ABSTRACT

Although monochloramine has long been considered as an alternative disinfectant to chlorine, little is known about the by-products from its reactions with natural organics. In this study, the by-products of aqueous monochloraminated fulvic acid were characterized by Ames assays, Total Organic Halide (TOX) analyses and by fractionation on High Performance Liquid Chromatography (HPLC). In character and type, the by-products are very similar to those produced by chlorination. The mutagenic by-products were observed to be polar, acidic compounds that exhibited an increase in mutagenicity with increasing monochloramine dose. In addition, they were shown to be predominantly direct acting, base-pair substituting and electrophilic. The results of concurrent Ames assays and TOX analyses suggested that chlorinated compounds were responsible for the mutagenicity produced by monochloramination. Analyses of ether extracts by High Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS) resulted in the identification and quantification of the potent bacterial mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and its analogs E-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX), E-2-chloro-3-(dichloromethyl)-butenedioic acid (ox-EMX) and 2,3-dichloro-4-oxobutenoic acid (mucochloric acid). MX, EMX and ox-EMX respectively accounted for 11%, 26% and 2% of the mutagenic activity of the monochloramination extracts. Several short-chain (C_2-C_9) aliphatic chlorinated organic acids, alcohols and aldehydes have also been tentatively identified of which di- and

trichloroacetic acids, dichlorosuccinic acid and E-2-chloro-3-(dichloromethyl)-butenedioic acid (ox-EMX) have been confirmed and quantified. Of these, the alkenoic acids may be of toxicological significance due to their structural similarity to the open oxo-butenoic form of MX.

KEY WORDS

monochloramine

fulvic acid

mutagenicity

ames assay

by-products

total organic halide

gas chromatography

mass spectrometry

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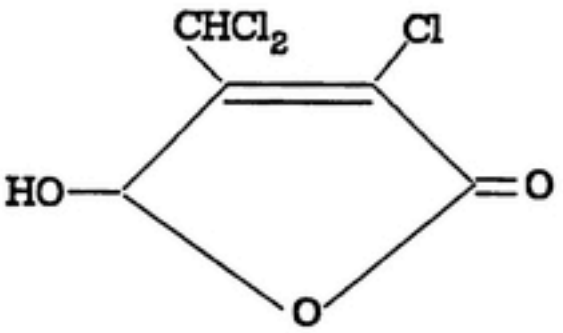
INTRODUCTION

The search for a suitable alternative disinfectant to chlorine has been active ever since Rook in 1974 demonstrated that chlorination of water containing natural humic substances led to the formation of trihalomethanes such as chloroform. Initial concern over the carcinogenic properties of chloroform was soon heightened by the discovery that large quantities of non-volatile chlorinated compounds are formed along with chloroform (Christman et al., 1983). Some of the former have also been shown to be mutagenic in the Ames assay. These compounds include chloroacetones, chloropropenals, 3,4-dichloro-5-(dichloromethyl)-5-hydroxy-2-furanone, 5,5,5-trichloro-4-oxopentanoic acid, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and E-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX), 3-chloro-4-(dichloromethyl)-2(5H)-furanone (red-MX) and 2-chloro-3-(dichloromethyl)-butenoic acid (ox-MX) (Coleman et al.,1987; Hemming et al., 1986; Holmbom et al., 1984; Kronberg and Christman, 1988; Meier et al., 1987; Munch et al., 1987). Of these, MX and to a lesser extent, its analogs (Figure 1) have become very significant due to their mutagenic potency. MX has been called "the most important single mutagen so far identified in drinking water" (Backlund et al., 1988). Activity in the Ames assay is cause for concern because the assay has been shown to be a reliable predictor of carcinogenicity for many classes of compounds (Mc Cann and Ames, 1976; Tennant et al., 1987).

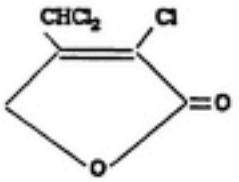
In view of the human health hazards posed by chlorine by-products,



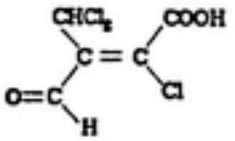
3-CHLORO-4-(DICHLOROMETHYL)-

5-HYDROXY-2(5H)-FURANONE

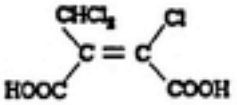
(MX)



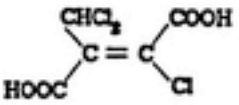
RED-MX



EMX



OX-MX



OX-EMX

Figure 1. MX and its Analogs.

monochloramine has gained importance as a possible alternative or secondary disinfectant in water treatment processes. Although it has poorer bacteriocidal and virucidal capabilities (Siders et al., 1973), it has been shown to have the advantage of producing much lower quantities (less than 3%) of trihalomethane than chlorine (Stevens et al., 1976). In addition, monochloramine produced Total Organic Halide (TOX) has been characterized by Jensen et al. (1984) to be smaller in quantity (9-49% as much as chlorine produced TOX) more hydrophilic and larger in molecular size than TOX produced from chlorine.

However, other information regarding the potential human health hazards associated with the use of monochloramine is very limited. Several researchers have identified mutagenicity in monochloraminated humic as well as drinking waters (Cheh et al., 1980; Miller et al., 1986; Backlund et al., 1988; Schenk et al., 1990). However, few have been able to identify by-products leading to this mutagenicity. It has been postulated that monochloramine will form substitution products over oxidation products in drinking waters due to its poor oxidant ability (Jensen, 1983). However, the only by-products identified to date are 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and E-2-chloro-3-(dichloromethyl)-4-oxobutenoic acid (EMX) (Backlund et al., 1988).

Clearly there exists a need to further investigate the effect of monochloramine on the formation of potentially toxic by-products, especially that of MX and its analogs. The objective of this research was thus to characterize and identify the by-products in mutagenic extracts of aqueous monochloraminated fulvic acid. Fulvic acid was chosen as a model precursor for by-product formation since it accounts

for approximately 45% of the organic carbon in natural water. In addition, the products of aqueous chlorination of fulvic acid and chlorination by-products found in drinking water have been found to be similar (Norwood, 1985).

The problem previously encountered with research on monochloramine by-products was the isolation of sufficient product for the identification of non-volatile compounds, owing to the poor oxidant abilities of monochloramine (Jensen, 1983). Accordingly, in this work, methodologies were developed to react monochloramine with fulvic acid and isolate significant amounts of mutagenically active product. The extracted product was then characterized by Ames Assays, High Performance Liquid Chromatography (HPLC) and Total Organic Halide (TOX) and Total Organic Carbon (TOC) analyses. High Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS) was used to identify and quantify the by-products. These techniques have previously been used in chlorination studies for the isolation and identification of several by-products including the strong mutagen MX (Horth et al., 1985; Horth et al., 1987; Kronberg et al, 1987; Becher et al., 1985; St. Aubin, 1985; Coleman et al., 1984; Meier et al., 1985).

EXPERIMENTAL METHODS

An overview of the procedures and reaction conditions used in the major portion of this study is shown in Figure 2.

Sample Preparation

Monochloramine solutions were prepared by the method of Johnson and Overby (1969). Ammonia and chlorine were reacted in 3:1 molar ratio. Equal volumes of desired concentrations of NaOCl and NH_4Cl in 0.05M phosphate buffer, pH 8.0 were mixed with stirring in an ice bath. Chlorine and monochloramine concentrations were determined throughout this work by the DPD Ferrous Titrimetric method (Standard Methods 408D, 1989).

The fulvic acid used in this study had previously been isolated from Lake Drummond, VA waters by the method of Thurman and Malcolm (1981). The fulvic acid has a low ash content and a carbon content of 48.81% (Jensen, 1983).

Monochloramine and fulvic acid were reacted at the desired Cl:C molar ratio at room temperature, with stirring. The pH of the reaction mixture was maintained at pH 8.0 over the reaction period of 96 hours to ensure that monochloramine was the only chloramine specie in solution. Monochloramine residuals measured at the end of the reaction period ranged from 0% to about 9% of the original dose. In preliminary experiments the reaction mixture was divided into two parts, one

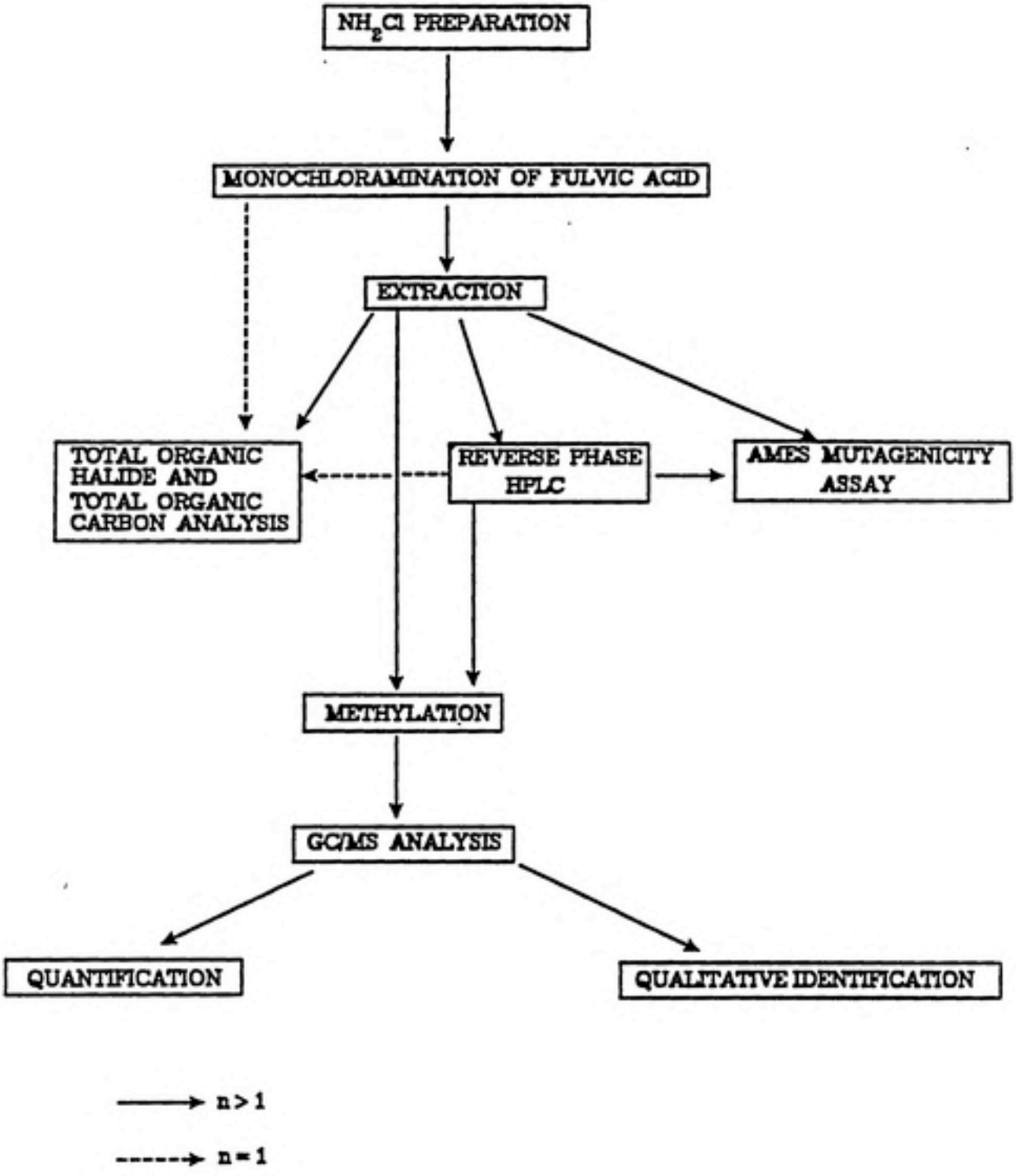


Figure 2. Overview of the Analytical Protocol

acidified with concentrated HCl to pH 2.0, the other maintained at pH 8.0. In the remainder of the work, the entire sample was acidified to pH 2.0 with HCl. Extraction of the sample was performed immediately after acidification.

Chlorination of fulvic acid was performed by reacting NaOCl (300mg/L as Cl_2) with fulvic acid at pH 7.0 for 48 hours at a molar Cl:C ratio of 1:5. The residual at the end of the reaction period was measured to be 40mg/L as Cl_2. Products in the resulting extracts were then quantified so that a comparison of the amounts of specific by-products of monochloramination and chlorination could be obtained at comparable doses (molar Cl:C ratio).

The techniques used for extraction and concentration of the by-products were macroreticular XAD-Resins (Rohm and Hass, Philadelphia, PA), freeze-drying and liquid-liquid extraction with ether. Resin experiments utilized XAD-8, a polar methyl methacrylate resin and XAD-4, a non-polar styrene-divinylbenzene resin, in series. After the 96 hour reaction period, 333mL of the sample (pH 2.0 or pH 8.0) was passed through the XAD-8 column at the rate of one bed volume per hour. The eluate was then acidified to pH 1.0 with HCl and passed through the XAD-4 resin at the same rate. Both resins were then soxhlet extracted with ether. The extract subsequently underwent a hundred fold concentration under nitrogen gas for mutagenicity testing. Freeze drying was performed on 333mL of the sample (pH 2.0 or pH 8.0) on a Labconco Freeze dryer. The lyophilized samples were then stored dry at -20°C. Liquid-liquid extractions with ether were performed on 250mL aliquots (pH 8.0 and 2.0) of the reaction mixture, the original volume of which varied

from 0.5L to 2.0L. Each aliquot underwent 3 successive extractions with 40mL, 20mL and 20mL of diethyl ether. The extracts were stored in ether at -20°C. No quenching agents were used prior to extraction or concentration due to the uncertainty of the effects of quenching agents on the extracted compounds.

TOX and TOC Analyses

TOC analyses were performed by the Persulfate UltraViolet Oxidation method with infrared analysis (Standard Methods, 5310C) on a Model 700 TOC Analyser from O-IV Corporation (College Station, TX). Carbon Concentration was obtained as mg/L as C.

TOX Analyses were performed in by the Adsorption-Pyrolysis-Titrimetric Method (Standard Methods, 5320B) on a Dohrman Xertex TOX Analyzer (Model M-1; Dohrmann Division, Xertex, Santa Clara, CA). Total Organic Chlorine concentration was obtained as mg/L as Cl^-.

HPLC Fractionation

The extracted samples were fractionated on a Varian 5000 HPLC (Palo Alto, CA) equipped with a Partisil-10 ODS 4.6x250mm analytical column (Whatman Inc., Maidstone, England), a Whatman 6mm ODS guard column, and a Rheodyne (Cotati, CA) injection port. The elution solvents used were distilled deionized water at ambient pH or $1x10^{-3}M$ phosphate buffer at pH 3.0 (Solvent A) and Acetonitrile (Solvent B). The HPLC effluent was monitored at 254 nm using a Waters (Milford, MA)

Model 440 spectrophotometric detector. The chromatograms were recorded on a Shimatzu Chromatopac CR2-AX integrator (Kyoto, Japan).

The ether extracts of the 250mL aliquots were prepared for HPLC by drying under N_2 and redissolving in 0.5mL of acetonitrile and 0.7mL of water. This was the smallest volume and ratio that would completely dissolve the sample present. The sample loop (500uL capacity) was injected with 500uL of sample. Three fractions were then collected manually based on the following step gradient separation scheme:

0 - 110 minutes : 90% Solvent A, 10% Solvent B (Fraction 1)
110 - 150 minutes : 50% Solvent A, 50% Solvent B (Fraction 2)
150 - 190 minutes : 100% Solvent B (Fraction 3)

The fractions were taken to dryness by rotary evaporation on a Rotavapor R-110 (Buchi, Switzerland) in a 50°C water bath. They were then extracted twice with 20mL of diethyl ether and stored at -20°C.

Ames Assay

The mutagenicity of the extracts of monochlormination sample and HPLC fractions was assayed in accordance with the standard plate incorporation assay by Maron and Ames (1983). Whole sample extracts were assayed in both TA98 and TA100 strains whereas the HPLC fractions were only tested in TA100. The only modification to the standard procedure was in the use of Biochemical Agar Agar due to the

unavailability of the Difco Bacto Agar. The slight increase in background caused in the TA100 plates by the new agar was compensated for by modifying the concentrations of agar and histidine biotin solutions used. The effect of exogenous xenobiotic metabolizing enzymes on the sample was tested by using Aroclor 1254 induced rat liver homogenate, S9. The effect of a strong nucleophilic compound on the mutagenicity of the extract was tested with glutathione (GSH). The effect of non-specific protein binding was observed by performing assays with Bovine Serum Albumin (BSA).

Assays on the extract involved the use of 50uL, 75uL, 100uL and 150uL doses of the sample dissolved in DMSO, whereas those performed on the HPLC fractions used 50uL and 100uL doses. All samples and controls were assayed in duplicate.

All the Ames assays in this work were performed by David Cozzie (Cozzie, 1990).

