levels of total organic halides. Previous work indicates that the formation of MX and mutagenicity

Table 6. Mean (SD) mutagenic activity and disinfection by-product concentration in repeated samples for seven eastern Massachusetts communities served by a single source and seven independent communities.

	No.	Spring 1997	Spring 1998	Fall 1998
Mutagenicity (rev/L)				
Eastern Massachusetts	7	1,000 (531)	1,000 (131)	1,350 (370)
Independent	7	2,700 (1,558)	1,950 (1,037)	850 (458)
Total	14	1,850 (1,432)	1,500 (867)	1,100 (467)
MX (ng/L)				
Eastern Massachusetts	7	20.0 (12.7)	17.0 (4.8)	37.9 (21.3)
Independent	7	48.7 (21.8)	32.7 (20.7)	41.6 (19.9)
Total	14	34.6 (22.7)	24.8 (16.6)	44.9 (20.1)
TTHM ($\mu\text{g/L}$)				
Eastern Massachusetts	7	17.4 (11.8)	19.1 (6.7)	64.3 (8.3)
Independent	7	47.4 (23.3)	42.4 (16.4)	57.0 (25.1)
Total	14	32.4 (23.6)	30.8 (17.0)	60.7 (18.3)

Table 5. Linear regression predictors of mutagenic activity and disinfection by-product concentration.

	Mutagenicity (rev/L)		MX (ng/L)		TTHM ($\mu\text{g/L}$)	
	Univariate slope (SE)	Multivariate slope (SE)	Univariate slope (SE)	Multivariate slope (SE)	Univariate slope (SE)	Multivariate slope (SE)
Chloramination (yes vs. no)	$-535 (219)^{**}$	$-818 (306)^{\#}$	$-10.0 (3.8)^{**}$	$-16.5 (5.3)^{\#}$	$-2.7 (5.5)$	$-14.5 (6.9)^{**}$
Filtration (yes vs. no)	171 (217)	$-662 (307)^{**}$	3.3 (3.8)	$-12.3 (5.4)^{**}$	0.4 (5.2)	$-9.6 (6.9)$
Multiple chlorine application ^a (yes vs. no)	520 (252) ^{**}	529 (289) [*]	7.5 (4.5) [*]	7.2 (5.1)	$-1.6 (6.2)$	3.6 (6.5)
Cl ₂ dose (mg/L)	375 (190)	522 (191) [#]	7.0 (3.3) ^{**}	7.6 (3.3) ^{**}	11.2 (4.5) ^{**}	8.4 (4.3) [*]
Cl ₂ residual (mg/L)	146 (246)		2.2 (4.3)		4.7 (5.8)	
Turbidity (NTU)	168 (462)		8.0 (8.0)		27.4 (10.8) [#]	
TOC ^b	411 (120) [#]	284 (142) ^{**}	7.5 (2.1) [#]	6.0 (2.5) ^{**}	4.2 (3.2)	8.8 (3.2) [#]
pH	$-226 (132)$	465 (169) [#]	$-1.2 (2.3)$	10.0 (2.9) [#]	9.6 (3.0) [#]	14.5 (3.8) ^{##}
Temperature ($^{\circ}\text{C}$)	$-40.2 (20.1)^{**}$		$-0.6 (0.4)$		1.5 (0.5) [#]	
Season (spring vs. fall)	784 (195) ^{##}	809 (243) [#]	9.0 (3.6) ^{**}	10.4 (4.2) ^{**}	$-16.9 (4.9)^{\#}$	$-15.3 (5.5)^*$
Year (1997 vs. 1998)	309 (206)		4.0 (3.6)		$-12.9 (5.2)^{**}$	

^aChlorine applied before and after filtration. ^bCoefficient explains the unit change from low-to-moderate or from moderate-to-high values. ^{*} $p < 0.10$. ^{**} $p < 0.05$. [#] $p < 0.01$. ^{##} $p < 0.001$.

are also inversely associated with pH (52). We observed a linear dependence of mutagenicity and MX on pH, similar to TTHM formation, after adjusting for other covariates. Similar to other reports (39), we found that other parameters are more influential in the formation of MX and mutagenicity than pH at levels typically found in distribution systems (pH 6–10).

Seasonal variation (i.e., peaks in the summer months) in trihalomethanes has been widely reported, but information is limited on the seasonality of nonvolatile species and mutagenic activity. Mutagenicity has been reported to be higher in spring due to increases in temperature and runoff due to rainfall (53) and slightly higher in winter (compared to summer) (39). Our data indicate that seasonal differences exist for both MX and mutagenicity beyond that associated with increases in water temperature and chlorine dosage (Table 5). Mutagenic levels were higher in the spring (vs. the fall) in repeated samples from seven independent water systems in which no major chlorination changes occurred (Table 6). Because of the changes in chlorination practices over this period in the eastern Massachusetts communities, we would not expect a similar pattern. MX concentrations were similar over time. Assumptions regarding seasonal and spatial variability are built into many exposure assessment constructs. Therefore, additional data on the sources of variability in mutagenic compounds would greatly enhance epidemiologic efforts.

