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## THE EFFECT OF CYCLOHEXENE, A PRESERVATIVE IN DICHLOROMETHANE ON THE LIQUID-LIQUID EXTRACTION AND ANALYSIS OF CHLORINATED DRINKING WATER

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### SUMMARY

Liquid-liquid extraction with dichloromethane is the Environmental Protection Agency's (EPA) method of choice for the analysis of acid or base neutral organic chemicals in water. Free radical chlorination and oxidation products of cyclohexene (the solvent preservative) in dichloromethane have been observed in extracts of chlorinated drinking water (after  $10^5$  fold concentration) by gas chromatography and gas chromatography-mass spectrometry. The chlorinated cyclohexene derivatives limit the quantitative and qualitative analysis of compounds eluting at Kovát's index < 700-1000 and the use of these extracts for organoleptic and mutagenic studies. The effect of residual free chlorine in water on the cyclohexene present in the solvent was studied. Over ten cyclohexene derivatives were produced in every case when any residual free chlorine was present in the water. When chlorine is reduced to chloramine, the cyclohexene derivatives were greatly reduced in number and amount.

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### INTRODUCTION

Nanogram to microgram per liter concentrations of organic chemicals are isolated and concentrated up to  $10^5$  fold to enable gas chromatographic (GC) and GC-mass spectrometric (MS) analysis of the extracts for evaluation of toxic, organoleptic (taste and odor) or mutagenic (Ames Bioassay) properties of drinking water. A major objective of the concentration methods is to minimize chemical alteration and artifact formation while efficiently isolating and concentrating the chemicals in the sample. This is extremely important for toxicity testing because artifacts can be identified during chemical analysis and can be incorrectly blamed for a toxic effect. Jolly and Suffet<sup>1</sup> indicate that two of the most practical isolation methods for large volumes of water (approximately 100 l) are resin accumulation and continuous solvent extraction. The solvents used for these isolation methods must be free of impurities at ppb levels for unambiguous analytical and toxicity studies. Distilled-in-glass solvents are the choice of analysts.

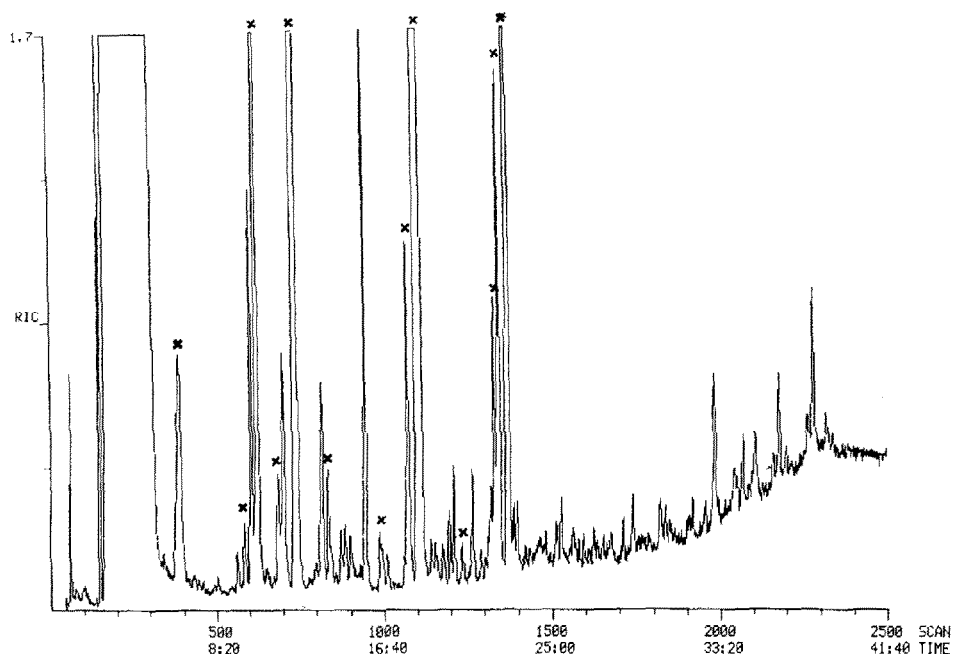


Fig. 1. A capillary GC-MS profile on Supelcowax-10 of a CLLE dichloromethane extract of 100 l of chlorinated drinking water sample. ×, Cyclohexene-related artifacts.

Liquid-liquid extraction (LLE) with dichloromethane is the EPA method of choice for the broad spectrum analysis of acid or base neutral organic chemicals in water samples<sup>2,3</sup>. Bowers *et al.*<sup>4</sup> have found trace impurities in dichloromethane. The compounds identified included tri- and tetrachloropropanes, tetrachloroethane, phthalic anhydride and dioctyl phthalate. In our studies of broad spectrum GC analysis and collecting samples for Ames mutagenicity testing, a continuous liquid-liquid extraction (CLLE) system has been developed to enable on-line composite sampling (2 l/h) and concentration of 100 l water samples to a 1-ml solvent concentrate<sup>5,6</sup>. For a 100-l water sample extracted by a continuous liquid-liquid extractor at a 10:1 water to solvent ratio, approximately 1.7 l dichloromethane are lost due to its solubility in water. Of the remaining 300 ml, 80 ml are left in the apparatus and are concentrated to 1 ml for toxicity testing and chemical analysis. Thus, if the original solvent contains as little as 100 ppb\* of cyclohexene, after concentrating 2 l of solvent, 200 µg/ml are present in the final extract. This is equivalent to 200 µg per 100 l water or equivalent to 2 ppb concentration in the water. Usually, the extracts of drinking water contain only a few peaks > 1 ppb.

During CLLE isolation and concentration of trace organic chemicals from chlorinated drinking water, many cyclohexene derivatives were observed by GC-MS. Fig. 1 shows the cyclohexene derivatives observed in a 10<sup>5</sup> fold concentration of 100 l of water. Keith *et al.*<sup>7</sup> have shown that these are reaction artifacts of the cyclohexene preservative in the dichloromethane and the chlorine residual in the water sample. The cyclohexene may be present in the original manufacturer's product or may be

\* Throughout this article the American billion (10<sup>9</sup>) is meant.

added by the solvent distributor to preserve dichloromethane to prevent decomposition to phosgene and hydrogen chloride. For GC analysis of a specific organic chemical, the chlorine can be reduced by sodium sulfite to eliminate the artifact formation. However, this reduction can not be utilized for mutagenic toxicity studies as sodium sulfite could react with electrophiles in the sample that can cause the mutagenic response. Also, reduction could not be used for broad spectrum analysis because it may change the chemicals in the sample. For example, chloropicrin which is formed during chlorination of water, is removed by sodium sulfite addition<sup>8</sup>. In a related study which uses XAD resin for isolating organics from drinking water, it has been shown that, when using dichloromethane, the resins react with free chlorine in the water sample and produce artifacts which are Ames positive. The production of these mutagenic artifacts could be suppressed at least ten fold by converting free chlorine to monochloramine<sup>9</sup>.