Derivatization and Internal Standardization

Diethyl ether extracts of 250mL aliquots of original sample mixture and HPLC fractions, containing $^{13}C_6$ benzoic acid (50pg/uL) added as an internal standard were taken to dryness under a stream of N_2 gas and then redissolved in 250uL of methanol containing 14% (v/v) boron trifluoride (BF_3). The mixture was then allowed to react for 12 hours in a 70°C mineral oil bath. The mixture was subsequently neutralized with 3mL of 2% v/v $NaHCO_3$, extracted twice with 250uL of hexane and concentrated to 100uL under a stream of N_2 gas. Either anthracene-$\underline{d}_{10}$

(25pg/uL) was added as a recovery standard or decafluorobiphenyl (10ng/uL) was added as an internal standard. BF_3 in methanol was chosen to be the derivatization agent because it methylates MX as well as its analogs. Other available methylating techniques were shown to be unsuitable. Diazomethane does not methylate MX. Although sulfuric acid in methanol does derivitize MX, it does not methylate its diacidic analogs (Kronberg, 1990).

Gas Chromatography / Electron Capture Detection

Gas Chromatography / Electron Capture Detection (GC/ECD) analyses were performed on a Hewlett-Packard 5890A gas chromatograph. A 30m, 0.25mm I.D., 0.25um film, DB-5 fused capillary column were used in all analyses. The carrier gas was helium at 1mL/min. The GC oven temperature was held at 50°C for 1 minute, and then programmed to rise at 2.5°C/min to 150°C, then at 5°C/min to 250°C. Decafluorobiphenyl was used at a concentration of 1ng/uL as an internal standard.

High Resolution Gas Chromatography / Mass Spectrometry

High Resolution Gas Chromatography:

High-resolution gas chromatography / mass spectrometry (HRGC/MS) analyses were performed on a Hewlett-Packard 5890 gas chromatograph interfaced to a VG70-250SEQ mass spectrometer operating at resolving powers of 1000 or 10000 (10% valley definition). A 30m, 0.25mm I.D.,

0.25um film, DB-5 fused capillary column was used in all the experiments (J & W Scientific, Folsom, CA). The carrier gas was helium at 1mL/min. The GC oven temperature was held at 50°C for 1 minute, then programmed to rise at 10°C/min to 300°C in analyses conducted to quantify MX, EMX, ox-EMX and mucochloric acid. In analyses performed for the purpose of identifying the organic acid by-products, the GC oven was held at 50°C for 1 minute, and then programmed to rise at 2.5°C/min to 150°C, then at 5°C/min to 300°C.

Full-scan Mass Spectrometry:

Full-scan mass spectra were acquired by using electron ionization (EI) at an electron energy of 70eV, 200uamp trap current, 250°C source temperature and resolving power of 1000. The magnet was scanned from 500-50 amu at 1 second/decade.

Full scan quantifications of di- and trichloroacetic acids, dichlorosuccinic acid and ox-EMX were performed based on the relative response of a specific ion for each of the compounds dichloroacetic acid (m/z 83), trichloroacetic acid (m/z 117), dichlorosuccinic acid (m/z 183) and ox-EMX (m/z 225) to the response of the m/z 334 ion of decafluorobiphenyl. The concentrations of both the analyte and internal standard in pure standards were 10ng/uL. Because the linearity of response over a range of analyte concentrations to the internal standard was not demonstrated, the analyses can only be considered semi-quantitative.

Selected Ion Monitoring Mass Spectrometry:

Analyses in Selected Ion Monitoring (SIM) mode were performed under similar instrument conditions as full scan analyses except that a resolving power of 10000 was used. Two or more ions were monitored for the quantification of methylated ions of MX (m/z 198.9121 and m/z 200.9094), EMX (m/z 244.9534 and m/z 246.9510), E-2-chloro-3-(dichloromethyl)-butenedioic acid (ox-EMX) (m/z 224.9721 and m/z 226.9691; m/z 228.9226 and m/z 230.9196) and mucochloric acid (m/z 146.9848 and m/z 148.9818). Ions monitored for internal standards are the M^+ ion (m/z 142.076) of $^{13}C_6$ methyl benzoate, the M^+ ion (m/z 188.1410) of anthracene-$\underline{d}_{10}$. The PFK lock-mass ion used for monitoring MX and mucochloric acid ions was m/z 142.9920 and that for EMX and ox-EMX ions was 192.9888.

MX quantification was based on a four point calibration curve constructed from the analysis of standard solutions containing 50pg/uL $^{13}C_6$ methyl benzoate, 25pg/uL anthracene $\underline{d}_{10}$ and either 50, 100 250 or 500pg/uL of methylated MX. Semi-quantitative analyses of EMX, ox-EMX and 3,4-dichloro-5-hydroxy furanone (mucochloric acid) were based on a relative response factor obtained from the analysis of a single standard of each analyte (250pg/uL, 50pg/uL and 250pg/uL, respectively) containing 50pg/uL $^{13}C_6$ benzoic acid.

It should be noted that sample extracts and internal standard were derivatized together whereas compound standards were derivatized individually and then mixed with previously methylated internal standard. The difference in response if any, between samples and standards has not been accounted for in calculations.

RESULTS AND DISCUSSION

Optimizing Reaction and Extraction Conditions

Fundamental to the development of a protocol for reaction of monochloramine and fulvic acid and subsequent extraction of the by-products was the examination of monochloramine reactivity with fulvic acid. Monochloramine was found to react very slowly with fulvic acid as evident by persistent residuals for Cl:C molar ratios greater than 1:40 even after 142 hours of reaction time (Table 1, Figure 3). It has been previously shown that the demand of fulvic acid for monochloramine is about four times lower than that for chlorine (Jensen, 1983).

High monochloramine doses and long reaction times therefore had to be used in order to obtain large amounts of product. The problem with the use of high monochloramine dose was the occurrence of persistent residuals in the extracts which were toxic to the Ames Salmonella Bacteria. The residual monochloramine was quenched in preliminary experiments with ferrous ammonium sulfate. However, the method was not adopted due to the uncertainties of the effect of sample handling on the by-products. Quenching was also found to be unacceptable because it caused precipitation of the sample.

Due to the problem of monochloramine residuals, the extraction method used not only had to effectively recover the mutagenic product but also had to separate the monochloramine residual from the extract. Of the three procedures initially used to isolate products for

Table 1. Monochloramine Residuals at Various Cl:C Ratios

Cl:C Ratio	Residual Concentration (mg/L as Cl_2) Reaction Time (hr)			
	0	48	96	142
1:2	712	69	35	22
1:5	286	56	36	22
1:10	143	24	19	11
1:20	71	8	4	2
1:40	35	0.8	ND*	ND

* ND = Not Detectable

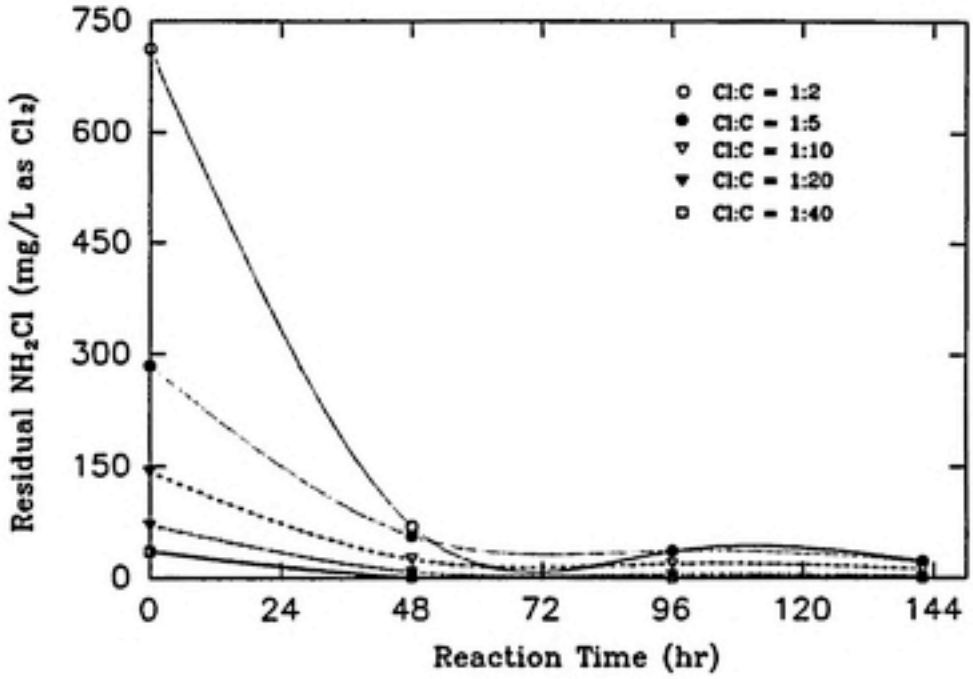


Figure 3. Effect of Reaction Time and Cl:C Ratios on NH_2Cl Consumption

mutagenicity testing, freeze-drying and XAD-resin adsorption failed to meet the criteria set for the extraction method of choice. Freeze drying samples at both pH 2.0 and pH 8.0 was ineffective in retaining products that were mutagenically active (Cozzie, 1990). This may have been due to product loss from volatilization or masking of the mutagenic activity due to the sheer bulk of the product. The XAD-resin procedure consistently isolated mutagenicity from pH 2.0 samples passed through the polar XAD-8 resin (Cozzie, 1990). However, sporadic mutagenic activity was observed in the blanks and XAD-4 resins suggesting possible artifact formation. An additional problem with the resin adsorption procedure was that it was unsuitable for separating monochloramine residual from the product in samples reacted with a Cl:C molar ratio higher than 1:40. The results from the lyophilization and resin adsorption experiments were surprising since previous studies on chlorination and monochloramination by-products have reported excellent recovery of mutagenicity from both procedures (Horth et al., 1987; Kool et al., 1981; Kronberg et al., 1987; Miller et al., 1986).

Liquid-liquid extraction with diethyl ether was found to be ideal for three reasons. First, mutagenic product was recovered consistently. Second, residuals were effectively separated even when high doses of NH_2Cl (up to 800mg/L as Cl_2) were used which enabled the study of dose-mutagenic response relationships. Finally, there was no artifact formation observed as evidenced by a lack of mutagenic activity in the blanks.

Although the liquid-liquid extraction method is not as commonly used as resin adsorption, it has been shown to produce levels of

mutagenicity comparable with the XAD procedure (Kronberg, 1987). In addition, the method has previously been found to extract essentially all of the mutagenicity produced in aqueous chlorinated fulvic acid (Meier et al., 1985).

The extraction of mutagenic by-products was found to be critically dependent on pH. Mutagenic activity was only observed in samples extracted at pH 2.0. Although previous chlorination studies have focused on mutagens with acidic properties they also found activity in the neutral extracts that was a factor of 10 less than that of the acidic extracts (Kronberg et al., 1988; Ringhand et al., 1987; Wigilus, 1985). In this study the mutagens produced by monochloramination were primarily acidic since there was a consistent lack of activity in the pH 8.0 extracts. These findings support an earlier hypothesis by Jensen et al (1983) that the products of monochloramination are more hydrophilic than those of chlorination.

Mutagenicity of the Extracts

Having established an extraction method that is consistent in its recovery of mutagenic product, the next step was to characterize the mutagenicity (Cozzie, 1990). This was performed through an investigation of the effect of bacterial strain used (TA100 and TA98), dose-mutagenic response relationships, effects of metabolizing enzymes and non-specific protein binding.

Studies with the bacterial strains TA100 and TA98 showed that TA100 was more responsive to monochloramine produced mutagenicity than

TA98. A limited number of assays with TA98 showed that about 70% less activity was observed in TA98 as in TA100 (Cozzie, 1990). The TA100 activity indicates that the mutagens are predominantly base-pair substituting rather than frame-shift mutagens. Due to the much greater sensitivity of the TA100 strain to the mutagens in the extract, all subsequent mutagenicity experiments utilized this strain.

A dose-mutagenic response relationship was explored over a monochloramine dose range of 40mg/L - 800mg/L (Cl:C molar ratios of 1:40 - 1:2) using the TA100 strain (Table 2, Figure 4). In the upper plot mutagenic activity seems to increase linearly up to a maximum at a monochloramine consumed dose of 415mg/L (Cl:C of 1:5) after which a plateau appears to have been reached. This may be due to the lethal effects of either the high concentrations of products or the residual monochloramine on the Ames Salmonella bacteria. Toxicity was in fact observed in the plates (evidenced by a clearing of the bacterial lawn), when the sample extract corresponding to a NH_2Cl dose of 1160mg/L as Cl_2 was assayed. Although the lower plot in Figure 4 shows that mutagenicity may be weakly related to carbon dose, it appears far more likely that the total mutagenic activity is dependant in some manner on the amount of monochloramine consumed in reaction (NH_2Cl consumed = NH_2Cl dose - residual after 96 hours).

The mean mutagenic activity was calculated to be 79+/-35 revertants/mg Cl_2 consumed (44% RSD). The Ames assay replicates were found to have a relative standard deviation of only about 10% which indicates that most of the variability in mean mutagenic activity can be attributed to the reaction and extraction procedure. The quantity of

Table 2. TA100 Mutagenicity of Monochloramination Extracts at Various Cl:C Ratios

Expt.#[a]	Cl:C Ratio	NH_2Cl Measurements (mg/L as Cl_2)			Carbon Dose (mg/L as C)	TA100 Activity[a]		Mean Rev/L +/- SD (%RSD)
		Dose	Residual	Consumed		Rev/L	Rev/mg Cl_2[c] Consumed	
1	1:40	40	0	40	269	NA[b]	0	3964/-3443
2		50	0	50	333	5672	113	(87% RSD)
3		60	0	60	400	6220	104	
4	1:20	80	4	76	271	10688	141	10688
5	1:10	160	18	142	270	15131	107	18639+/-5497
6		170	19	151	283	16946		(30% RSD)
7		180	17	163	300	13840	85	
8		200	18	182	333	27680	152	
9		225	13	212	375	19600	93	
10	1:5	250	31	219	208	10066	46	20572+/-6432
11		250	31	219	208	11739	54	(31% RSD)
12a		270	26	244	225	21841+/-6353	90	
12b		270	26	244	225	(29% RSD)		
12c		270	26	244	225			
13		270	25	245	225	17216	70	
14a		300	32	268	254	25588+/-4605	96	
14b		300	32	268	254	(18% RSD)		
14c		300	32	268	254			
15a		300	30	270	254	17476+/-3640	65	
15b		300	30	270	254	(21% RSD)		

Table 2. (Continued)

Expt.#	Cl:C Ratio	NH_2Cl Measurements (mg/L as Cl_2)			Carbon Dose (mg/L as C)	TA100 Activity*		Mean Rev/L +/- SD (%RSD)
		Dose	Residual	Consumed		Rev/L	Rev/mg Cl_2 Consumed	
16a	1:5	320	34	286	267	21292+/-16	75	
16b		320	34	286	267	(0.08% RSD)		
17a		320	37	283	267	12873+/-1525	46	
17b		320	37	283	267	(12% RSD)		
18a		325	36	289	275	23772+/-1319	82	
18b		325	36	289	275	(6% RSD)		
19a		325	36	289	275	23640+/-4740	82	
19b		325	36	289	275	(20% RSD)		
20a		350	30	320	292	21836+/-388	68	
20b		350	30	320	292	(2% RSD)		
21		360	36	324	300	9257	29	
22		360	36	324	300	34377	106	
23		445	30	415	371	27600	67	
24	1:2	750	65	685	250	33040	48	30650+/-3380
25		800	61	739	271	28260	38	(11% RSD)
26		1160	68	1092	386	T[d]		

[a] All samples labelled a, b, and c are aliquots from the same reaction vessel
[b] NA - No Activity above background
[c] Mean Rev/mg Cl_2 - 79 +/- 35
[d] T - Toxic
[e] Ref: David Cozzie, 1990