The analysis of the water quality parameters should be interpreted with caution because the data provided by the water departments cannot be independently verified. Data were not always available on the sample collection dates, so we collected data that were closest in time and location to our samples. We were more interested in evaluating the between-town variability, so limited daily within-town variability would have minimal impact on our findings. The TOC measurements were an exception because data were not available at the time of sample collection. Therefore, the data may not correspond to levels of organics at the time of our sampling. However, we assume that the relative TOC rankings are valid and that temporal variations would be similar across towns within a given geographic region.

The U.S. EPA has recently established a maximum contaminant level (MCL) of 60 µg/L for HAA₅ and has revised the MCL to 80 µg/L for TTHMs (54). MCLs were also established for bromate (10 µg/L) and chlorite (1 mg/L). These standards are effective December 2001 for public water systems serving populations over 10,000 and December 2003 for smaller systems. Other

DBPs are not currently regulated in the United States, although the World Health Organization has recommended that the formation of MX be limited in drinking water (55). We have demonstrated that MX and mutagenic activity are found in significant concentrations in Massachusetts surface water and that TTHM may not be a good surrogate for nonvolatile DBPs or mutagenic activity. Although the impending regulations are an important step in limiting exposure to DBPs in drinking water, they are unlikely to provide much information on the occurrence of mutagenic compounds.