This study defines the cyclohexene problem. In particular, the problem is addressed for use with a CLLE where 2 l of solvent are concentrated  $2 \cdot 10^3$  fold to 1 ml. Different methods to eliminate the cyclohexene problem while minimizing electrophilic reduction within the sample are studied, to enable the extracts to be used for Ames mutagenicity testing and broad spectrum GC analysis.

## EXPERIMENTAL

An illustration of the problem showing the cyclohexene artifacts is seen in Fig. 1. A set of experiments were designed to evaluate the effects of residual free chlorine in water on the cyclohexene present in the extracting solvent (dichloromethane) and to devise methods to eliminate the problem.

### *Solvents*

Two commercially available samples and one undistilled sample of dichloromethane (b.p. 39.75°C) were used. These samples were: (1) lot No. 4502 from EM Science (Cherry Hill, NJ, U.S.A.); (2) lot No. AK 847 from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.); (3) undistilled dichloromethane from EM Science which had about ten times higher amount of cyclohexene than the Burdick and Jackson or the solvent from EM Science, lot No. 4502.

### *GC and GC-MS*

The concentrated extracts were analyzed by capillary GC using a 60-m DB-1 column and GC-MS using a 30-m Supelcowax-10 column and a 30-m DB-5 column. The experimental conditions are listed in Table I.

### *Continuous liquid-liquid extraction (CLLE)*

The continuous liquid-liquid extraction (CLLE) apparatus<sup>4,5</sup> was utilized for extraction of 90-100 l of sample with a total of 2 l solvent. The volume of solvent recycled in the apparatus through the evaporative concentration system (EVACS)<sup>10</sup> is 300 ml. The final volume of solvent (80 ml) is concentrated to 1 ml by the EVACS.

The first series of experiments includes three CLLE runs at a flow-rate of 2 l/h to develop blanks. These CLLE runs represent: (1) a 22-h recycled solvent blank (2 l) (Fig. 2a); (2) a 45-l Milli-Q water extract (1 l solvent) (Fig. 2b); (3) a 90-l Milli-Q

TABLE I  
GC AND GC-MS CONDITIONS

	GC	GC-MS
Instrument	2150 Carlo Erba	Finnigan 1020
GC column	DB-1, 60 m, 0.25 mm I.D.	(A) Supelcowax-10, 30 m × 0.32 mm I.D. (B) DB-5, 30 m, 0.32 mm I.D.
Type of injection	Splitting ratio 20:1	45 s Splitless
Injection size	2.5 $\mu$ l	3 $\mu$ l
Injection temperature	250°C	250°C
<i>Temperature program</i>		
Start temperature	40°C, 8-min hold	40°C, 1-min hold
Ramp 1	3°/min to 150°C	6°/min to 250°C
Ramp 2	50°/min to 275°C	
Final temperature	275°C	250°C
<i>MS conditions</i>		
Mass range	—	33–333 a.m.u.
Scan rate	—	300 a.m.u./s
EM multiplier voltage	—	1800 V
Manifold temperature	—	80°C

water extract with a 2.7 mg/l free chlorine residual added (2 l solvent) (Fig. 2c). The three extracts were concentrated to 1 ml using the EVACS and analyzed to determine the effects of chlorine on the cyclohexene in circumstances where relatively large amounts of chlorine residual (243 mg of chlorine as Cl) were free to react over a long period of time (45 h or more).

#### *Batch extraction*

One to one batch extraction in a separatory funnel was utilized to see the immediate effect of chlorination on the dichloromethane. Undistilled dichloromethane was used since it contained the highest concentration of cyclohexene. A series of laboratory experiments were conducted to show the difference between extracting chlorinated and chloraminated water with dichloromethane. (1) A blank of 200 ml of Milli-Q water, pH 4.0, was batch extracted with 200 ml of dichloromethane (Figs. 3a and 4a). (2) Another 200 ml of the same batch of Milli-Q water were chlorinated at a level of 5 mg/l free chlorine residual. Chlorinated Milli-Q water was batch extracted immediately with 200 ml of dichloromethane (Figs. 3b and 4b). (3) Experiment 2 was repeated after adding a slight excess of ammonium chloride to change the free chlorine into chloramine (Figs. 3c and 4c). Attempts were made to optimize the pH and the amount of ammonium chloride to be added for chloramination. It was found that converting free chlorine into chloramine is best done at pH higher than 6. A 600-mg/l amount of ammonium chloride is needed to change all the free chlorine (5 mg/l as Cl) into chloramine. The pH was 6 after adding the ammonium chloride.

The three extracts were concentrated, using the EVACS, from 200 to 1 ml and analyzed by capillary GC and GC-MS analysis (Figs. 3 and 4).

The continuous liquid-liquid extraction requires long periods of contact (20–50 h or more) between the water sample and the extracting solvent to collect detectable amounts of the trace organic compounds. For a 100-l sample extracted at a flow-rate of 2 l/h in the CLLE, the contact time between the sample and the solvent is 50 h. The following experiments were conducted to understand the chlorine/chloramine effect on dichloromethane (preserved with cyclohexene) over 20–50 h contact times. (1) Batch extraction of 1 l of milli-Q water with 5 mg/l free chlorine residual and 1 l of dichloromethane (lot No. 4502). This 1:1 batch extraction was allowed to stay in a shaker for 22 h before separating the phases (Fig. 5a). (2) Batch extraction of 1 l of Milli-Q water with 5 mg/l free chlorine residual, 100 ppm ammonium chloride and 1 l of the same lot of dichloromethane. This 1:1 batch extraction was allowed to stay in a shaker for 22 h before separating the phases (Fig. 5b).

#### *Purification of dichloromethane*

One liter of dichloromethane (lot No. 4502, Spectral grade, EM Science) was added to the pot of a PTFE helix spinning band still (Model 40 T/CL; B/R Instrument Corp, Pasadena, CA, U.S.A.). A PTFE band was put in a 21-mm bore hole by 24-in. long adiabatic column. The pot and head temperature was adjusted to 39.6°C and the spinning band speed to 1000 rpm. These settings generate about 30 theoretical plates for the distillation. A reflux ratio of 10:1 was used. The first 50 ml of distillate were discarded to eliminate the possibility of any presence of dichloromethane cyclohexene azeotrope at the beginning of the process. The middle cut was collected for testing. When 1 l of solvent was collected, the solvent was concentrated to 0.5 ml in the EVACS and analyzed by capillary GC profile analysis. An 800-ml volume of dichloromethane was reclaimed by condensation during the concentration step, 500 ml of which were subjected to a 48-h, one to one batch extraction in the presence of 5 ppm concentration of chlorine as Cl. The dichloromethane extracts were concentrated by the EVACS to a final volume of 0.5 ml (1000 fold) and analyzed by capillary GC profile analysis.