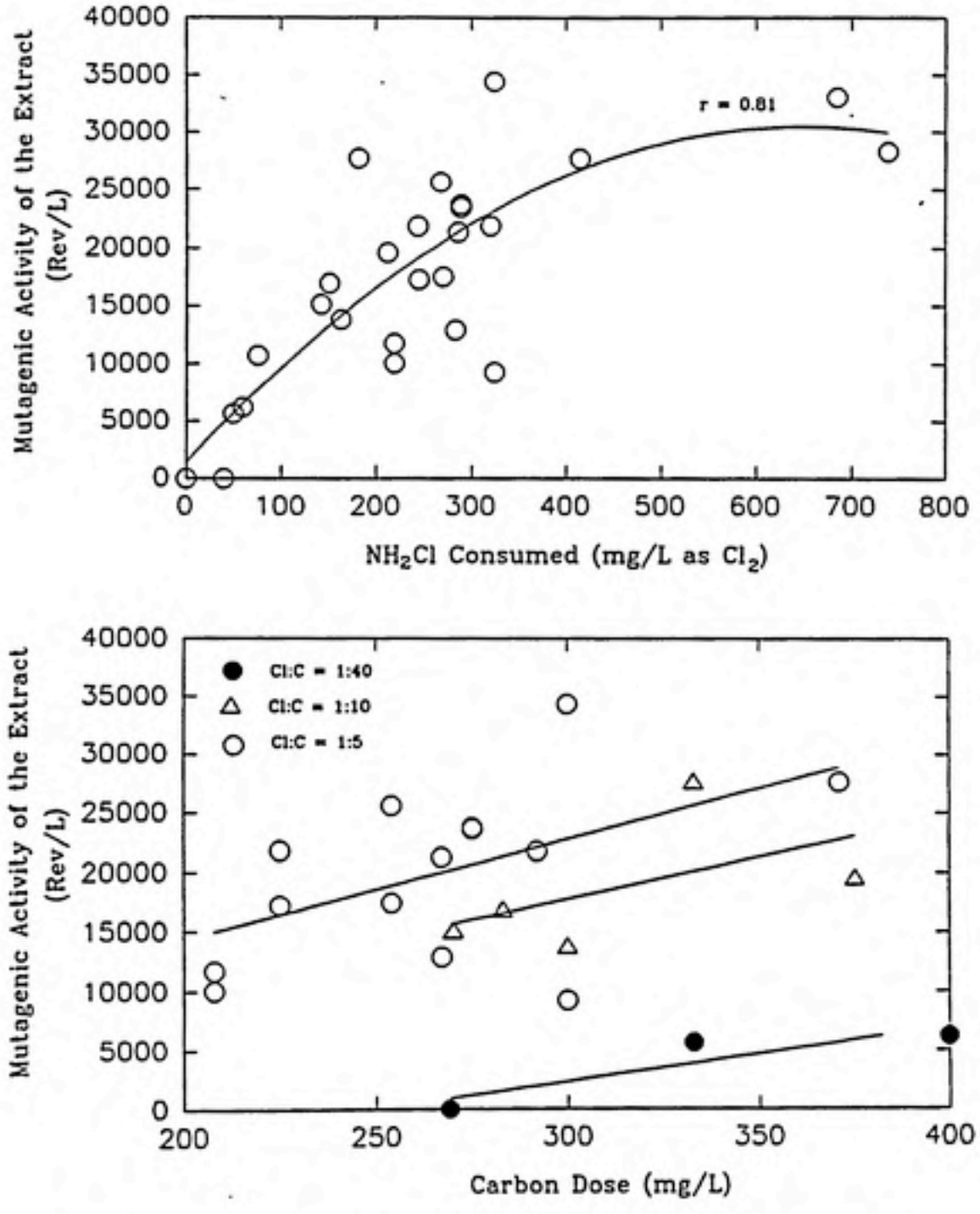


Figure 4. Effect of NH_2Cl Consumed (upper) and Carbon Dose (lower) on the Production of TA100 Mutagenicity

mutagenicity observed in this study must be interpreted with caution due to the possibility of mutagenic artifacts formed from the interactions of the by-products in the extracts with the solvent dimethyl sulfoxide (DMSO) in the Ames Assay. DMSO has previously been found to enhance the mutagenicities of some chlorinated compounds (Nestmann et al., 1985).

All three proteins (S9, Glutathione and Bovine Serum Albumin) assayed appeared to have an inactivating effect on the mutagenicity of the extract (Table 3, Figure 5). Inactivation by these three proteins shows that the mutagens of interest are likely to be direct acting, electrophilic and inactivated by non-specific protein binding, respectively. Experiments with pure MX showed that its mutagenic activity was also decreased by the action of the proteins, although the decrease was quantitatively greater than that observed in the extract. This finding suggested that MX and similar compounds may be important contributors to the mutagenicity of the extract.

The results of the mutagenicity experiments compare well with the findings of previous chlorination studies as summerized in Table 4. Historically, chlorination by-products have been predominantly base-pair substituting (TA100 active), direct acting (S9 inactivated) and electrophilic (GSH inactivated). However, the decrease in the mutagenicity of the extracts or of MX due to non-specific protein binding (inactivation by bovine serum albumin) observed in this study is not supported by previous studies. No such decreases have previously been reported. Nevertheless, the close agreement of the remainder of the results with previous studies indicates that the mutagens produced by monochloramination are very similar to those produced by chlorination.

Table 3. Comparison of the Effect of S9, Bovine Serum Albumin and Glutathione on the Mutagenic Activity of the Monochloramination Extract and MX

Protein	% Decrease in Activity[a,b,c,d]	
	Extract	MX
S9	34.6 +/- 16.5 (47.7% RSD)	63.2 +/- 1.0 (1.6% RSD)
Glutathione	62.8 +/- 12.2 (19.4% RSD)	83.4 +/- 0.6 (0.7% RSD)
Bovine Serum Albumin	8.6 +/- 3.0 (34.9% RSD)	15.2 +/- 1.9 (12.5% RSD)

[a] % Decrease in Activity = Activity (TA100+proteins)/Activity(TA100)

[b] n=2 in all cases except S9 (extract) where n=10

[c] Cl:C = 1:5 in all extracts assayed with the proteins

[d] ref: David Cozzie, 1990

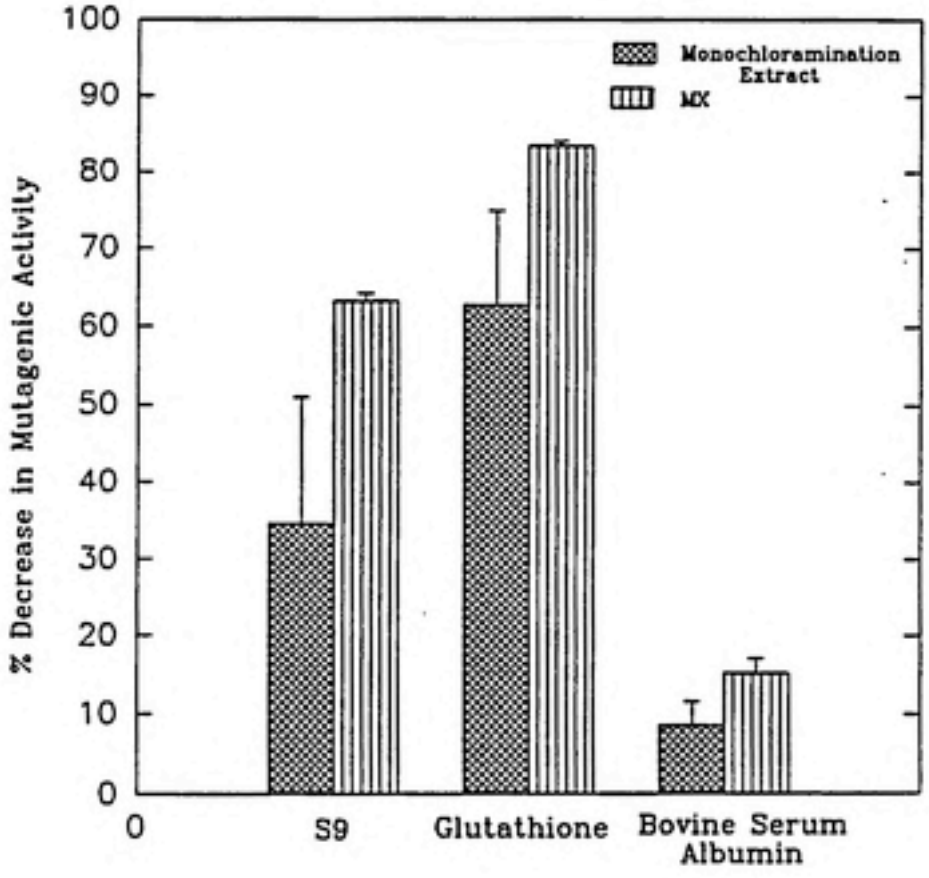


Figure 5. Comparison of the Effect of Exogenous Proteins on TA100 Mutagenicity of Monochloramination Extract and MX

Table 4. Comparison of Monochloramination and Chlorination Products and their Characteristics

Property	Monochloramination	Chlorination
Predominant characteristics mutagens	acidic and polar (acid pH extractables only; elute in most polar HPLC fraction)[a]	predominantly acidic and polar but also some non-polar species (acid and neutral pH extractables; elution in polar HPLC fractions)[a,b,o,p]
	base-pair substituting, direct acting and electrophilic	base-pair substituting direct acting and electrophilic[b,f,g,h,j,k,l,n]
Mean TA100 activity in acid extracts (Rev/mg Cl_2)	455[c] 79+/-35	769+/-353[c] 1050[i] 1667[m]
Major contributors to mutagenicity found to date	MX, EMX	MX, EMX, 2,3,3-trichloropropenal[c,i,l]
Mean MX Conc. (ng/mg Cl_2)	4.3[c] 0.3+/-0.01	14.6+/-6.9[c] 13.0[i] 4.7[m] 0.5+/-0.05
% Contribution of MX to total activity	24[c] 11+/-0.6	49[c] 36[i] 41[m]
Mean EMX Conc. (ng/mg Cl_2)	5.9[c] 11.5+/-6.7	22.0+/-3.3[c] 26.3[i] 51.9+/-4.1
% Contribution of EMX to total activity	2[c] 26+/-15	4[c] 4[i]
Major products identified to date	chlorinated aliphatic (C_2-C_9) acids, aldehydes and alcohols	chlorinated and non-chlorinated aliphatic and aromatic- acids, aldehydes ketones nitriles and alcohols[d,e,l]

References for Table 4

[a] Highlighted portions represent results from the present work.
[b] Backlund et al., 1985
[c] Backlund et al., 1988
[d] Christman et al., 1983
[e] de Leer et al., 1985
[f] Holmbom et al., 1984
[g] Horth et al., 1987
[h] Kronberg et al., 1985
[i] Kronberg et al., 1988b
[j] Loper, 1990
[k] Meier et al., 1983
[l] Meier et al., 1985b
[m] Meier et al., 1987
[n] Nestmann et al., 1979
[o] Ringhand et al., 1987
[p] Wigilus et al., 1985

The mutagenicity produced in this study is low compared to that found in previous studies. For instance, the mean activity in the present study is only about 10% of that of chlorination and 17% of that of monochloramination in a study by Backlund et al. (1987) (Table 4). This may be due to differences in experimental conditions or other effects that cannot be explained at present.

TOX and TOC Analyses

Total Organic Halide has previously been used to gauge the degree of chlorine substitution in chlorination and monochloramination by-products (Fleishacker and Randtke,1983; Jensen et al., 1985; Meier et al., 1985). TOX information is vital since chlorine substitution products have been implicated in being primarily responsible for mutagenic activity in extracts (Christman et al., 1983). This study found chlorine incorporation (the degree of chlorine substitution into the organic matrix) to show a slight increase with increasing Cl:C ratios (Table 5). Fleishacker and Randtke (1983) found a chlorine incorporation of about 2% for monochloramination and about 7% for chlorination samples. This compares well to the 2.5% in our study at a comparable Cl:C ratio.

The formation of extractable TOX was also observed to increase with increasing Cl:C ratio (Table 5, Figure 6, upper). Mutagenic activity shows a similar increase but the mutagenic response reaches a plateau after a Cl:C ratio of 1:10 (Table 5, Figure 6, middle). It was not surprising therefore, to find that TOX and mutagenic activity are

Table 5. TOX Analyses of Monochloramination Samples at Various Cl:C Ratios

Cl:C Ratio	NH_2Cl Consumed (mg/L as Cl_2)	TOX (mg/L as Cl^-) Non-Extracted Samples	Extracted Samples	Mean+/-SD	% Extraction[a,b] Recovery	% Chlorine[c] Incorp.	TA100 Activity[d] (Rev/L)
Blank	0	0.00	0.00	0.00	0.0	0.00	0
1:40	30	0.49	0.11	0.095+/-0.02	22.5	1.64	-
	40	-	0.08		-	-	0
1:20	57	1.12	0.29	0.31+/-0.02	25.0	1.96	-
	76	-	0.32		-	-	10688
1:10	106	2.57	0.67	0.89+/-0.30	26.3	2.42	-
	142	-	1.10		-	-	15131
1:5	212	5.68	1.54	1.78+/-0.34	26.9	2.68	-
	270	-	2.02		-	-	20385
1:2	571	14.54	3.46	3.1+/-0.51	23.7	2.54	-
	739	-	2.74		-	-	26260

[a] % Extraction Recovery = (TOX in Extracts / TOX in Non-Extracted Samples) x 100
[b] Mean % Extraction Recovery = 24.9% +/- 1.81
[c] % Chlorine Incorporation = (TOX in Non-Extracted Samples / NH_2Cl Consumed) x 100
[d] ref.: David Cozzie, 1990

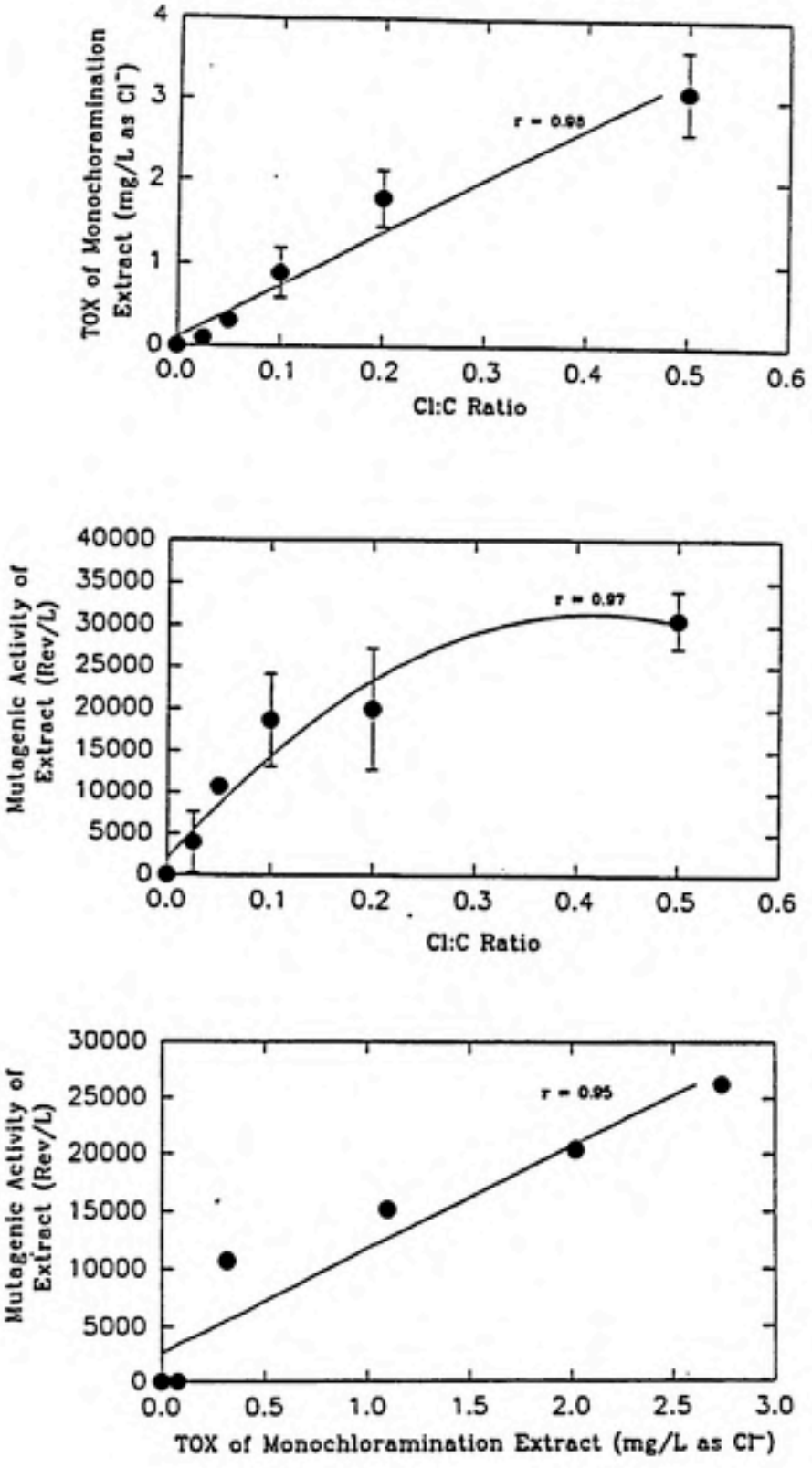


Figure 6. Effect of Cl:C Ratio on the Production of TOX (upper) and TA100 Mutagenicity (middle). Effect of TOX on the Production of TA100 Mutagenicity (lower)

positively correlated (Table 5, Figure 6, lower) with a linear correlation coefficient (r) of 0.95. However, even a strong correlation does not establish a cause-effect relationship. Oxidation products may also account for the mutagenicity of the monochloramination extract.

Unlike TOX, the TOC of the sample prior to extraction shows a decrease with increasing NH_2Cl dose (Table 6). This decrease is probably due to oxidation of the fulvic acid by NH_2Cl resulting in the formation of carbonates and subsequently carbon dioxide.

Extraction recoveries of TOX and TOC (Tables 5 and 6) did not vary with dose. The mean extraction recovery of TOX was 24.9% +/- 1.8% which compares well to the work of Jensen (1983) and Meier (1985) who found that 20% and 25% respectively of the TOX was extractable by the liquid-liquid extraction procedure. The mean TOC extraction recovery was only 10.1% +/- 1.0%. The low extraction recoveries need not be of concern if all of the mutagenic product were extracted by the liquid-liquid extraction procedure. However, no recovery studies of this sort were performed in this study.