REFERENCES AND NOTES

- Mughal FH. Chlorination of drinking water and cancer: a review. *J Environ Pathol Toxicol Oncol* 11:287–292 (1992).
- National Cancer Institute. Report on the Carcinogenesis Bioassay of Chloroform. NTIS PB-264-018. Bethesda, MD:National Institutes of Health, 1976.
- Bull RJ, Sanchez IM, Nelson MA, Larson JL, Lansing AJ. Liver tumor induction in B6C3F₁ mice by dichloroacetate and trichloroacetate. *Toxicology* 63:341–359 (1990).
- Bull RJ, Meier JR, Robinson M, Ringhand HP, Laurie D, Stober J. Evaluation of mutagenic and carcinogenic properties of brominated and chlorinated acetonitriles: by-products of chlorination. *Fundam Appl Toxicol* 5:1065–1074 (1985).
- Kurokawa Y, Aoki S, Matsushima Y, Takamura N, Imazawa T, Hayashi Y. Dose-response studies on the carcinogenicity of potassium bromate in F344 rats after long-term oral administration. *J Natl Cancer Inst* 77:977–982 (1986).
- Komulainen H, Kosma V-M, Vartiainen S-L, Vartiainen T, Kaliste-Korhonen E, Lötjönen S, Tuominen RK, Tuomisto J. Carcinogenicity of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone in the rat. *J Natl Cancer Inst* 89(12):848–856 (1997).
- NTP. Toxicology and Carcinogenesis Studies of Tribromomethane (Bromoform) (CAS No. 75-25-2) in F344/N Rats and B6C3F₁ Mice (Gavage Studies). TR 350. Research Triangle Park, NC:National Toxicology Program, 1989.
- NTP. Toxicology and Carcinogenesis Studies of Bromodichloromethane (CAS No. 75-27-4) in F344/N Rats and B6C3F₁ Mice. TR 321. Research Triangle Park, NC:National Toxicology Program, 1989.
- Morimoto K, Koizumi A. Trihalomethanes induce sister chromatid sister exchanges in human lymphocytes *in vitro* and mouse bone marrow cells *in vivo*. *Environ Res* 32:72–79 (1983).
- Le Curieux F, Gauthier L, Erb F, Marzin D. Use of the SOS chromotest, the Ames-fluctuation test and the newt micronucleus test to study the genotoxicity of four trihalomethanes. *Mutagenesis* 10:333–341 (1995).
- Meier JR. Genotoxic activity of organic chemicals in drinking water. *Mutat Res* 196:211–245 (1988).
- Krasner SW, McGuire MJ, Jacangelo JG, Patania NL, Reagan KM, Aietta EM. The occurrence of disinfection by-products in US drinking water. *J Am Water Works Assoc* 81(8):41–53 (1989).
- DeMarini DM, Perry E, Shelton ML. Dichloroacetic acid and related compounds: induction of prophage in *E. coli* and mutagenicity and mutation spectra in TA100. *Mutagenesis* 9:429–437 (1994).
- Fusco JC, Afshari AJ, George MH, DeAngelo AB, Tice RR, Salzman T, Allen JW. *In vivo* genotoxicity of dichloroacetic acid: evaluation with the mouse peripheral blood micronucleus assay and the single cell gel assay. *Environ Mol Mutagen* 27:1–9 (1996).
- Harrington-Brock K, Doerr CL, Moore MM. Mutagenicity of three disinfection by-products: di- and trichloroacetic acid and chloral hydrate in L5178Y/TK^{+/+}-3.7.2C mouse lymphoma cells. *Mutat Res* 413:265–276 (1998).
- Meier JR, Knohl RB, Coleman WE, Ringhand HP, Munch JW, Kaylor WH, Streicher RP, Kopfier FC. Studies on the potent bacterial mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: aqueous stability, XAD recovery and analytical determination in drinking water and in chlorinated humic acid solutions. *Mutat Res* 189:363–373 (1987).
- Daniel FB, Olson GR, Stober JA. Induction of gastrointestinal tract nuclear anomalies in B6C3F₁ mice by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and 3,4-(dichloro)-5-hydroxy-2(5H)-furanone, mutagenic byproducts of chlorine disinfection. *Environ Mol Mutagen* 17:32–39 (1991).
- Sasaki YF, Nishidate E, Izumiyama F, Watanabe-Akanuma M, Kinai N, Matsusaka N, Tsuda S. Detection of *in vivo* genotoxicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) by the alkaline single cell gel electrophoresis (Comet) assay in multiple mouse organs. *Mutat Res* 393:47–53 (1997).
- Furihata C, Yamashita M, Kinai N, Matsushima T. Genotoxicity and cell proliferative activity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone [MX] in rat glandular stomach. *Water Sci Technol* 25:341–345 (1992).
- Mäki-Paakkanen J, Jansson K. Cytogenic effects in the peripheral lymphocytes and kidney cells of rats exposed to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) orally on three consecutive days. *Mutat Res* 343:151–156 (1995).
- Jansson K, Mäki-Paakkanen J, Vartiainen S-L, Vartiainen T, Komulainen H, Tuomisto J. Cytogenetic effects of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in rat peripheral lymphocytes *in vitro* and *in vivo*. *Mutat Res* 299:25–28 (1993).
- Meier JR, Blazak WF, Knohl RB. Mutagenic and clastogenic properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone: a potent bacterial mutagen in drinking water. *Environ Mol Mutagen* 10:411–427 (1987).
- Brunborg G, Holme JA, Söderlund EJ, Hongso JK, Vartiainen T, Lötjönen S, Becher G. Genotoxic effects of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in mammalian cells *in vitro* and in rats *in vivo*. *Mutat Res* 260:55–64 (1991).
- Matsumura H, Watanabe M, Matsumoto K, Ohta T. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) induces gene mutations and inhibits metabolic cooperation in cultured Chinese hamster cells. *J Toxicol Environ Health* 43:65–72 (1994).
- Mäki-Paakkanen J, Jansson K, Vartiainen T. Induction of mutation, sister-chromatid exchanges, and chromosome aberrations by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone in Chinese hamster ovary cells. *Mutat Res* 310:117–123 (1994).
- Harrington-Brock K, Doerr CL, Moore MM. Mutagenicity and clastogenicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in L5178Y/TK^{+/+}-3.7.2C mouse lymphoma cells. *Mutat Res* 348:105–110 (1995).
- Le Curieux F, Nesslany F, Munter T, Kronberg L, Marzin D. Genotoxic activity of chlorohydroxyfuranones in the microscale micronucleus test on mouse lymphoma cells and the unscheduled DNA synthesis assay in rat hepatocytes. *Mutagenesis* 14(5):457–462 (1999).
- Melnick RL, Boorman GA, Dellarco V. Water chlorination, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), and potential cancer risk. *J Natl Cancer Inst* 89(12):832–833 (1997).
- Boorman GA, Dellarco V, Dunnick JK, Chapin RE, Hunter S, Hauchman F, Gardner H, Cox M, Sills RC. Drinking water disinfection byproducts: review and approach to toxicity evaluation. *Environ Health Perspect* 107(suppl 1):207–217 (1999).
- Morris RD, Audet AM, Angelillo IF, Chalmers TC, Mosteller F. Chlorination, chlorination by-products, and cancer: a meta-analysis. *Am J Public Health* 82:955–963 (1992).
- Koivusalo M, Hakulinen T, Vartiainen T, Pukkala E, Jaakkola JJK, Tuomisto J. Drinking water mutagenicity and urinary tract cancers: a population-based case-control study in Finland. *Am J Epidemiol* 148(7):704–712 (1998).
- Koivusalo M, Jaakkola JJK, Vartiainen T, Hakulinen T, Karjalainen S, Pukkala E, Tuomisto J. Drinking water mutagenicity and gastrointestinal and urinary tract cancers: an ecological study in Finland. *Am J Public Health* 84(8):1223–1228 (1994).
- Koivusalo M, Pukkala E, Vartiainen T, Jaakkola JJK, Hakulinen T. Drinking water chlorination and cancer—a historical cohort study in Finland. *Cancer Causes Control* 8:192–200 (1997).