The following experiments were conducted to remove the cyclohexene before the spinning band distillation. (1) Prewashed alumina in the pot of the spinning band still. It was hoped that the cyclohexene would adsorb to the solid and affect the separation of the two component system. (2) An azeotropic mixture of freon (FC-113) and dichloromethane (1:1) was prepared. The boiling point of the azeotropic mixture was 36°C. (3) A saturated solution of potassium permanganate was mixed with dichloromethane and stirred for 3 h in a covered container. The slight miscibility of water in dichloromethane (about 1.4%) allowed the oxidant to stay suspended in solution and in intimate contact with the reactant. The solution was then added to the pot of the spinning band still and distilled. When 1 l of solvent was collected, a 2000-fold concentration was completed in the EVACS to a final volume of 0.5 ml. An 800-ml volume of dichloromethane was reclaimed by the EVACS during the concentration step, 500 ml of which were subjected to a 1:1, 48-h batch extraction in the presence of a 5 ppm concentration of chlorine as Cl. The extract was separated, concentrated 1000 fold by the EVACS and analyzed by capillary GC profile analysis. (4) Two saturated solutions of bromine and iodine were allowed to react with cy-

clohexene in dichloromethane for 48 h at 25°C. After spinning band distillation, 500 ml of the product were subjected to batch extraction of chlorinated Milli-Q water for 48 h. The extract was separated, concentrated to a final volume of 0.5 ml and analyzed by capillary GC profile analysis.

## RESULTS

The effect of cyclohexene present in distilled in glass dichloromethane on the continuous liquid-liquid extraction and analysis of chlorinated drinking water was studied. Different methods to define and eliminate the cyclohexene problem were performed.

### *Extraction of chlorinated water*

Fig. 2 shows the chromatograms of the CLLE solvent blank (a), the 45-l Milli-Q water blank (b) and the 90-l chlorinated Milli-Q water blank (c). Table II lists the compounds identified by GC-MS profile analysis in the three extracts. Confirmed analysis by GC retention time data are shown by a star for most cyclohexene derivatives.

The presence of the compounds listed as impurities can be attributed to original solvent impurities or apparatus artifacts. Cyclohexene, cyclohexene oxide, cyclohexen-1-one and cyclohexen-1-ol appear in the Milli-Q water blank (Fig. 2b). The appearance of cyclohexene related compounds in the CLLE Milli-Q water extract can be attributed primarily to air oxidation of the cyclohexene during the course of the CLLE run. Even when chlorine is not present (Fig. 2a and b) chlorocyclohexanol and chlorocyclohexenes appear in the sample. It is not known how these chlorinated

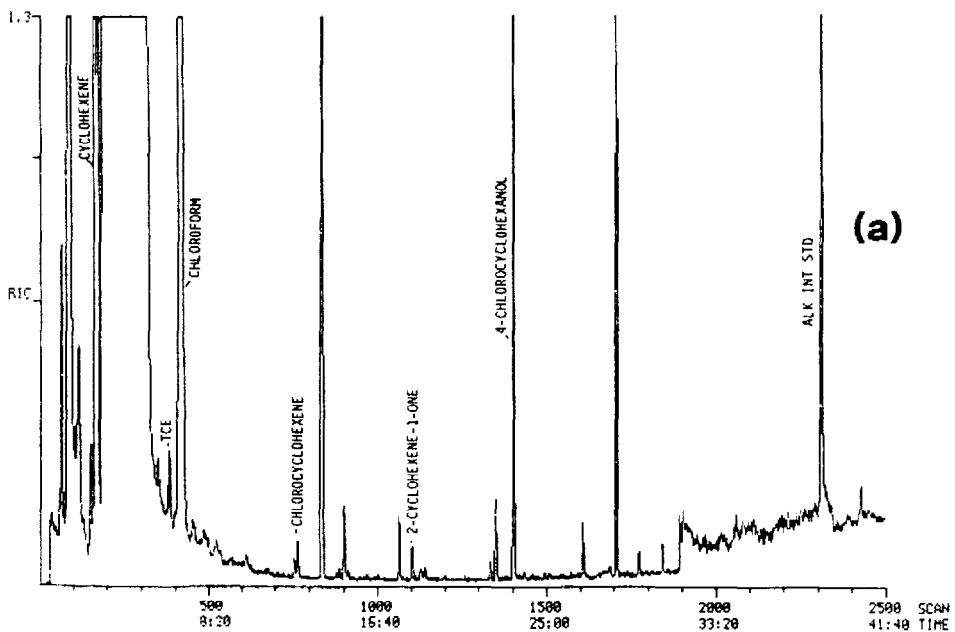


Fig. 2.