HPLC Fractionation of By-products

HPLC has frequently been used in previous studies to obtain fractions of chlorinated water for mutagenicity testing (Horth et al., 1985; Kronberg, 1987). Previous success in isolating relatively polar compounds such as MX with reverse phase HPLC (Kronberg et al., 1987) led us to use the same technique. In order to maximize the recovery of the mutagenic product and improve separation of acidic species, we examined

Table 6. TOC Analyses of Monochloramination Extracts at Various Cl:C Ratios

Cl:C Ratio	Carbon Dose (mg/L as C)	TOC (mg/L as C) Non-Extracted Samples	TOC (mg/L as C) Extracts	Mean+/-SD	% Extraction[a,b] Recovery
Blank	203	185.8	16.9	-	9.1
1:40	203	196.3	17.9	18.4+/-0.6	9.1
	269	-	18.8		-
1:20	203	168.7	19.0	20.9+/-2.7	11.2
	269	-	22.8		-
1:10	203	142.8	13.5	21.4+/-11.1	9.5
	269	-	29.2		-
1:5	203	125.2	13.6	16.8+/-4.5	10.9
	269	-	20.0		-
1:2	203	116.5	12.7	14.8+/-2.9	10.9
	269	-	16.9		-

[a] % Extraction Recovery = (TOC in Extracts / TOC in Non-Ext. Samples) x 100
[b] Mean % Extraction Recovery = 10.1% +/- 1.0%

the use of solvents buffered to low pH. The use of phosphate buffer at pH 3.0 in the solvents seemed to promote a greater overall mutagenic recovery of about 73 +/- 21% compared with 48 +/- 20% recovery when unbuffered solvents were used (Table 7). Most of this previously unrecovered activity appeared to be in Fraction 1. To ensure that the presence of the buffer itself was not enhancing activity, extracts dissolved in buffer and in water were assayed. No difference in mutagenic activity was observed indicating that the higher recovery was not due to artifact formation.

Figure 7 shows a chromatogram that was obtained by a stepwise elution scheme. The most polar fraction (Fraction 1) consistently contained the greatest portion of recovered mutagenic material (52%). Fraction 2 contained the residual mutagenicity (21%) and Fraction 3 was devoid of activity in every experiment. These results suggest that the most mutagenically active products were polar. In addition the fact that the use of the buffer enhanced recovery of activity in Fraction 1 shows that the products are acidic. These findings again support the earlier hypothesis that the mutagenic by-products of monochloramination are polar and acidic. A previous chlorination study utilizing HPLC to isolate mutagenic fractions (Kronberg et al., 1988) also reported recovering most of the activity in the most polar fraction. No further fractionation of Fraction 1 was possible due to limitations imposed by the column used.

TOX and TOC studies on the HPLC fractions show that, similar to the mutagenic activity, most of the TOX and TOC (55% and 60% respectively) were recovered on the first two more polar fractions

Table 7. Distribution of Mutagenic Activity in HPLC With and Without Buffered Solvents

Solvent System	Mutagenic Equivalents (Revertants)* (% Recovery)			
	Injected	Fraction 1	Fraction 2	Fraction 3	Total
Unbuffered	2379	1012 (42.5%)	464 (19.5%)	-	1476 (62.0%)
	2462	424 (17.2%)	396 (16.1%)	-	820 (33.3%)
Mean:	2420	718 (29.9+/-17.9%)	430 (17.8+/-2.4%)	-	1148 (47.7+/-20.3%)
Buffered with $1x10^{-3}$ M Phosphate	2379	1872 (78.7%)	584 (24.5%)	-	2456 (103.2%)
	2462	1072 (43.5%)	596 (24.2%)	-	1688 (67.7%)
	2300	908 (39.5%)	340 (14.8%)	-	1248 (54.3%)
	2760	1280 (46.4%)	605 (21.9%)	-	1885 (68.3%)
Mean:	2530	1283 (52.0+/-18.0%)	531 (21.4+/-4.5%)	-	1819 (73.4+/-20.9%)

* Ref.: David Cozzie, 1990

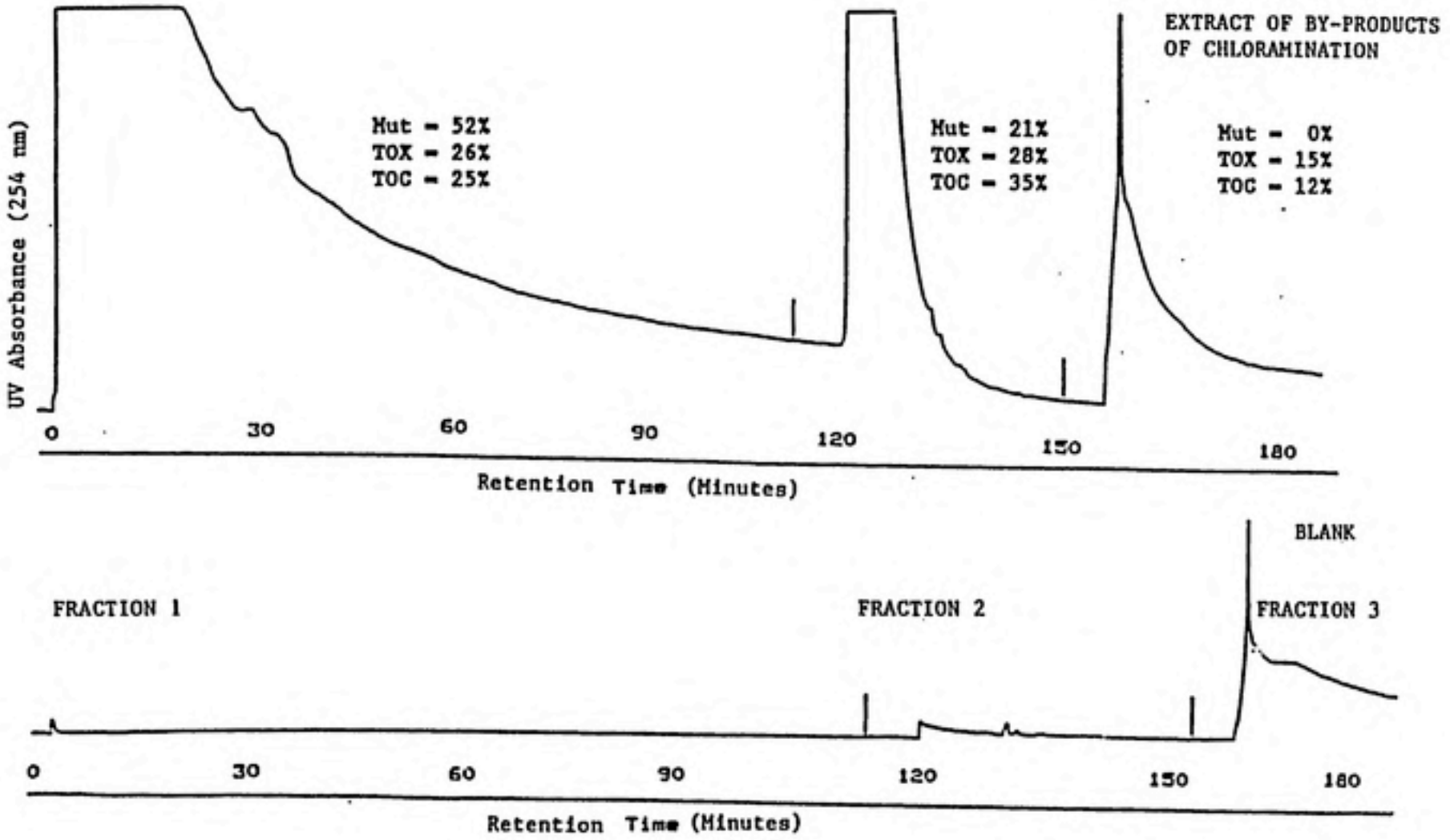


Figure 7. C_{18} Column HPLC Separation of Monochloramination Extract (upper) and Blank (lower). Mut, TOX and TOC are % recoveries of mutagenicity, TOX and TOC respectively.

(Table 8). This finding again suggests that the mutagenicity may be due to chlorine substituted products.

GC/MS Identification of By-Products

The total ion chromatogram of the methylated pH 2.0 ether-extractable monochloramination products is shown in Figure 8. Forty-five compounds have been identified (Table 9) in full-scan EI mode. These compounds may be present in isomeric forms other than that suggested due to the tentative nature of the identification.

The majority of the compounds listed were identified only on the basis of structural assignments to EI spectra. A few were confirmed by performing analyses on authentic spectra. Comparison with published spectra (Mc Lafferty and Stouffer, 1988) strengthened a few other identifications. Due to problems related to the instrument, neither negative nor positive chemical ionization analyses were successful in providing molecular weight confirmations to the majority of the compounds listed. However, comparison of the total ion chromatogram to a chromatogram of the extract obtained by using Gas Chromatography / Electron Capture Detection (GC/ECD) indicated that the majority of the compounds were halogenated (Figure 9).

The products identified in this experiment are all ethers and esters derived from the methylation of chlorinated mono- and diacids, mono-, di- and trialcohols and aldehydes (which may prove to be diols if analyses were repeated using different conditions). These include saturated and unsaturated compounds. Non-chlorinated compounds are not

Table 8. Distribution of TOX and TOC in HPLC Fractions

Sample*	TOX (mg/L as Cl^-)	% TOX Recovery	TOC (mg/L as C)	% TOC Recovery
Extract	2.0	-	19.9	-
Fraction 1	0.5	26.2	5.0	25.2
Fraction 2	0.6	28.2	6.9	34.9
Fraction 3	0.3	14.9	2.4	12.1
Total	1.4	69.3	14.4	72.2

* Values are mean values from two determinations of one sample

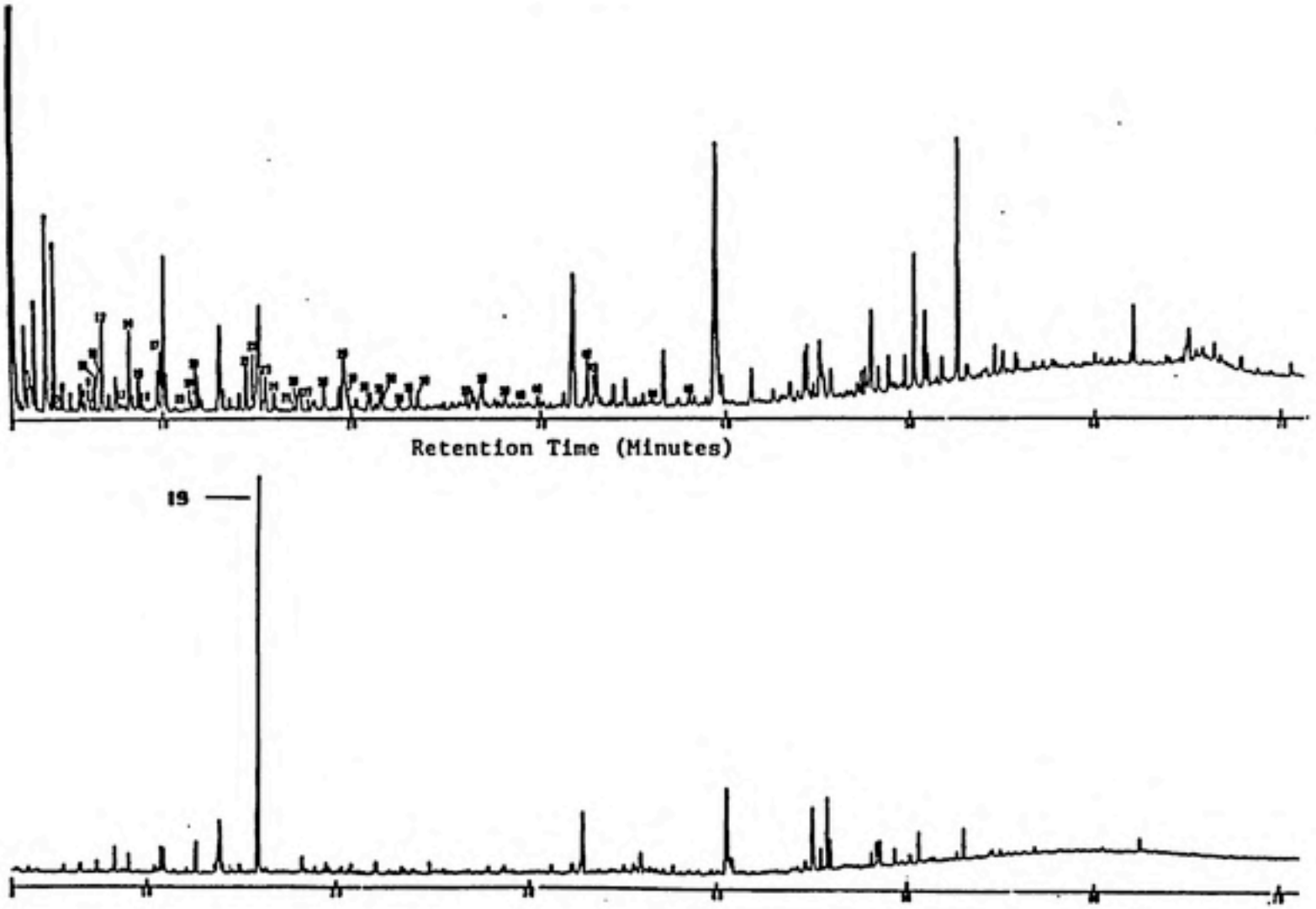


Figure 8. Total Ion Chromatograms of Derivitized Monochloramination Extract (upper) and Fulvic Acid Containing Blank (lower). Numbered GC peaks refer to identifications in Table 9. IS = Internal Standard. The chromatograms are normalized to the largest peaks. The internal standard peak in the upper chromatogram is very small compared to the other peaks and is therefore not visible.

Table 9. Monochloramination Products Identified by GC/MS

GC Peak#	Mol. Wt.	Compound Structure	Possible Precursor
1	124	$CH_2Cl-CH(OCH_3)_2$[a,c,d,e]	2-chloro-1,1-ethanediol or chloroactaldehyde[h]
2	142	$CHCl_2-COOCH_3$[b,c,d,e,f]	dichloroethanoic acid (dichloroacetic acid)[h,g]
3	138	$CH_2Cl-CH_2-CH(OCH_3)_2$[a,c]	3-chloro-1,1-propanediol or 3-chloropropanal[h]
4	138	$CH_2Cl-C(OCH_3)_2-CH_3$[a,d,e,f]	3-chloro-2,2-propanediol
5	156	$CH_3-CCl_2-COOCH_3$[a,c]	2,2-dichloropropanoic acid[h]
6	152	$CH_3-CHCl-CH_2-CH(OCH_3)_2$[a]	2-chloro-1,1-butanediol or 2-chlorobutanal
7	152	$CHCl(OCH_3)-(CH_2)_2-CH_2(OCH_3)$[a]	1-chloro-1,4-butanediol
8	176	$CCl_3-COOCH_3$[b,c]	trichloroethanoic acid (trichloroacetic acid)[h,g]
9	158	$CHCl_2-CH(OCH_3)_2$[a]	2,2-dichloro-1,1-ethanediol or dichloroethanal (dichloroacetaldehyde)
10	152	$CH_2Cl-CH(CH_3)-CH(OCH_3)_2$[a]	3-chloro-2-methyl-1,1-propanediol or 3-chloro-2-methylpropanal
11	154	$CCl_2=CH-COOCH_3$[a]	3,3-dichloropropenoic acid
12	152	$CH_3-CHCl-CH_2-CH(OCH_3)_2$[a]	3-chloro-1,1-butanediol or 3-chlorobutanal
13	172	$CHCl(OCH_3)-CH_2-CHCl(OCH_3)$[a]	1,3-dichloro-1,3-propanediol
14	172	$CH_2(OCH_3)-CHCl-CHCl(OCH_3)$[a]	2,3-dichloro-1,2-propanediol
15	172	$CHCl_2-CH_2-CH(OCH_3)_2$[a]	3,3-dichloro-1,1-propanediol or 3,3-dichloropropanal
16	156	$CH_2Cl-CCl(OCH_3)-CH_2CH_3$[a]	1,2-dichloro-2-butanol
17	172	$CH_2Cl-CCl(OCH_3)-CH_2(OCH_3)$[a]	1,2-dichloro-3,4-propanediol
18	216	$COOCH_3-CHCl-CCl(OCH_3)_2$[a]	2,3-dichloro-3,3-dihydroxypropanoic acid[h]
19	164	$CH_3-CH_2-CCl(OCH_3)-CH_2-CH(CH_3)_2$[a]	3-chloro-5,5-dimethyl-3-pentanol
20	166	$COOCH_3-CHCl-CH_2-CH_2(OCH_3)$[a]	2-chloro-4-hydroxybutanoic acid
21	178	$CH_2(OCH_3)-CCl=C(CH_3)-COOCH_3$[a,d,e]	3-chloro-4-hydroxy-2-methylbutenoic acid
22	198	$CH_2(OCH_3)-CH_2-O-CH_2)-CHCl-CCl-CH_2$[a]	2,3-dichlorobutene-2-hydroxyethylether
23	200	$COOCH_3-CCl_2-COOCH_3$[a,d]	dichloropropanedioic acid (dichloromalonic acid)[h]
24	202	$COOCH_3-CCl=CCl-CH_2Cl$[a]	2,3,4-trichlorobutenoic acid[h]
25	248	$CHCl_2-(CH_2)_4-CCl(OCH_3)_2$[a]	1,6,6,-trichloro-1,1-hexanediol or 1,6,6-trichlorohexanal