34. Koivusalo M, Vartiainen T, Hakulinen T, Pukkala E, Jaakkola JJK. Drinking water mutagenicity and leukemia, lymphomas and cancers of the liver, pancreas, and soft tissue. *Arch Environ Health* 50(4):269–276 (1995).
35. Kronberg L, Vartiainen T. Ames mutagenicity and concentration of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone and its geometric isomer E-2-chloro-3-(dichloromethyl)-4-oxo-butenoic acid in chlorine treated tap water. *Mutat Res* 206:177–182 (1988).
36. Suzuki N, Nakanishi J. The determination of strong mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone, in drinking water in Japan. *Chemosphere* 21(3):387–392 (1990).
37. Fawell JK, Horth H. Assessment and identification of genotoxic compounds in water. In: *Genetic Toxicology of Complex Mixtures* (Waters MD, ed). New York: Plenum Press, 1990;197–214.
38. Singer PC. Control of disinfection by-products in drinking water. *J Environ Eng* 4:727–744 (1994).
39. Vartiainen T, Liimatainen A, Kauranen P, Hiisvirta L. Relations between drinking water mutagenicity and water quality parameters. *Chemosphere* 17:189–202 (1988).
40. Cheh AM, Skochdopole J, Koski P, Cole L. Nonvolatile mutagens in drinking water: production by chlorination and destruction by sulfite. *Science* 207:90–92 (1980).
41. Kowbel DJ, Ramaswamy S, Malaiyandi M, Nestmann ER. Mutagenicity studies in *Salmonella*: residues of ozonated and/or chlorinated water fulvic acids. *Environ Mutagen* 8:253–262 (1986).
42. Kronberg L, Vartiainen T. Ames mutagenicity and concentration of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone and its geometric isomer E-2-chloro-3-(dichloromethyl)-4-oxo-butenoic acid in chlorine-treated tap water. *Mutat Res* 206:177–182 (1988).
43. Symons JM, Stevens AA, Clark RM, Geldreich EF, Love OT Jr, DeMarco J. Treatment techniques for controlling trihalomethanes in drinking water. EPA-600/2-81-156. Washington, DC:U.S. Environmental Protection Agency, 1981.
44. U.S. EPA. Providing Safe Drinking Water in America, 1997 National Public Water Systems Compliance Report and Update on Implementation of the 1996 Safe Drinking Water Act Amendments. EPA 305-R-99-002. Washington DC:U.S. Environmental Protection Agency, 1999.
45. Guterma D. Personal communication.
46. U.S. Environmental Protection Agency. Information collection rule. *Fed Reg* 61(94):24354–24388 (1996).
47. Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. *Mutat Res* 113:173–215 (1983).
48. U.S. EPA. Methods for the Determination of Organic Compounds in Drinking Water. EPA/600/R-95-131-Supplement III. Washington DC:U.S. Environmental Protection Agency, 1995.
49. Smeds A, Vartiainen T, Mäki-Paakkanen J, Kronberg L. Concentrations of Ames mutagenic chlorohydroxyfuranones and related compounds in drinking waters. *Environ Sci Technol* 31(4):1033–1039 (1997).
50. Nestmann ER, Otson R, LeBel BL, Williams DT, Lee EGH, Biggs DC. Correlation of water quality parameters with mutagenicity of drinking water extracts. In: *Water Chlorination, Vol 4(2)* (Jolley RL, Brungs WA, Cotruvo JA, Cummings RB, Mattice JS, Jacobs VA, eds). Ann Arbor, MI:Ann Arbor Science Publishers, 1983;1151–1164.
51. Singer P. Formation and characterization of disinfection by-products. In: *Safety of Water Disinfection: Balancing Chemical and Microbial Risk* (Craun G, ed). Washington DC:ILSI, 1986;201–219.
52. Meier JR, Lingg RD, Bull RJ. Formation of mutagens following chlorination of humic acid. A model for mutagen formation during drinking water treatment. *Mutat Res* 118:25–41 (1983).
53. Grabow WOK, Van Rossum PG, Grabow NA, Denkhaus R. Relationship of the raw water quality to mutagens detectable by the Ames *Salmonella*/microsome assay in a drinking-water supply. *Water Res* 15:1037–1043 (1981).
54. U.S. Environmental Protection Agency. National primary drinking water regulations: disinfectants and disinfection byproducts; final rule. *Fed Reg* 63(241):69390–69476 (1998).
55. WHO. *Guidelines for Drinking Water Quality, Vol 1, Recommendations*. Geneva:World Health Organization, 1993.