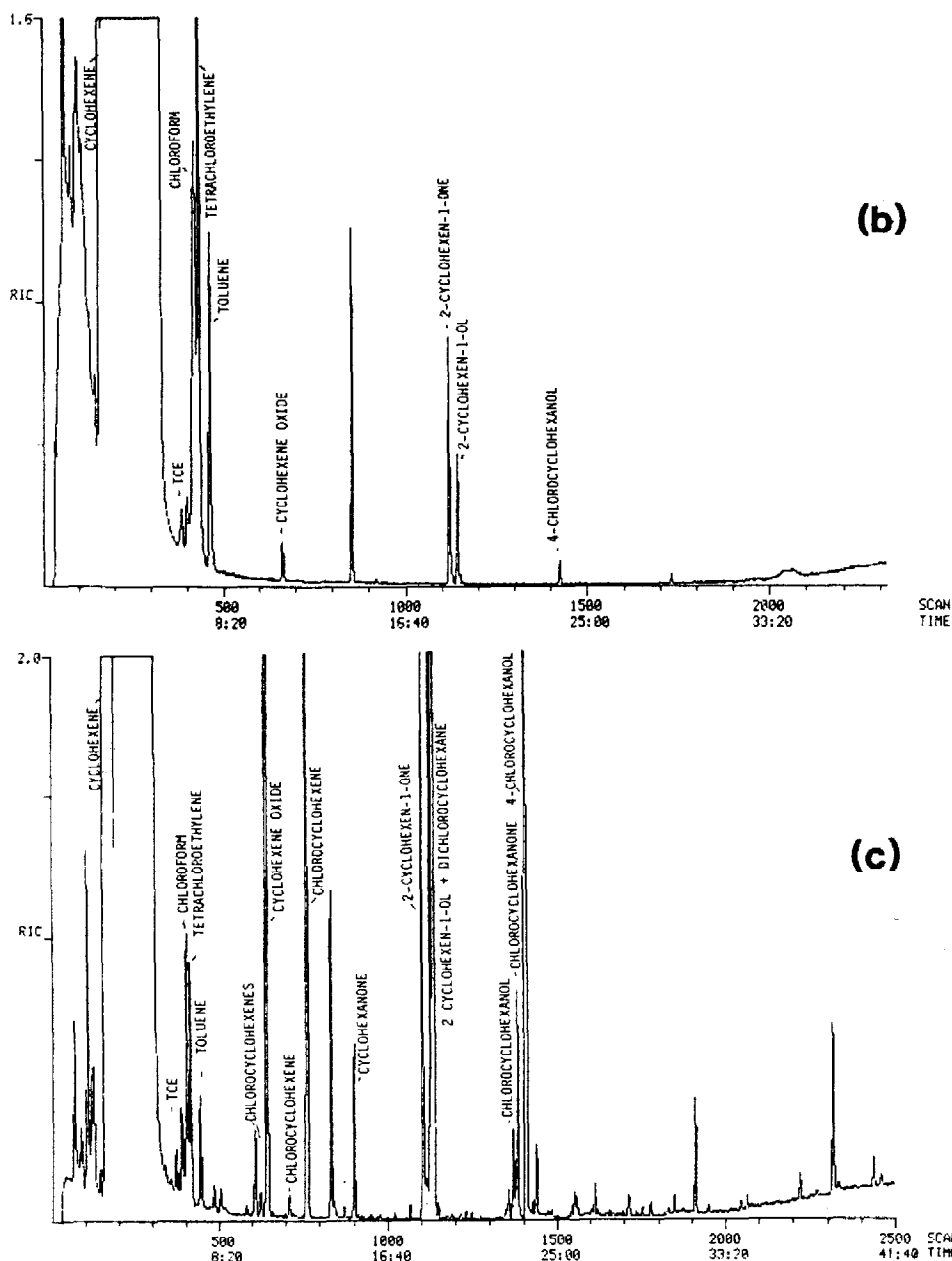


Fig. 2. Capillary GC-MS profiles on Supelcowax-10 of CLLE dichloromethane extracts of: (a) recycled dichloromethane after 2000-fold concentration, (b) Milli-Q water blank (45 l) after 1000-fold concentration, (c) chlorinated Milli-Q blank (90 l) after 2000-fold concentration. TCE = Trichloroethylene; ALK INT STD = alkane internal standard.

impurities are produced. Chlorine may be photochemically formed from dichloromethane to react with the cyclohexene and form chlorinated compounds. Only a small amount of chlorine is sufficient to initiate the reaction.

TABLE II  
COMPOUNDS IDENTIFIED IN CLLE EXTRACTS (FIG. 2)

	<i>Extract (concentration fold)</i>		
	<i>(a)</i> <i>Recycled</i> <i>solvent</i> <i>( × 2000 )</i>	<i>(b)</i> <i>Milli-Q</i> <i>water</i> <i>( × 1000 )</i>	<i>(c)</i> <i>Milli-Q</i> <i>water + chlorine</i> <i>( × 2000 )</i>
<i>Impurities</i>			
1,2-Dichloroethylene			×
Acetone	×		
1,1-Dichloroethylene		×	×
Benzene	×		
Trichloroethylene		×	×
Chloroform	×	×	×
Tetrachloroethylene		×	×
Toluene		×	×
<i>Cyclohexene-related compounds</i>			
Cyclohexane*			×
Cyclohexene*	×	×	×
Cyclohexene oxide*		×	×
Cyclohexanone*		×	×
2-Cyclohexen-1-one*	×	×	×
2-Cyclohexen-1-ol*		×	×
<i>Chlorinated cyclohexene derivatives</i>			
Chlorocyclohexene (s)*,**	×		4
Dichlorocyclohexane (s)*,**			2
Chlorocyclohexanone*			×
Chlorocyclohexanol (s)**	×	×	2

\* Identity verified with authentic compound.

\*\* Isomers.

In the presence of free chlorine residual, oxidation of the cyclohexene is enhanced as well as the chlorinated substitution products. Whereas one compound (chlorocyclohexanol) is detected in a Milli-Q water extract, nine chlorinated compounds are detected in the chlorinated sample. The most significant impurities that are shown in presence of chlorine (Fig. 2c) are listed in the order of decreasing concentration as dichlorocyclohexane, cyclohexene oxide, chlorocyclohexene, cyclohexen-1-one, cyclohexen-1-ol, chlorocyclohexanol and chlorocyclohexanone.

Kuraciose<sup>11,12</sup> reported that cyclohexene reacts with chlorine in carbon tetrachloride solution to produce 30% substitution (chlorocyclohexenes), and 70% addition products (dichlorocyclohexanes). As seen in Figs. 1 and 5, the sum of the dichlorocyclohexanes is approximately twice the sum of the substituted chlorocyclohexenes. Subsequent experiments employing both batch and continuous liquid-liquid extractions yielded essentially the same results. Fig. 6 summarizes the reactions of cyclohexene that are described in the literature. Each reaction has been marked for reference (A-H<sub>2</sub>) to produce the various oxidation and chlorination products. The reaction products that occur in the samples are listed in Table III. The reactions



TABLE III  
CYCLOHEXENE REACTION PRODUCTS IN THE SAMPLES

<i>Sample</i>	<i>Reaction</i>
CLLE solvent recycle (Fig. 2a)	H <sub>1</sub> , B, E
CLLE milli-Q water extract (Fig. 2b)	G, C <sub>2</sub> , H <sub>1</sub> , H <sub>2</sub> , A, B, E
CLLE milli-Q water + 2.7 ppm chlorine (Fig. 2c)	G, C <sub>2</sub> , H <sub>1</sub> , H <sub>2</sub> , A, B, E, F
1:1 Batch + 5 ppm chlorine (Fig. 5a)	G, C <sub>2</sub> , H <sub>1</sub> , H <sub>2</sub> , A, B, E, F
1:1 Batch + 5 ppm chlorine + ammonium chloride (Fig. 5b)	G, H <sub>1</sub> , H <sub>2</sub> , A, B, E, F

of trace materials (ppb concentration) in a solvent saturated with water has not been studied except in cases like this where artifacts disappear.