Table 9. (Continued)

GC Peak#	Mol. Wt.	Compound Structure	Possible Precursor
26	234	CCl_3-CH(OCH_3)-CH_2-$COOCH_3$[a]	1,1,1-trichloro-3-hydroxybutanoic acid[b]
27	198	$CHCl_2$-$(CH_2)_4$-$COOCH_3$[a]	6,6-dichlorohexanoic acid
28	178	$CH_2(OCH_3)$-CCl=CH-CH_2-$COOCH_3$[a,d,e]	4-chloro-5-hydroxypentenoic acid[b]
29	214	$COOCH_3$-CHCl-CHCl-$COOCH_3$[b,d,e]	2,3-dichlorobutanedioic acid (2,3-dichlorosuccinic acid)[b]
30	192	$COOCH_3$-C(CH_3)=CCl-$COOCH_3$[a,d,e]	2-chloro-3-methylbutenedioic acid (cis or trans)[b]
31	198	$CHCl_2$-$(CH_2)_2$-CH(CH_3)-$COOCH_3$[a]	5,5-dichloro-2-methylpentanoic acid[b]
32	198	CH_2Cl-$(CH_2)_2$-CCl(CH_3)-$COOCH_3$[a]	2,5-dichloro-2-methylpentanoic acid[b]
33	212	$COOCH_3$-CCl=CCl-$COOCH_3$[a,d]	2,3-dichlorobutenedioic acid (cis or trans)[b]
34	228	$CH_2(OCH_3)$-CH_2-CCl=CCl-CH(OCH_3)$_2$[a]	2,3-dichloro-2-pentene-1,1,5-triol or 2,3-dichloro-5-hydroxypentenal
35	252	$COOCH_3$-CH_2-CH=CH-$(CH_2)_3$-CO-$CHCl_2$[a]	9,9-dichloro-8-oxononenoic acid
36	210	C(CH_3)$_2$Cl-C(OCH_3)$_2$-CH_3[a,d,e]	1-chloro-1,1-dimethyl-2,3,3-butanetriol
37	210	CH_2Cl-CH_2-CH(CH_3)-CH(OCH_3)-CH(OCH_3)$_2$[a]	1-chloro-3-methyl-4,5,5-pentanetriol or 5-chloro-2-hydroxy-3-methylpentanal
38	240	$CH_2(OCH_3)$-C(CH_2Cl)=C(CH_2Cl)-CH_2-$COOCH_3$[a,d]	3,4-di(chloromethyl)-5-hydroxypentenoic acid
39	240	CH_2Cl-C(CH_3)=C[CHCl(OCH_3)]-CH_2-$COOCH_3$[a,d]	3-chlorohydroxymethyl-5-chloromethyl-4-methylpentenoic acid
40	240	CHCl(OCH_3)-C(CH_2Cl)=C(CH_3)-CH_2-$COOCH_3$[a]	5-chlorohydroxymethyl-4-chloromethyl-3-methylpentenoic acid
41	240	CH_2Cl-CCl=C(OCH_3)-CH(CH_3)-CH_2-$COOCH_3$[a]	5,6-dichloro-4-hydroxy-3-methylhexenoic acid
42	244	$COOCH_3$)-CH(CH_2Cl)-CH_2-CCl(OCH_3)$_2$[a]	4-chloro-2-chloromethyl-4,4-hydroxybutanoic acid
43	260	$COOCH_3$-C($CHCl_2$)=CCl-$COOCH_3$[b]	3-chloro-2-dichloromethylbutenedioic acid (ox-EMX)[g,h]
44	332	$COOCH_3$-$(CH_2)_2$-C(C_2H_5)=C(Cl_3)-CH(OCH_3)$_2$[a]	5,5,5-trichloro-6,6-dihydroxy-4-ethylhexanoic acid or 5,5,5-trichloro-4-ethyl-6-oxo-hexanoic acid
45	284	CH(OCH_3)$_2$-CCl=CCl-$(CH_2)_4$-$COOCH_3$[a]	6,7-dichloro-8,8-dihydroxyoctanoic acid or 6,7-dichloro-8-oxo-octanoic acid

Footnotes to Table 9

[a] Tentative identification based on interpretation of EI spectra

[b] Identification confirmed by comparison with authentic standard

[c] Spectrum matched with published spectrum (McLafferty and Stouffer, 1988)

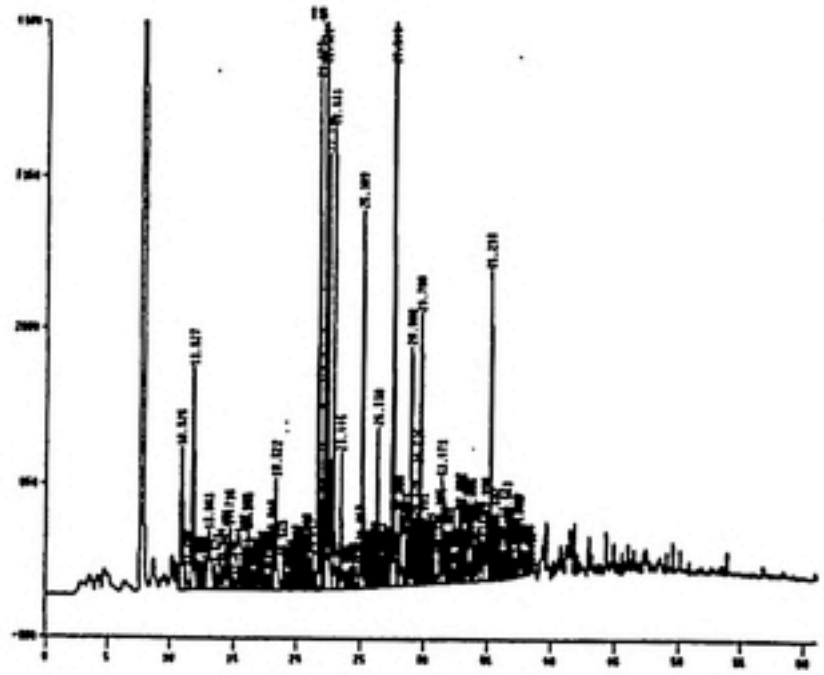
[d] Compound also tentatively identified in GC/MS trace of HPLC Fraction 1

[e] Compound also tentatively identified in GC/MS trace of HPLC Fraction 2

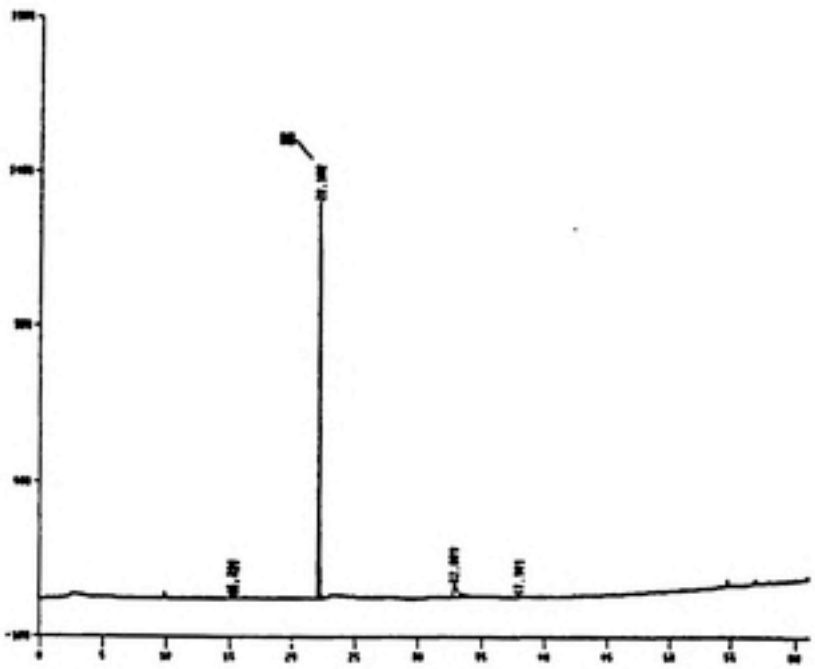
[f] Compound also tentatively identified in GC/MS trace of HPLC Fraction 3

[g] Compound is known mutagen or is known to exhibit other toxic properties (Herren-Freund et al., 1987; Kronberg et al., 1990)

[h] Compound or its isomer has been previously observed as a chlorination by-product (Becher et al., 1990; Christman et al., 1983; Coleman et al., 1984; Coleman et al., 1987; de Leer et al., 1985; Johnson et al., 1982; Meier et al., 1985)



Retention Time



Retention Time

Figure 9. GC/ECD Trace of Derivitized Monochloramination Extract (upper) and Fulvic Acid Containing Blank (lower)

listed because they were also identified in the fulvic acid blank (Figure 8, upper trace). Only quantification can determine the contribution of monochloramine oxidation in the formation of these products. It is also to be noted that no nitrogen compounds were detected although one would have expected to have observed nitriles and amino compounds as monochoramination by-products. It may be that they occurred in concentrations that were below the detection limit of full-scan EI mode. An alternative explanation for the dearth of identified nitrogenated organics in this work is the rapid conversion and oxidation of low molecular weight nitriles to the corresponding acid and nitrogen gas during reaction (de Leer et al., 1985).

The compounds listed are all aliphatic acids, alcohols and aldehydes. No aromatic compounds were identified. In addition, no compounds that eluted after 40 minutes were identified because of the degree of uncertainty involved in making structural assignments to the complex spectra of high molecular weight compounds. The majority of chlorinated compounds however, were observed to have eluted in the first 40 minutes of the analysis.

GC/MS analyses were also performed (in full scan mode) on the methylated extracts of the three HPLC fractions. Only a few compounds were tentatively identified in the fractions (Table 9). Identifications were limited by the low concentrations of the compounds and high background noise. Most of the compounds were identified in both Fractions 1 and 2. Dichloroacetic acid was identified in all three fractions. This makes it evident that relatively poor resolution was achieved by HPLC fractionation.

The products identified in this study are similar to those previously identified in chlorination studies although a number of additional compounds are reported here. Only fifteen of the forty-five compounds listed, most of which are chlorinated acids, may have previously been identified as chlorination by-products (Table 9)(Becher et al., 1990; Christman et al., 1983; Coleman et al., 1984; de Leer et al., 1985; Johnson et al., 1982; Kronberg et al., 1990). Of these compounds di- and trichloroacetic acids, dichlorosuccinic acid and ox-EMX have been confirmed with pure standards. Most of the compounds that are unique to this work are alcohols which may prove to be characteristic of monochloramination products. This is not surprising since monochloramine, being a weaker oxidant than chlorine will produce many products that are not as highly oxidized as chlorination products.

It is difficult to determine the relative health hazards of the chemicals identified as literature data only exists on the few chlorination products that have received the most attention. Recent work has shown that ox-EMX is weakly mutagenic (0.03 net revertants/nmole) in the Ames assay (Singh, 1989); di- and trichloroacetic acids, although not mutagenic (Waskell et al., 1978), have been shown to induce hepatic tumors in B6C3F1 mice (Herren-Freund et al., 1987). The several alkenoic acids identified may be of toxicological significance because of their structural similarity to the open and oxidized forms of MX.

Structural Assignments to EI Spectra

The identifications of the 45 listed compounds was based on a priori interpretations of the EI spectra. Primary to spectral interpretation and identification of possible precursor (Table 9) was knowledge of the methylation reactions of BF_3 in methanol which are as follows:

R-OH	------>	R-*OCH_3*	(Darbe, 1978)
R-$(OH)_2$	------>	R-*$(OCH_3)_2$*	(Darbe, 1978)
R-COOH	------>	R-*$COOCH_3$*	(Darbe, 1978)
R-$(COOH)_2$	------>	R-*$(COOCH_3)_2$*	(Darbe, 1978)
R-COH	------>	R-*$CH(OCH_3)_2$*	(M^{c}Murry, 1984; Kronberg, 1988)

The methylated portions (in italics) often provide characteristic losses or fragments ions in the mass spectrum. The difficulty encountered with the final identification of the precursor however, lies in the fact that a compound with the group $-CH(OCH_3)_2$ may have originated from an aldehyde or a diol. The following discussion of 4 spectra of compounds with different moieties will exemplify structural assignments.

The identification of 3,3-dichloropropenoic acid (Figure 10, upper) was based on the appearance of a molecular ion at m/z 154, fragment ions at m/z 123 ($M-OCH_3$) and m/z 95 ($M-COOCH_3$) and a two

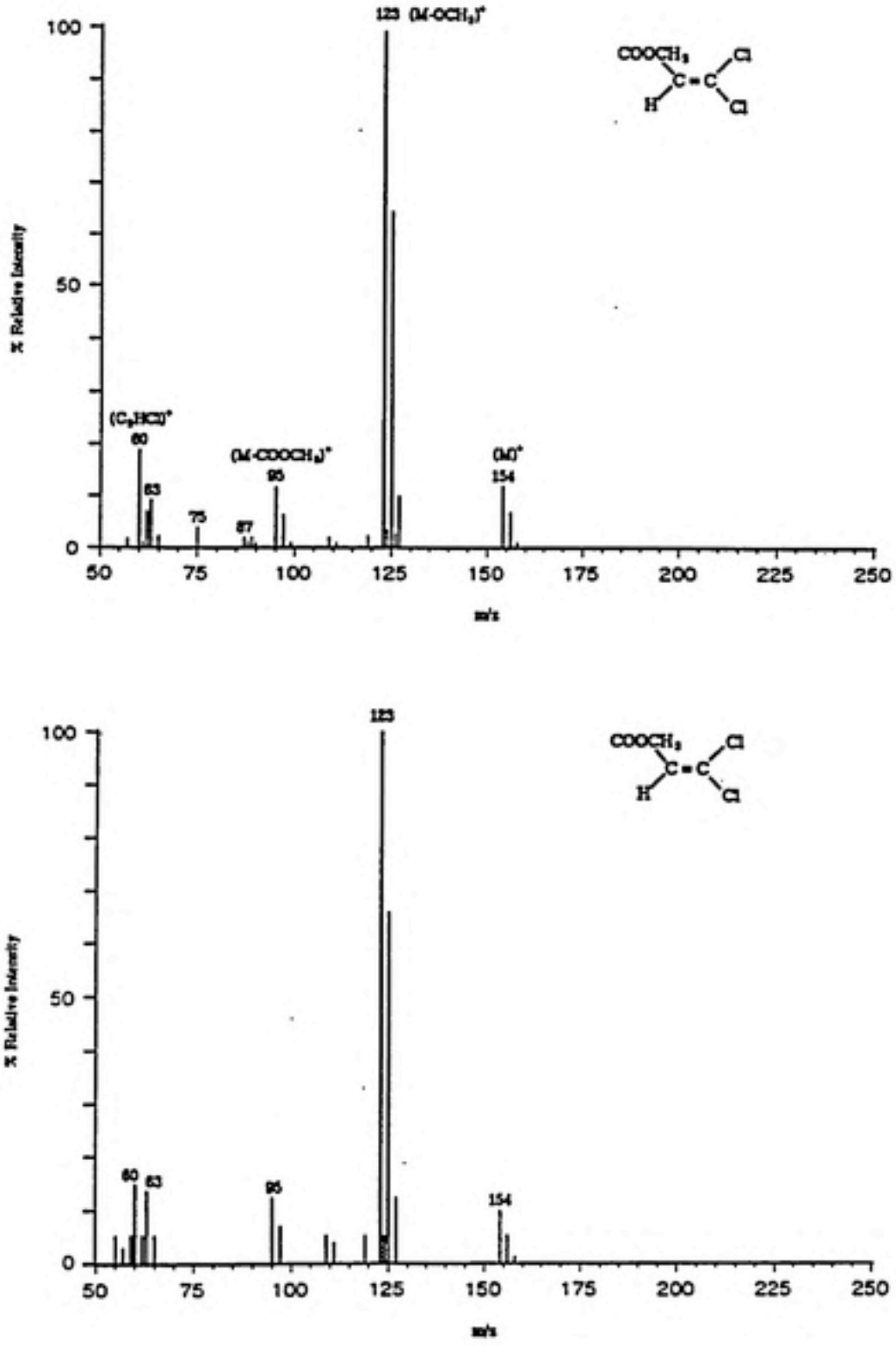
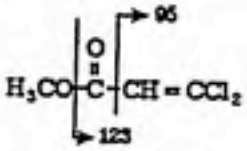
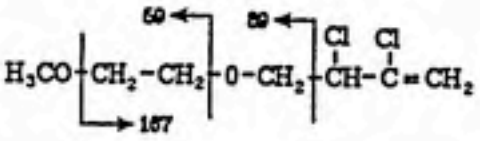


Figure 10. EI Spectrum of Methylated 3,3-Dichloropropenoic Acid in Monochloramination Extract (upper; GC peak# 11) and Published Spectrum of the Compound (lower)



chlorine isotope pattern at all three ions. The structure of this compound was proposed to be 3,3-dichloropropenic acid. Good agreement was found between this mass spectrum and a published spectrum of this compound (Mc Lafferty and Stouffer, 1988) also shown in Figure 10, lower.