#### *Removal of cyclohexene and its artifacts*

Attempts to remove the cyclohexene from the dichloromethane showed the following results. (1) Spinning band distillation and a 1000-fold concentration showed only cyclohexene and one small peak. After chlorination, spinning band distillation and a 1000-fold concentration all the chlorinated and oxidative cyclohexene products were produced. This indicates that there was an appreciable concentration of cyclohexene (b.p. 83°C) still remaining in the dichloromethane (b.p. 39.75°C) after the spinning band distillation. (2) After oxidation of dichloromethane by potassium permanganate, spinning band distillation and a 1000-fold concentration, all the chlorinated and oxidative cyclohexene products were again produced upon chlorination. This means that potassium permanganate is not a suitable oxidizing agent for complete removal of the cyclohexene in dichloromethane. The presence of K<sup>+</sup> in solution apparently decreases the reaction rate. Complexation of the K<sup>+</sup> in solution, e.g., crown ether, can help the oxidation process to some extent, however yields are seldom better than 50%<sup>13</sup>. (3) The cyclohexene artifacts were produced after using the azeotropic mixture of dichloromethane and freon (1:1) for extraction of chlorinated Milli-Q water. (4) Soaking the dichloromethane in pre-washed alumina also failed to completely eliminate the cyclohexene preservative. (5) The addition of I<sub>2</sub> or Br<sub>2</sub> to the solvent before spinning band distillation did not help to remove the cyclohexene.

#### *Effect of chloramination on cyclohexene derivatives*

Figs. 3 and 4 show the GC-MS chromatograms of the Milli-Q water 3-min batch extraction before and after treatment with chlorine and chloramine. Two GC columns were used: polar Supelcowax-10 (Fig. 3) and non-polar DB-5 (Fig. 4). Table IV lists the compounds found in the Milli-Q water extracts, with chlorination and chloramination.

The CLLE Milli-Q water blank (Table II) shows a number of cyclohexene compounds, chlorinated compounds and some impurities including benzaldehyde. Curiously, even though there is no chlorine present, low concentrations of two chlorine reaction products of cyclohexene are present. Benzaldehyde disappeared after chlorination or chloramination and thus may be a low level contaminant in the Milli-Q water.

The impurities present in dichloromethane show cyclohexene and cyclohexene

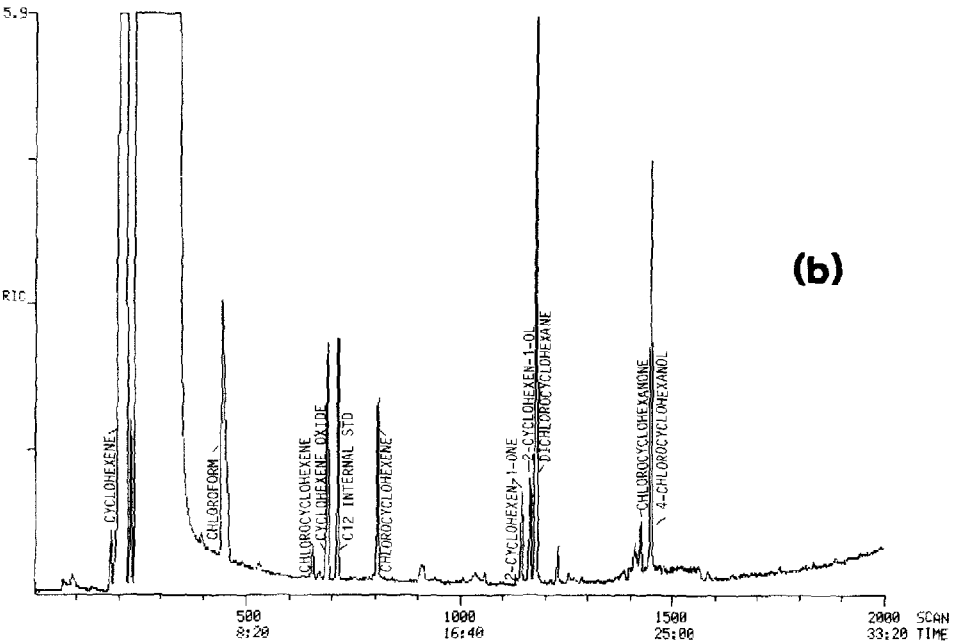
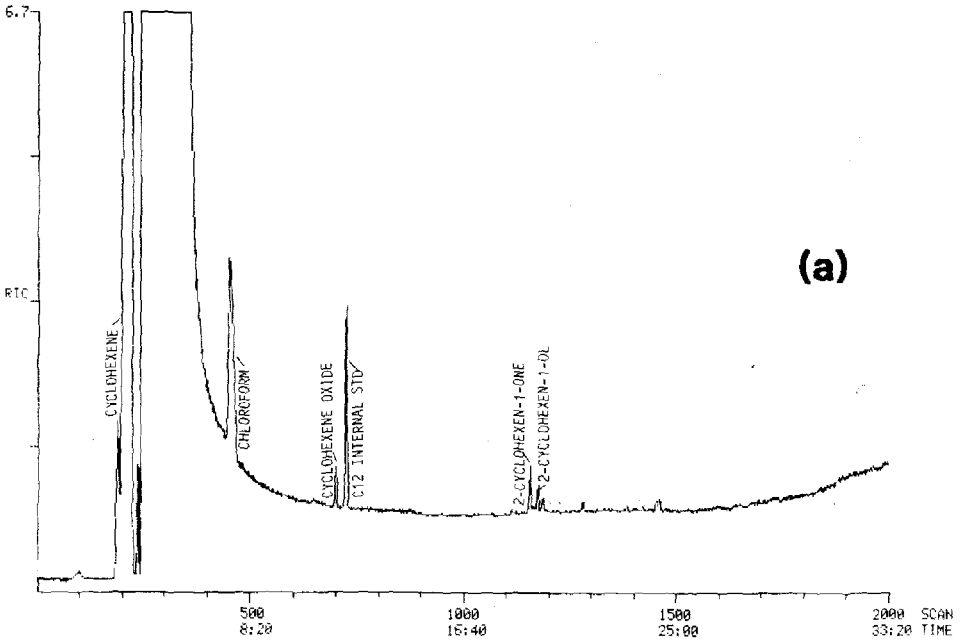


Fig. 3.