In Figure 11, a weak molecular ion, showing a 2-chlorine isotope can be observed at m/z 198. This fragment at m/z 167 (also with a 2 chlorine pattern) arises from the loss of $-OCH_3$. The fragments at m/z 112 and m/z 131 appear to arise from complicated fragmentations and recombinations and therefore do not readily contribute to knowledge of the compound structure. The fragments that determine the structure of



the molecule are those at m/z 59 and m/z 89 (no chlorine pattern observed). The only plausible structure that explains both these ions of high intensity is that of 2,3-dichlorobutene,2-hydroxyethyl ether.

In Figure 12, although no molecular ion is apparent, the molecular weight was deduced from the fragment at m/z 143 by proposing that it arises from simple alpha cleavage and subsequent loss of $-COOCH_3$. This fragment can also be observed as the base peak (m/z 59) indicating that the parent compound was a methyl ester of an acid. The observation of a

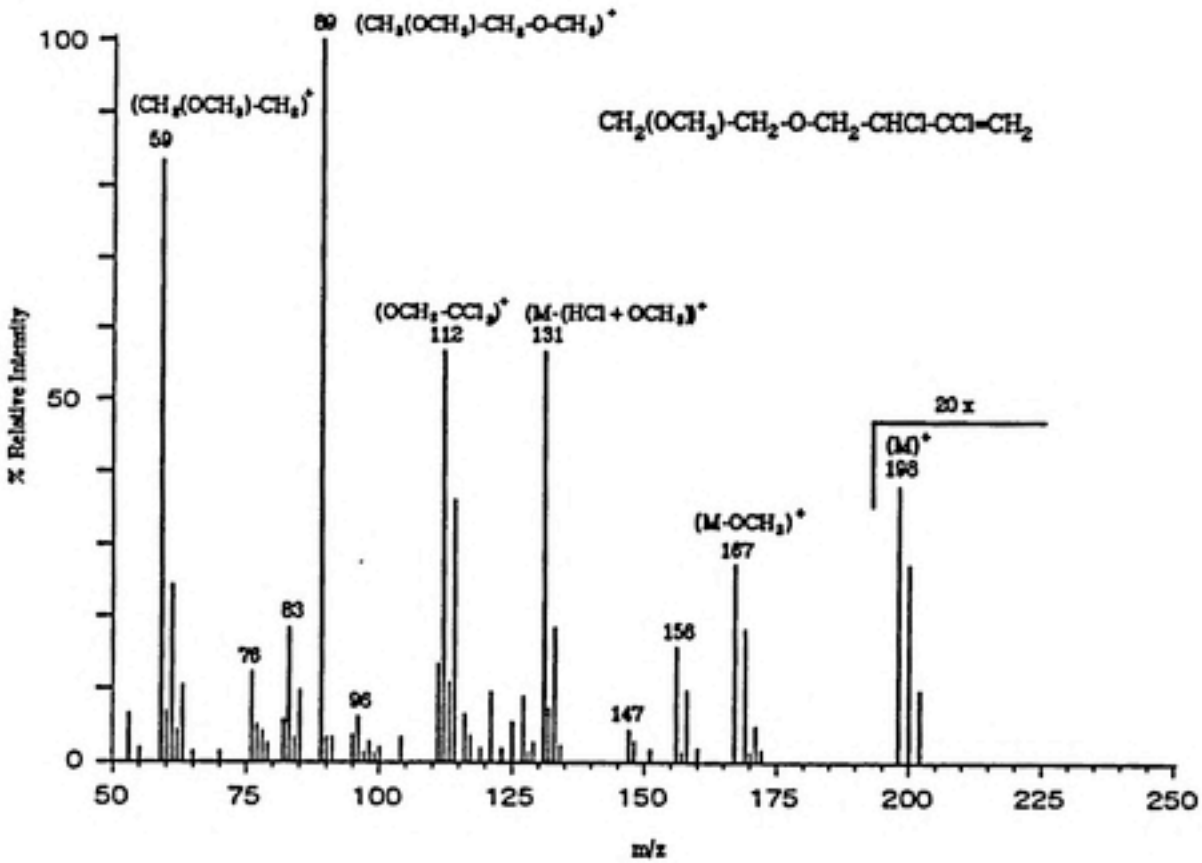


Figure 11. EI Spectrum of Methylated 2,3-Dichlorobutene,2-hydroxyethyl Ether in Monochloramination Extract (GC peak# 22)

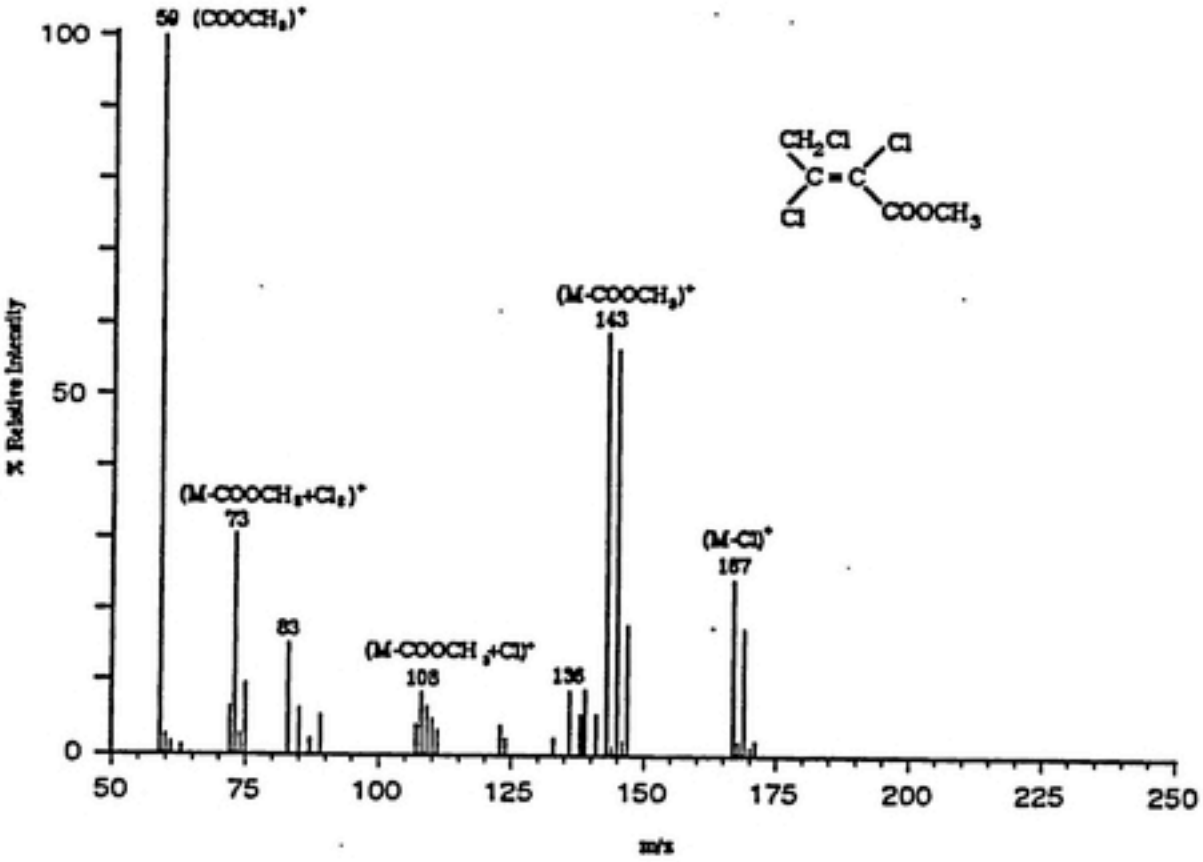
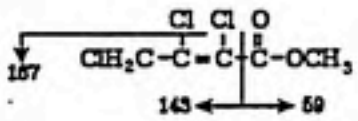
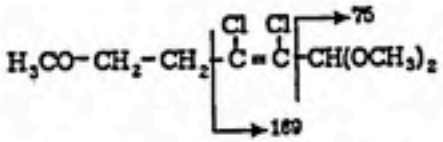


Figure 12. EI Spectrum of Methylated 2,3,4-Trichlorobutenoic Acid in Monochloramination Extract (GC peak# 24)



3-chlorine isotope pattern at m/z 143 indicates that the compound contained 3 chlorines. The ion at m/z 167 showing a 2-chlorine isotope pattern was thus postulated to occur due to a loss of a -Cl from the molecular ion. Thus the compound was tentatively identified as 2,3,4-trichlorobutenoic acid.

The spectrum presented in Figure 13 was interpreted on the basis of the ions at m/z 75, m/z 169 and the 2 chlorine isotope pattern at m/z 169. The base peak at m/z 75 is characteristic of the $-CH(OCH_3)_2$ fragment. Such a fragment has previously been observed in the spectrum



of methylated EMX and resulted from the replacement of the oxygen with two OCH_3 groups on the aldehyde group during methylation. In this case however, the fragment may have also originated from the methylation of two diol groups on the same carbon due to the nature of methylation by BF_3 in methanol. This makes the identification of the compound ambiguous as the underivatized compound could be either 2,3-dichloro-2-pentene-1,1,5-triol or 2,3-dichloro-5-hydroxypentenal.

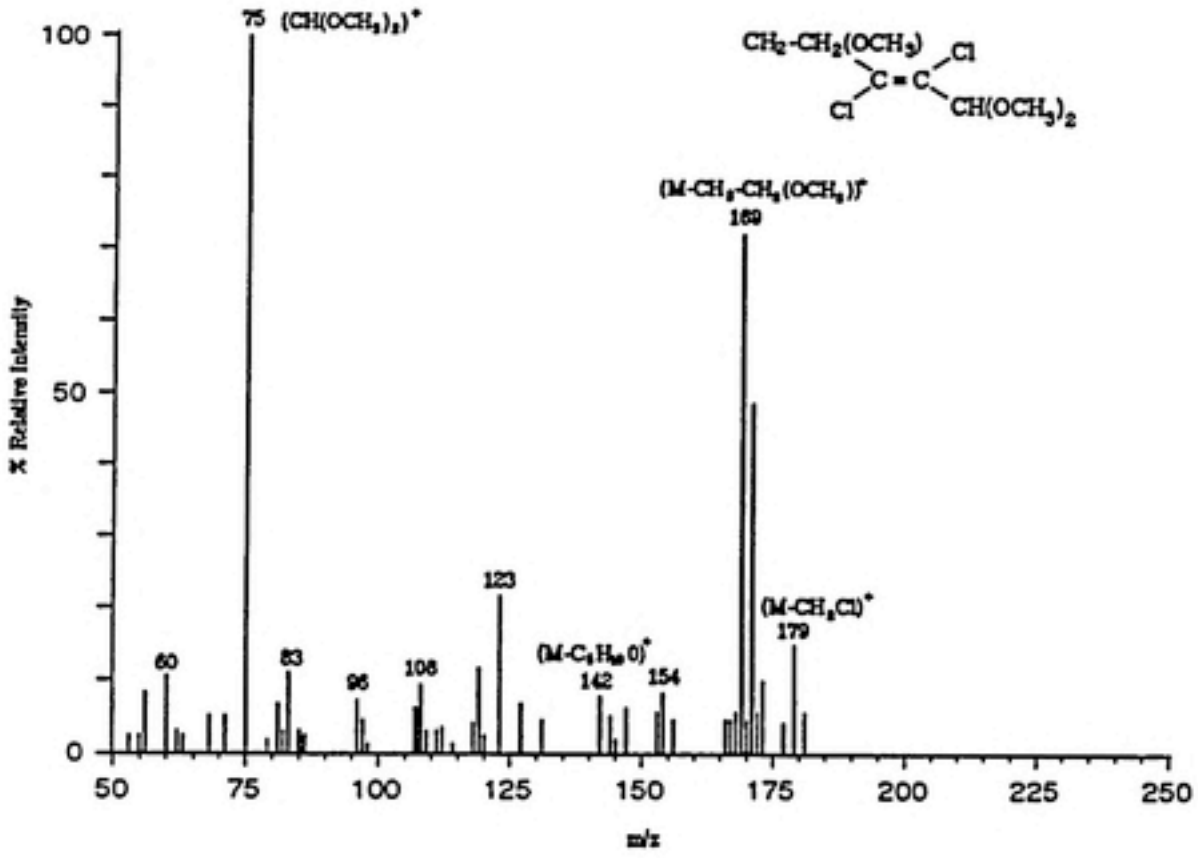


Figure 12. EI Spectrum of Methylated 2,3-Dichloro-2-pentene-1,1,5-triol or 2,3-Dichloro-5-hydroxypentanal in Monochloramination Extract (GC peak# 34)

GC/MS Quantitative Analyses

An instrument resolving power of 10000 was found to be necessary for the quantification of MX and its analogs because compounds exhibiting either molecular or fragment ions that interfered with the identification and quantification of MX were present under low resolution conditions (Table 10, Figure 14). The low signal to noise ratio of 1:1 at low resolution introduced great uncertainty into identification and quantification. Under high-resolution conditions, the ions arising from the loss of $-OCH_3$ and -OCH are resolved thus producing a theoretical ratio of 100:98 between the two ions at m/z 199 and m/z 201 rather than a ratio of 62:100 (Charles et al., 1990).

The standard curve data for the quantification of MX is shown in Table 11 (Figure 15, curve a). The response of methylated MX to $^{13}C_6$ methyl benzoate was found to be linear ($r = 0.98$). The percent relative standard deviation of the relative response factor of 36% demonstrates an unappealing variability in the results. This may have been due to the effect of retention of sample extract on the GC capillary column, which may have subsequently caused an increase in methylated MX peak area readings in the standards. This hypothesis was supported by the low percent relative standard deviation of about 15% for the mean relative response factor of a standard curve that had been previously obtained without the use of any sample extracts (Table 12; Figure 15, curve b). The variability in response in replicates of each standard curve point is shown in Table 12 to be about 11% - 21% in the range

Table 10. Comparison of MX Ion Ratios (m/z 199/201) at Resolving Power = 1000 and 10000

Resolving Power	Theoretical Ratio $[M]^{\cdot}/[M+2]^{\cdot}$	Measured Ratio $[M]^{\cdot}/[M+2]^{\cdot}$	%R.S.D.
1000	62:100*	82:100	20
10000	100:98	100:101	3

* At a resolving power = 1000, ion at m/z 200.9094 is a doublet which results in an observed ratio of 62:100 between ions at m/z 198.9121 and 200.9094.

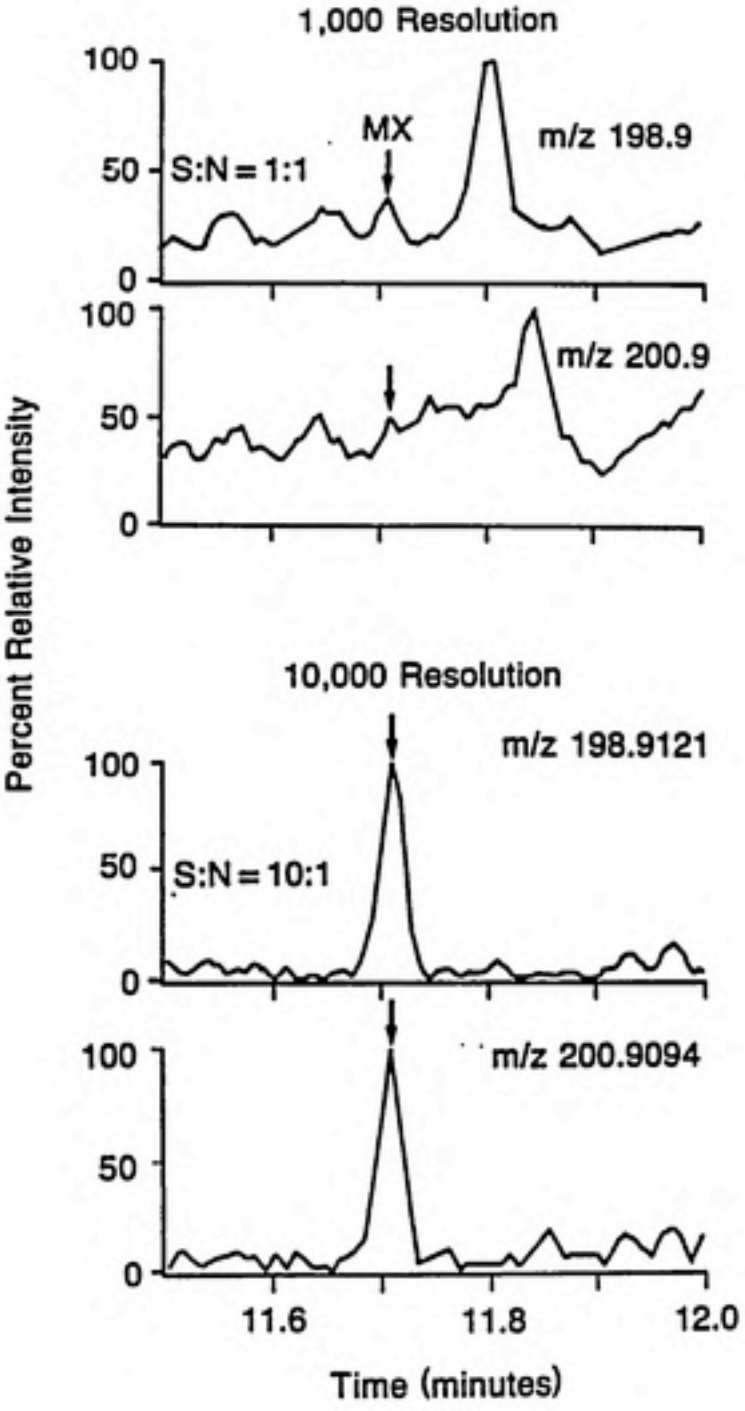


Figure 14. Comparison of Mass Chromatograms of MX Fragment Ions Recorded at 1000 (upper) and 10000 (lower) Resolution During Analysis of a Methylated Monochloramination Extract

Table 11. Standard Data for MX and Mucochloric Acid (MCA) Quantification by SIM-Mode

MX Std. Conc. (pg/uL)	Area MX[a]	Area MCA[b]	Area BA[c]	Area Anth.[d]	RF[e] MX/BA ($x10^{-2}$)	RRF:MX[f,g] ($x10^{-2}$)	RF MCA/BA	RRF:MCA[h] ($x10^{-2}$)	RF BA:Anth.	RRF:BA[i]
50	1.01	121.05	149.31	87.73	0.68	0.68	0.81	0.16	1.70	0.85
100	1.82	88.98	104.08	90.01	1.75	0.88	0.86	0.17	1.16	0.58
100	2.08	91.95	91.97	134.48	2.26	1.13	1.00	0.20	0.68	0.34
100	2.45	99.42	94.30	105.81	2.59	1.30	1.05	0.21	0.89	0.45
100	2.71	111.12	114.04	142.33	2.37	1.19	0.97	0.19	0.80	0.40
100	4.30	130.72	125.49	158.30	3.42	1.71	1.04	0.21	0.79	0.40
250	4.14	100.45	113.60	102.23	3.64	0.73	0.88	0.17	1.11	0.56
500	7.35	89.09	111.56	71.66	6.59	0.66	0.80	0.16	1.56	0.78

[a] Area MX - Area (m/z (199+201)/2)
[b] Area MCA - Area (m/z (147+149)/2)
[c] Area $^{13}C_6$ Benzoic Acid (BA) - Area m/z 142
[d] Area Anthracene (Anth.) - Area m/z 188
[e] RF - Response Factor
[f] RRF - Relative Response Factor
[g] Mean RRF for MX - 1.04×10^{-2} +/- 0.37×10^{-2} (35.6% RSD)
[h] Mean RRF for MCA - 0.18 +/- 0.02 (11.1% RSD)
[i] Mean RRF for BA - 0.55 +/- 0.19 (34.6% RSD)

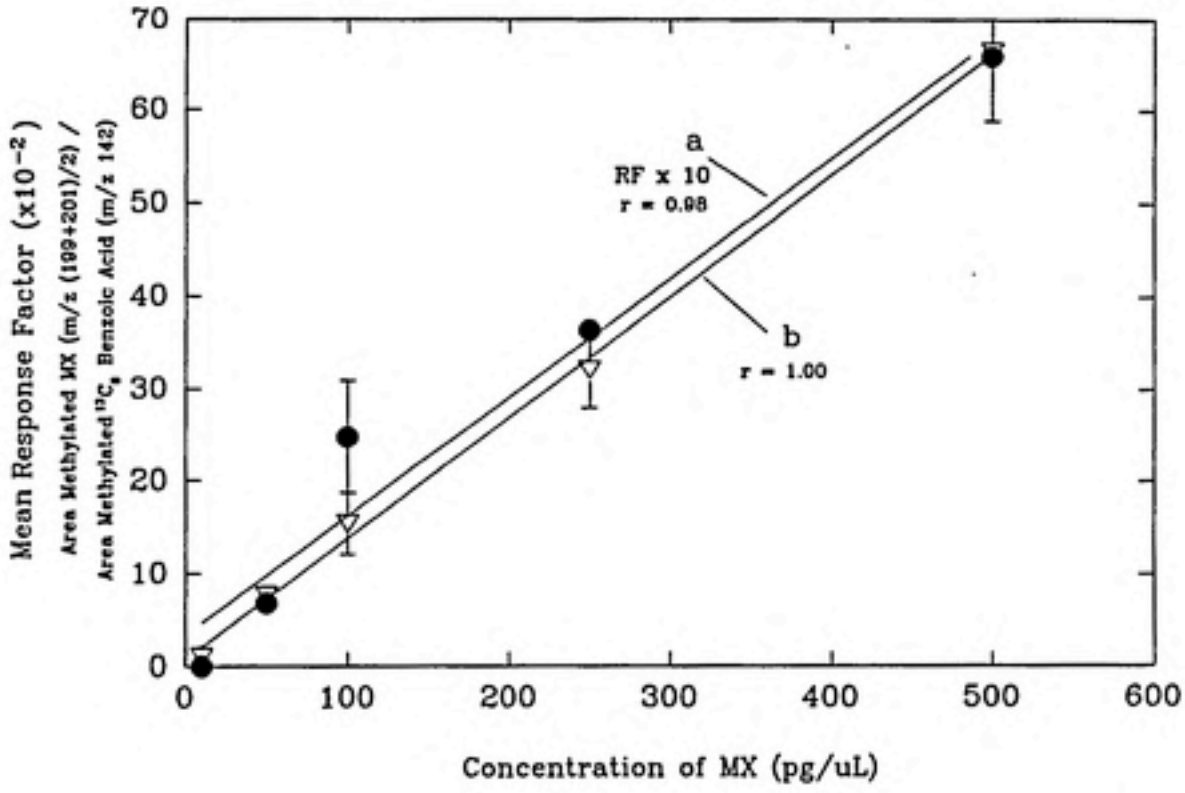


Figure 15. Standard Curves of the Response Factor of Methylated MX to Methylated $^{13}C_6$ Benzoic Acid ("a" was recorded five days after "b" and was used for MX quantification in monochloramination and chlorination extracts; "b" demonstrates that the response factors are reproducible.)

Table 12. MX Standard Data Demonstrating Reproducibility of Response Factors (analyses performed 5 days before quantification of MX in monochloramination extract)

MX Std. Conc. (pg/uL)	Area MX (m/z(199+201)/2)	Area $^{13}C_6$ BA (m/z 142)	RF Area Ratio MX/BA ($x10^{-2}$)	Mean+/-S.D. ($x10^{-2}$)	RRF[a] ($x10^{-2}$)
0	N.D.[b]	56.67	0.00	0.00	5.25
10	0.46	32.71	1.41	1.05+/-0.35	7.70
10	0.48	47.60	1.01	(33.3% RSD)	
10	0.58	41.95	0.72		
50	4.03	46.52	8.66	7.70+/-1.01	7.72
50	2.64	33.79	7.81	(13.1% RSD)	
50	3.10	46.68	6.64		
100	7.89	39.16	20.15	15.43+/-3.28	6.42
100	5.07	35.96	14.10	(21.3% RSD)	
100	7.48	50.36	14.85		
100	7.23	57.34	12.61		
250	17.06	46.79	36.46	32.10+/-4.13	6.64
250	12.55	39.70	31.61	(12.8 % RSD)	
250	11.17	31.55	28.24		
500	37.32	50.39	74.06	66.35+/-7.50	
500	31.72	48.13	65.90	(11.3% RSD)	
500	26.49	44.84	59.08		

[a] Mean RRF = $6.75x10^{-2}$ +/- $1.03x10^{-2}$ (15.3% RSD)
[b] N.D. = None Detected

50pg/uL - 500pg/uL. The variability in response in the four replicates of the 100pg/uL methylated MX standard in Table 11 is about 24%, which is in fairly close agreement with the the former. However the relative response factors obtained from the two curves differ by a factor of 7. This phenomenon of irreproducibility of the relative response factor was repeatedly observed in this work (data not shown) and is not fully understood at present. However, it seems reasonable to assume that the variability is due to nature of the instrument response to methyl MX because Horth et al. (1989) have reported similar problems.

The response between $^{13}C_6$ methyl benzoate and anthracene-d_{10} was monitored as a means to estimate the %recovery of MX, assuming that $^{13}C_6$ benzoic acid is a good analog of MX. A mean recovery of 75.0% +/-14.0% (19% RSD) was calculated (Table 13). The derivatization yields for both compounds have been determined in this laboratory to be about 80% and the methyl esters of MX and $^{13}C_6$ benzoic acid appear to be stable in hexane (Chen, G., unpublished work).

Preliminary SIM mode quantification work was performed on extracts of HPLC fractions (Tables 14 and 15; Figure 16). The results of this quantification must be interpreted with caution because the standard curve was only linear in the range shown (250pg/uL - 750pg/uL). This range falls outside the concentrations of MX in the extract and HPLC fractions, making calculations inaccurate. Nevertheless, it can be observed that the majority of the MX appears to have eluted in Fraction 1 and the remainder in Fraction 2. These findings are consistent with the distribution of mutagenic activity in the fractions (Table 7, Figure 7) and again demonstrates that inadequate separation was achieved by

Table 13. Sample Data for MX and Mucochloric Acid (MCA) Quantification by SIM-Mode

Extract	Area MX[a]	Area MCA[b]	Area BA[c]	Area Anth.[d]	RF[e] MX/BA ($x10^{-2}$)	Conc. MX[f] (pg/uL)	RF MCA/BA	Conc.MCA (pg/uL)	RF BA/Anth.	%Recovery
Monochlo-.	1.31	426.74	129.41	155.37	1.01	36.6	3.30	691.2	0.83	75.4
ramination	1.39	188.19	195.67	180.10	0.71	33.9	0.96	264.5	1.09	99.2
Blank	N.D.[g]	187.26								
Chlor-	1.65	327.42	159.72	191.88	1.03	37.3	2.05	429.4	0.83	75.4
ination	1.95	331.21	162 67	267.42	1.20	32.0	2.04	313.9	0.61	55.4
Blank	N.D.	145.92	193.53	244.60	0.00	0.0	0.75	149.6	0.79	57.2

[a] Area MX - Area (m/z (199+201)/2)
[b] Area MCA - Area (m/z (147+149)/2)
[c] Area $^{13}C_6$ Benzoic Acid (BA) - Area m/z 142
[d] Area Anthracene (Anth.) - Area m/z 188
[e] RF - Response Factor

Table 14. Standard Data for MX Quantification in Monochloramination Extract and HPLC Fractions Recorded in Preliminary Experiments

MX Std. Conc. (pg/uL)	Area MX m/z (199+201)/2	Area $^{13}C_6$ BA m/z 142	RF Area Ratio MX/BA ($x10^{-2}$)	Mean+/-S.D. ($x10^{-2}$)	RRF* ($x10^{-2}$)
250	6.89	585.00	1.18	1.65+/-0.42	0.66
250	5.53	276.00	2.00	(25.5% RSD)	
250	2.39	136.00	1.75		
500	14.87	402.00	3.70	4.70+/-0.88	0.94
500	12.55	226.00	5.55	(18.7% RSD)	
500	5.11	96.00	5.32		
500	2.46	58.00	4.24		
750	19.25	286.00	6.73	9.44+/-2.41	1.26
750	27.40	224.00	12.23	(25.5% RSD)	
750	5.76	55.00	10.46		
750	6.17	74.00	8.33		

* Mean RRF = 9.53 +/- 3.01 (31.6% RSD)

Table 15. Sample Data for MX Quantification in Monochloramination Extract and HPLC Fractions Recorded in Preliminary Experiments

Sample	Area MX[a] m/z (199+201)/2	Area $^{13}C_6$ BA m/z 142	MX/BA (x10^{-2})	MX Conc. (pg/uL)	MX[b,c] Conc. (ng/L)	Mean+/-S.D.
Extract	13.65	104.1	13.10	137.5	55	73+/-17
	24.89	120.8	20.60	216.2	87	(23% RSD)
	26.99	146.0	18.50	194.1	78	
Fraction 1	2.88	110.5	2.60	27.3	26	32+/-8
	4.38	116.8	3.70	38.8	37	(24% RSD)
Fraction 2	1.29	137.5	0.90	9.4	9	10+/-1
	1.37	139.2	1.00	10.4	10	(10% RSD)
Fraction 3	N.D.[d]	194.7	0.00	0.0	0	
	N.D.	128.8	0.00	0.0	0	

[a] Data for 3 determinations of one sample in the extract and two determinations of one sample in the HPLC fractions

[b] Conc. of MX (ng/L) in Extract = Conc. (pg/uL) x 100uL x Extraction Volume Correction Factor of 4 (250 mL aliquot extract)

[c] Conc. of MX (ng/L) in HPLC Fractions = Conc. (pg/uL) x 100uL x 4 x HPLC injection volume correction of 2.4 (0.5 mL out of 1.2 mL of extract redissolved in acetonitrile and water)

[d] N.D. = None Detected

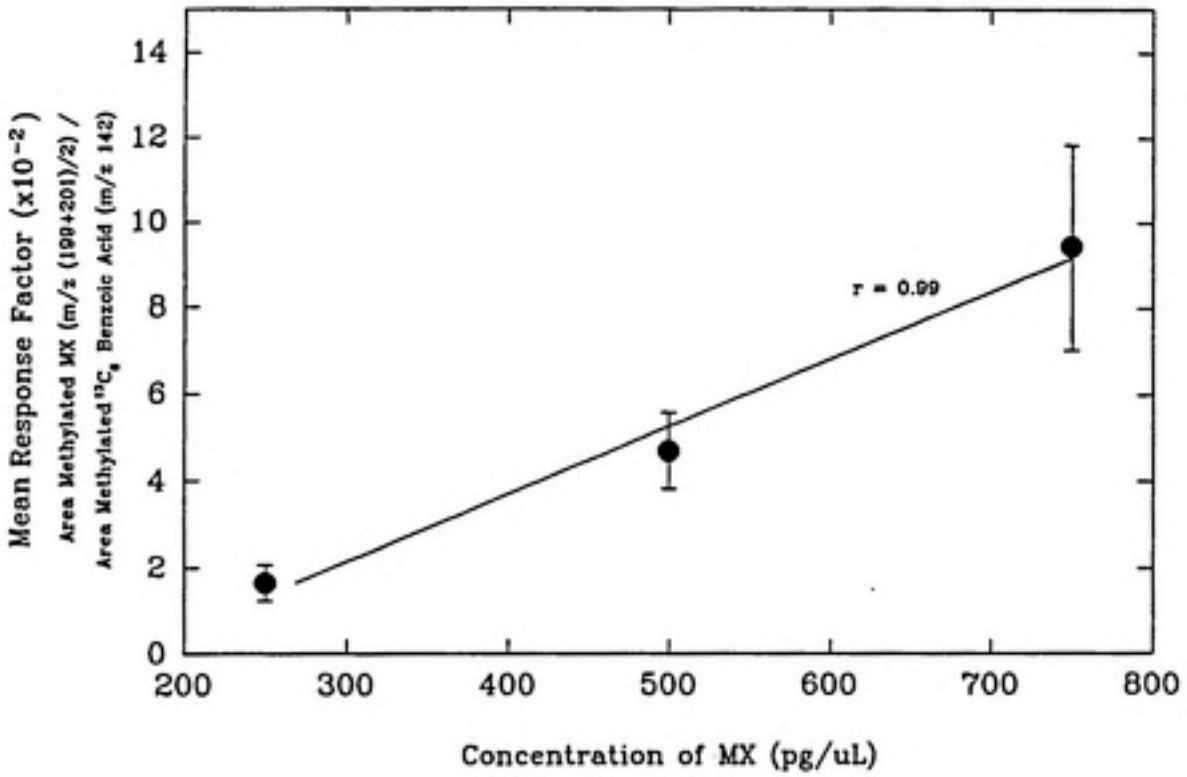


Figure 16. Standard Curve of the Response Factor of Methylated MX to Methylated $^{13}C_6$ Benzoic Acid Recorded in Preliminary Experiments

Identification of the MX, EMX, ox-EMX and mucochloric acid was based on matching of relative retention times and relative ion peak area ratios between standards and samples (Table 16, Figure 17). The matching was fairly accurate in most cases. Data for the quantification of EMX and ox-EMX, and concentrations of all four compounds in the monochloramination and chlorination extracts are shown in Tables 17 and 18.