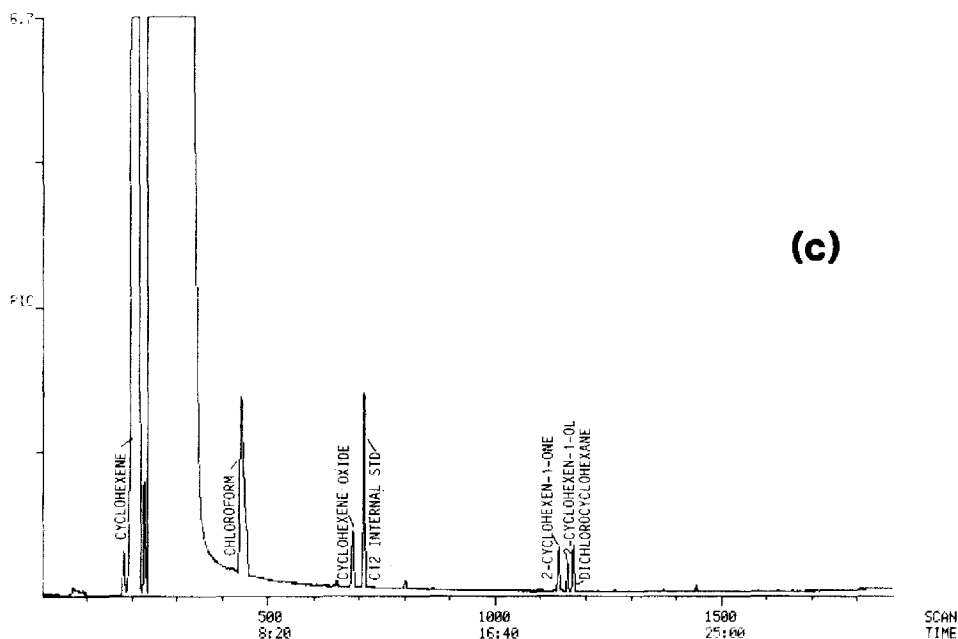


Fig. 3. Capillary GC-MS profiles on Supelcowax-10 column of 1:1 batch extractions with dichloromethane (3 min contact time) and 1000-fold concentration of: (a) Milli-Q water; (b) chlorinated Milli-Q water; (c) chloraminated Milli-Q water.

artifacts. When chlorine is added, a multitude of intense chlorine-cyclohexene products are formed (Table II). The intensity and number clearly shows that chlorine should not come into contact with the cyclohexene preservative in the dichloromethane.

When chloramines are used instead of chlorine, cyclohexene artifacts are greatly reduced to near the background level. Figs. 3c and 4c show GC-MS chromatograms of the chloraminated samples. In fact, on Supelcowax-10, the chloramination (Fig. 3c) looks almost identical to the original solvent (Fig. 3a). The only compounds which increased in concentration are 3-chlorocyclohexene and 1,3-dichlorocyclohexane. The chromatograms show numerous cyclohexene oxidation products (ketones, alcohols). A comparison of the two chromatograms (Figs. 3 and 4) shows that chloramination inhibits the formation of chlorination and oxidation products of cyclohexene.

Fig. 5 shows GC-MS chromatograms from 1:1 batch extraction of 500 ml of Milli-Q water (chlorinated and chloraminated) concentrated to 1 ml after 22 h contact time. As in CLLE extracts, several compounds are substantially reduced in the chloraminated sample. Comparison of Figs. 3a and 5a shows that the extended contact time between dichloromethane chlorinated Milli-Q water resulted in increase of concentration of all of the reaction products but no new compounds. Thus the reaction pathways listed in Fig. 6 occur almost instantaneously.

Table V lists the percent reduction in concentration of some of the reaction products based on total peak areas after chloramination. As confirmation of these findings, Fig. 7 shows a CLLE concentrate of 45 l chloraminated drinking water sample (22.5 h contact time) exhibiting fewer cyclohexene artifacts.

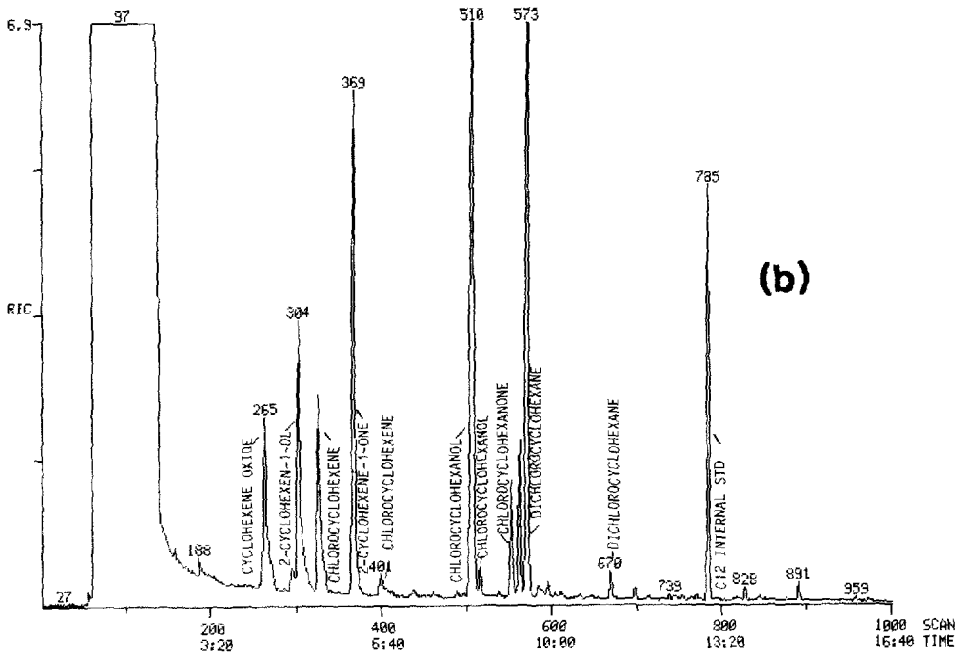
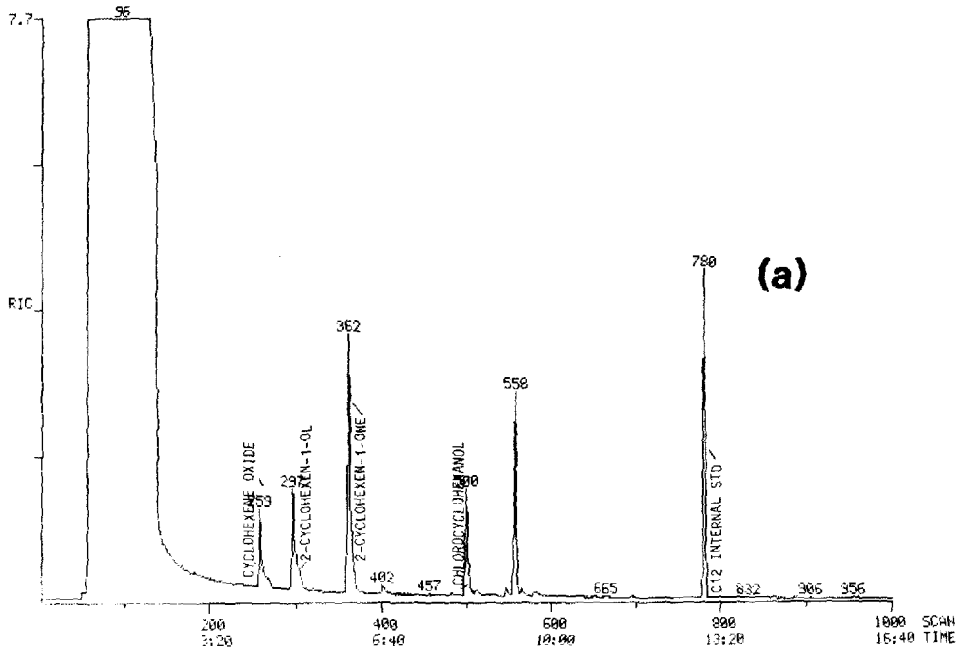


Fig. 4.

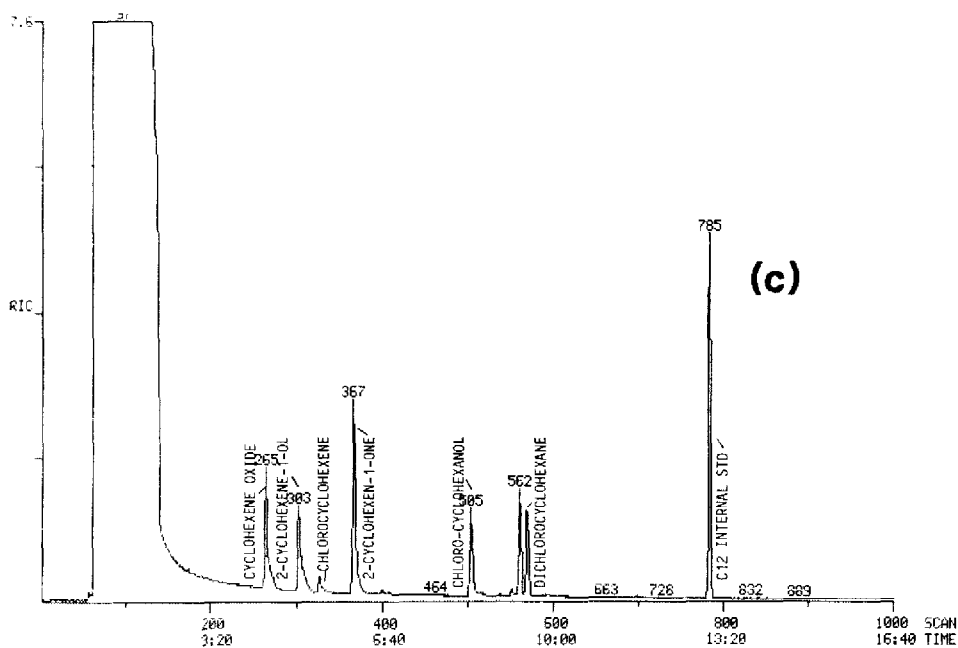


Fig. 4. Capillary GC-MS profiles on DB-5 column of 1:1 batch extractions with dichloromethane (3 min contact time) and 1000-fold concentration of: (a) Milli-Q water; (b) chlorinated Milli-Q water; (c) chloraminated Milli-Q water.

TABLE IV

GC-MS ANALYSIS OF SAMPLES FOR CHLORINE/CHLORAMINE EFFECT

Columns: 1, Supelcowax-10 (Fig. 3); 2, DB-5 (Fig. 4).

	<i>Milli-Q</i> <i>water</i>		<i>Milli-Q</i> + <i>chlorine</i>		<i>Milli-Q</i> + <i>chloramine</i>	
	1	2	1	2	1	2
<i>Cyclohexene-related compounds</i>						
Cyclohexene	×	×*	×	×*	×	×*
Cyclohexene oxide	×	×	×	×	×	×
2-Cyclohexen-1-one	×	×	×	×	×	×
2-Cyclohexen-1-ol	×	×	×	×	×	×
4-Chlorocyclohexanol		×		×		×
Dichlorocyclohexane (s)		×		2		×
Chlorocyclohexene (s)			2	2		×
2-Chlorocyclohexanone			×	×		
Bi-2-cyclohexen-1-yl						×
<i>Impurities</i>						
1,2-Dichloroethylene	×		×		×	
Chloroform	×		×		×	
Benzaldehyde	×	×				
Heptyl hydroperoxide	×	×	×	×	×	×
Hexane			×			
2,5-Hexanedione			×			
Diethyl phthalate			×			

\* Under solvent peak at scan 117 on DB-5.

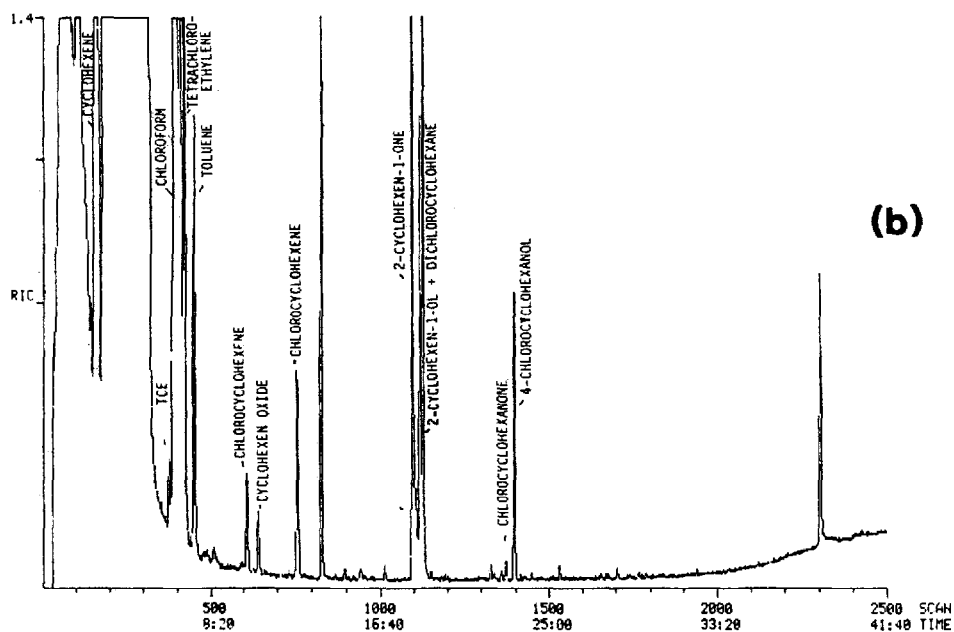
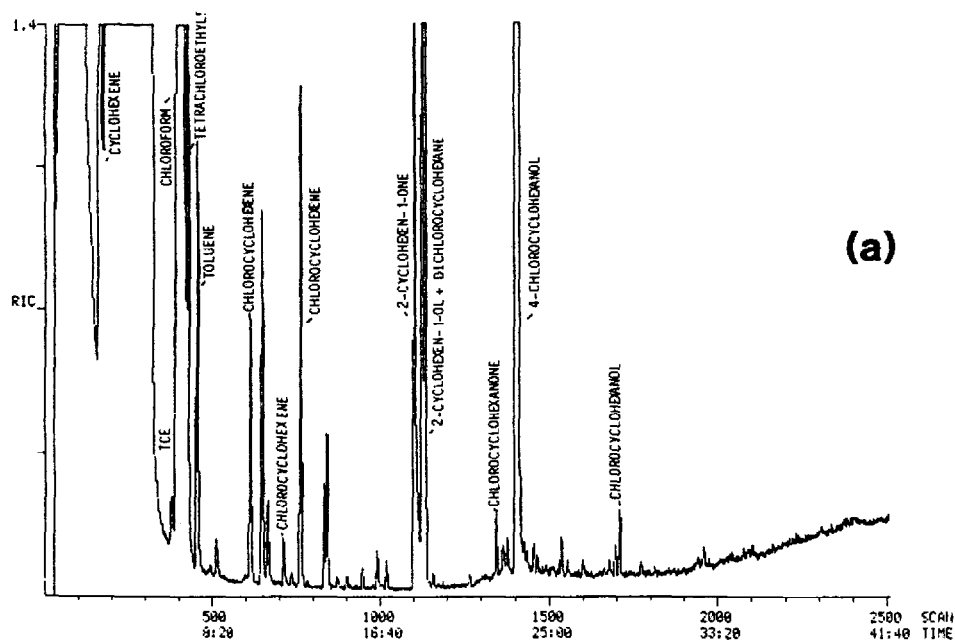