Confirmation of di- and trichloroacetic acids, dichlorosuccinic acid and ox-EMX in full scan mode was based on matching of relative retention times and of the mass spectra of the compound identified in extract and HPLC fractions to spectra of pure standards. The quantification data for these compounds is shown in Tables 19 and 20.

The mean concentration of MX obtained from analyses of two replicates of monochloramination extracts is 71 +/- 4 ng/L. Chlorination was observed to produce approximately twice as much MX as monochloramination. A previous study (Table 4) has shown that chlorination produces up to 4 times as much MX as chloramination (Backlund et al., 1988). The study also reports higher MX levels for both oxidants than are reported here. In addition MX was found to contribute 24% to the activity of the extract, more than twice that (11%) reported in the present work.

The remainder of the compounds quantified occurred in concentrations that were 8 to 63 times higher than the concentration of MX in monochloramination extracts and 10 to 653 times higher than those in chlorination extracts.

Monochloramination produced 3.1 +/- 1.8 ng/L of EMX, a quantity

Table 16. Ion Peaks Used for SIM Identification and Quantification of MX, EMX, ox-EMX and Mucochloric Acid (MCA) in Monochloramination and Chlorination Extracts

Compound	Fragmentation	m/z	Relative Peak Area Ratios				
			Std.	Monochlor. Extracts		Chlor. Extracts	
MX	M-OCH,	198.9121	1.00[1]	1.00	1.00	1.00	1.00
		200.9094	0.94	1.37[2]	1.14[2]	0.90	0.75
EMX	M-OCH,	244.9539	1.00[1]	1.00	1.00	1.00	1.00
		246.9510	1.14	0.76	0.91	0.85	0.99
ox-EMX	M-Cl	224.9721	1.00[3]	1.00	1.00	1.00	1.00
		226.9691	0.65	0.64	0.68	0.71	0.68
	M-OCH,	228.9226	1.00[4]	1.00	1.00	1.00	1.00
		230.9196	0.89	1.02	0.97	0.95	0.95
MCA	M-Cl	146.9848	1.00[5]	1.00[7]	1.00	1.00	1.00
		148.9818	0.33	0.32	0.33	0.37	0.32

[1] theoretical ratio = 1:0.98

[2] interference from background

[3] theoretical ratio = 1:0.67

[4] theoretical ratio = 1:0.98

[5] theoretical ratio = 1:0.98

[6] theoretical ratio = 1:0.33

[7] all samples were spiked with 250pg/uL MCA standard

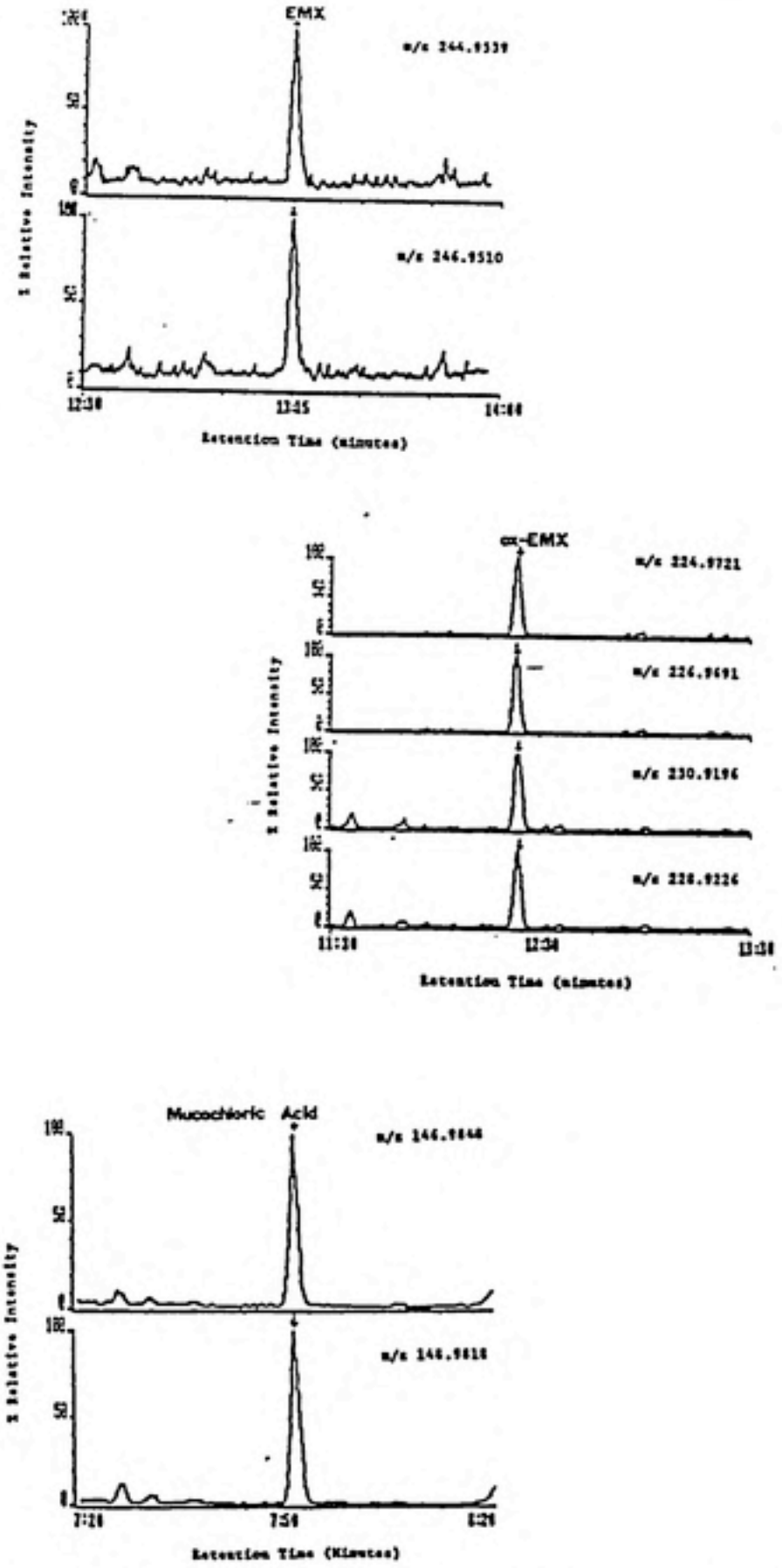


Figure 17. Mass Chromatograms of EMX (upper), ox-EMX (middle) and Mucochloric Acid (lower) Fragment Ions Recorded During Analysis of Methylated Monochloramination Extract

Table 17. Sample Data for Quantification of EMX and ox-EMX in Monochloramination and Chlorination Extracts

Extract	Area $^{13}C_6$ BA	Area EMX	EMX/BA (x10^{-2})	Conc. EMX (pg/uL)	ox-EMX : M - Cl* Area	ox-EMX/BA	Conc. (pg/uL)	ox-EMX : M - OCH_3 Area	ox-EMX/BA	Conc. (pg/uL)
Monochl.	93.17	3.79	3.32	2184.4	83.54	0.73	2510.3	53.72	47.14	2223.6
	113.97	1.51	1.43	940.8	74.10	0.73	2512.3	44.05	41.83	1973.1
Blank	105.31	N.D.	0.00	0.0	N.D.	0.00	0.0	N.D.	0.00	0.0
Chlor.	127.69	4.92	4.86	3197.4	729.80	7.21	24704.1	328.11	324.32	15298.1
	101.17	5.24	5.46	3592.1	735.37	7.67	26266.1	339.82	354.42	16717.9
Blank	95.88	N.D.	0.00	0.0	N.D.	0.00	0.0	0.00	0.00	0.0

* Only the quantification using the M-Cl ions of ox-EMX was reported in the text of this paper

Table 18. Concentration of MX, EMX, ox-EMX and Mucochloric Acid (MCA) in Monochloramination and Chlorination Extracts and Mutagenicity Contribution of the Most Active Compounds : SIM Mode Quantification

Extract[a]	Concentration [b]								Mutagenicity Contr.I[c]	
	MX	Mean+/-S.D.	EMX	Mean+/-S.D.	ox-EMX	Mean+/-S.D.	MCA	Mean+/-S.D.	MX	EMX
	(ng/L)		(ug/L)		(ug/L)		(ug/L)			
Chloram.	73	71+/-4	4.4	3.1+/-1.8	5.0	5.0+/-0	930	500+/-600	11+/-0.6	26+/-15
	68		1.9		5.0		76			
Blank	0		0		0		0			
Chlorin.	150	140+/-14	13	14+/-1.1	99	105+/-7.8	1100	880+/-310	NA[d]	NA
	130		14		110		660			
Blank	0		0		0		0			

[a] The extracts were produced from equivalent monochloramine and chlorine dose and Cl:C ratio
[b] MX concentrations presented have been corrected for recovery.
[c] Contribution to mutagenicity is calculated on the basis of 5600 and 320 net rev/nmole (Kronberg et al., 1990) specific MX and EMX TA100-S9 mutagenicity respectively. The TA100 activity of the monochloramination extract was determined to be 17476 rev/L (David Cozzie, 1990).
[d] No assays were performed on chlorination extracts.

Table 19. Data for Full-Scan Quantification of Compounds in Monochloramination Extract and HPLC Fractions

Compound[a]	Sample[b]	Area C_{std}[c] ($x10^6$)	Area IS_{std} ($x10^8$)	RRF ($x10^{-2}$)	Area C_{sam} ($x10^5$)	Area IS_{sam} ($x10^7$)	C/IS_{sam} ($x10^{-2}$)	Conc. C_{sam} (ng/uL)
DCA (m/z 83)	Extract	7.53	1.32	5.70	694.00	6.07	114.33	200.6
	Frac. 1	7.53	1.32	5.70	89.30	2.58	34.61	60.7
	Frac. 2	7.53	1.32	5.70	9.16	5.42	1.69	3.0
	Frac. 3	7.53	1.32	5.70	1.59	4.73	0.34	0.6
TCA (m/z117)	Extract	1.03	1.32	0.78	13.10	6.07	2.16	27.7
DCSA (m/z 183)	Extract	0.15	1.32	0.11	39.80	6.07	6.56	596.4
	Frac. 1	0.15	1.32	0.11	2.79	2.58	1.08	98.2
ox-EMX (m/z 225)	Extract	1.95	1.69	1.15	18.30	6.07	3.02	26.3

[a] DCA - Dichloroacetic acid
TCA - Trichloroacetic acid
DCSA - Dichlorosuccinic acid
the ion monitered is given in parentheses

[b] Frac. - HPLC Fraction

[c] C - Compound or analyte being quantified (10ng/uL in standard)
IS - Internal standard, decafluorobiphenyl (10ng/uL in standards and samples; m/z 334 monitered)

Table 20. Concentration of Di- and Trichloroacetic Acids, Dichlorosuccinic Acid and ox-EMX in Monochloramination Extract and HPLC Fractions: Full Scan Quantification

Compound	Ion Monitored[a] (m/z)	Concentration (ug/L)			
		Ether Extract	HPLC Fraction 1	HPLC Fraction 2	HPLC Fraction 3
Dichloroacetic Acid	83	80	58	2.9	0.6
Trichloroacetic Acid	117	11	ND[b]	ND	ND
Dichlorosuccinic Acid	183	240	94	ND	ND
ox-EMX	225	11	ND	ND	ND

[a] Performed in full scan GC/MS mode with internal standard decaflorobiphenyl (10ng/uL) and pure standards of the compounds (10ng/uL)

[b] ND = Not Detectable

that was a factor of 4 less than that produced by chlorination in this study. Backlund et al, (1987) observed the same ratio between chloramination and chlorination, but found lower concentrations of EMX than were found in this study (Table 4). In addition, they found EMX to contribute only 2% to the total activity of the extract in contrast to the 21% found in this study. The fact that a greater quantity of EMX (ring-open isomer of MX, predominant at pH > 6.5; Holmbolm et al., 1984; Meier et al., 1987) was formed in this study may explain the lower concentrations of MX and consequently the lower mutagenic activity observed in this study in comparison to the study by Backlund et al., (1987). The reason for the above may be due to the fact that the Backlund study utilized a reaction pH of 7.0, one pH unit lower than that used in this study. A difference of one pH unit may have caused the production of a ten fold greater quantity of EMX in the present work.

ox-EMX was quantified in both SIM and full-scan modes. The amount quantified by full-scan (Table 20)(11 ug/L) was approximately twice the amount quantified by SIM (5 ug/L). This difference in concentration indicates that the assumption of linearity made for SIM-mode quantification did not hold true. However, the results must be interpreted with caution since both analyses were only semi-quantitative.

Mucochloric acid was observed in both monochloramination and chlorination extracts (500 +/- 600 ng/L and 880 +/- 310 ng/L, respectively). However, the high variability in the results render the semi-quantitative results unreliable.

Full-scan determinations (Table 20) yielded high concentrations of dichloroacetic acid (80 ug/L), trichloroacetic acid (11 ug/L) and dichlorosuccinic acid (239 ug/L). Dichloroacetic acid and dichlorosuccinic acid were also quantified in the HPLC fractions. About 76% of dichloroacetic acid and 40% dichlorosuccinic acid quantified in the extracts were recovered in the fractions. The recovery of the former compares fairly well with that of the mean recovery of approximately 71%, of mutagenicity, TOX and TOC from HPLC.

ox-EMX and mucochloric acid have both been found to be weakly mutagenic (7.8 net revertants/nmole and 1.3 net revertants/nmole respectively; Singh, 1989; Meier et al., 1986). Despite its occurrence in large concentration in the extracts, ox-EMX only contributes about 2% to the mutagenic activity of the monochloramination extract. The contribution of mucochloric acid is negligible.

CONCLUSIONS AND RECOMMENDATIONS

Monochloramination of aqueous fulvic acid was consistently shown to produce mutagenic by-products that show similar characteristics to those produced by chlorination (Table 4). The preferential extraction of the mutagens at acid pH and elution in the most polar HPLC fraction shows them to be polar and acidic. In addition, the strong correlation between TOX and mutagenic activity implicates chlorinated compounds in the production of mutagenicity. Mutagenic activity of the extracts appears to increase linearly up to a NH_2Cl dose (consumed) of 415 mg/L. Higher doses did not result in an increase in activity possibly due to the lethality of the products at high concentrations. The mutagens produced are most likely to be direct acting, base-pair substituting and electrophilic.

Forty-eight compounds were identified by HRGC/MS analyses. Of these MX and its structural analogs EMX, ox-EMX and mucochloric acid were quantified or semi-quantified by SIM-mode analyses. Chloramination produced about half as much MX, about a fourth as much EMX, about a twentieth as much ox-EMX and about the same concentration of mucochloric acid as chlorination under the same conditions. These compounds together account for about 39% of the total mutagenic activity of the monochloramination extract.

The 44 other compounds tentatively identified (except for di- and trichloroacetic acids and dichlorosuccinic acid which were standard confirmed) are short-chain (C_2-C_9) chlorinated aliphatic acids, alcohols

and aldehydes. These include alkenoic acids, compounds structurally similar to EMX, the geometric isomer of MX.

Additional research is greatly needed before definitive conclusions can be reached about the relative health hazards of monochloramination. Many recommendations can be made for future study. First, HPLC and derivitization methods must be developed to further isolate the mutagenic fraction and to identify compounds that account for the residual 61% of the activity. Second, the pH dependent production of MX and EMX in monochloramination extracts must be further investigated as the equilibrium concentrations of both determine the level of mutagenic activity observed. Third, the mutagenic activity of the identified compounds that are structurally similar to EMX must be determined and pH dependent structural changes must be examined. Fourth, product identification work needs to be continued not only with model reaction systems but also with real drinking water extracts. Last, animal studies must be conducted to evaluate toxicology and carcinogenicity of MX and related compounds in mammalian systems.

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APPENDIX

Equations Used for Calculations in the Quantification Work:

1. Relative Response Factor (RRF) = (AreaC x Conc.IS)/(AreaIS x Conc.C)

AreaC represents an average area of 2 ion peak areas for each compound quantified in the SIM mode and a single ion peak area for compounds quantified in full-scan mode. AreaIS represents single ion peak area of the internal standard.

2. $Conc.C_s = ((AreaC_s \times Conc.IS)/(AreaIS_s \times RRF)) \times \%Recovery$

The subscript "s" represents sample values.

3. $\%Recovery = ((AreaBA_s \times Conc.A)/(AreaA \times RRF) / Conc.BA_{std.}) \times 100$

BA is $^{13}C_6$ methyl benzoate, A is anthracene-d_{10} and RRF is the relative response factor of $^{13}C_6$ methyl benzoate to anthracene-d_{10} in the standards.

4. $Conc.\ MCA_{corr.} = Conc.\ MCA_{sample} - Conc.\ MCA_{blank}$

MCA is mucochloric acid. The correction is necessary because all samples and blanks were spiked with 250pg/uL of mucochloric acid.

5. %Contribution to mutagenic activity =

$(Conc.M_{ng/L} \times Activity\ M_{rev/ng}) / Total\ Activity\ of\ Extract$

M is the mutagen whose contribution is being calculated