Fig. 5. Capillary GC-MS profiles on DB-5 column of 1:1 batch extractions with dichloromethane (22 h contact time) and 1000-fold concentration of: (a) chlorinated Milli-Q water; (b) chloraminated Milli-Q water.

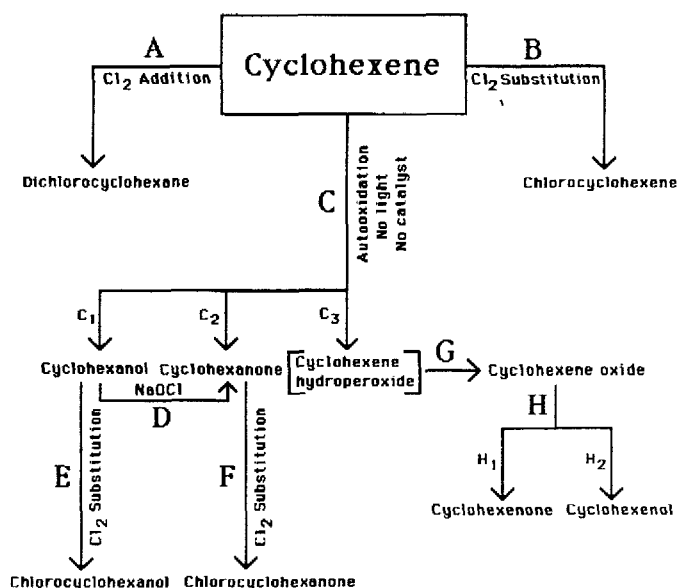


Fig. 6. The chemical reactions of chlorine and cyclohexene.

## DISCUSSION

Chlorine substitution, addition and oxidation products of cyclohexene have been detected by GC and GC-MS in dichloromethane extracts of chlorinated drinking water samples. These compounds come from the cyclohexene that is used as a preservative in commercially available dichloromethane to inhibit the formation of free radicals which produces degradation products such as phosgene and hydrogen chloride. Solvent decomposition is caused by heat, water, air or metals<sup>14</sup>. Even when 20 ppm amylene are added to dichloromethane as a preservative, phosgene appears at the 100 ng/ml level in 15 days exposure to sunlight<sup>15</sup>. The presence of chlorinated

TABLE V

EFFECT OF CHLORAMINATION ON CONCENTRATION OF CYCLOHEXENE-RELATED COMPOUNDS

Compound	Reduction (%)
1-Chlorocyclohexene	63
Cyclohexene oxide	83
3-Chlorocyclohexene	100
Chlorocyclohexene	56
2-Cyclohexen-1-one	39
2-Cyclohexen-1-ol	56
Dichlorocyclohexane	75
2-Chlorocyclohexanone	50
4-Chlorocyclohexanol	90
Chlorocyclohexanol	100

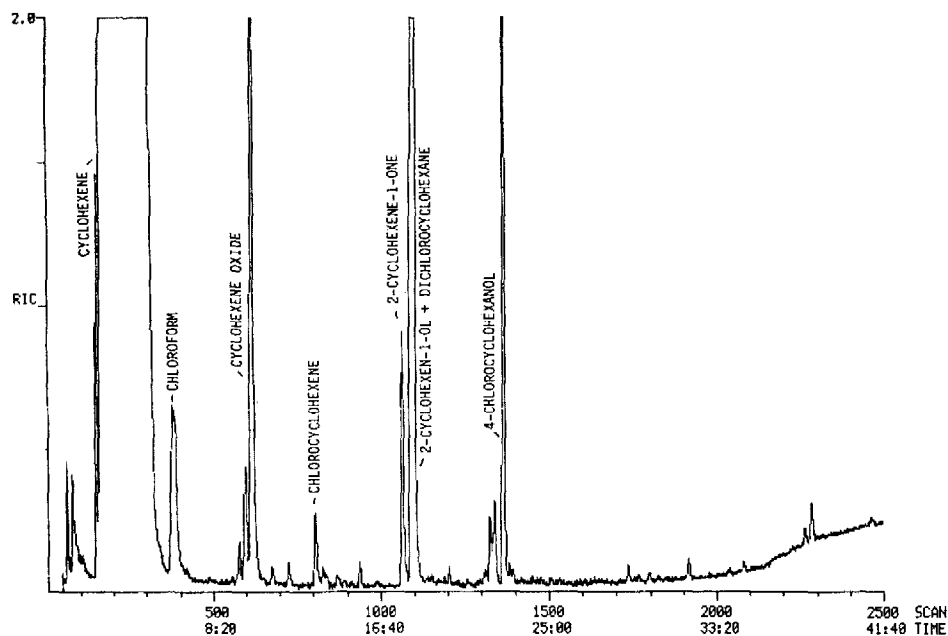


Fig. 7. A capillary GC-MS profile on Supelcowax-10 of a CLLE dichloromethane extract of 45 l of chloraminated drinking water sample.

and oxidized cyclohexene artifacts limits broad spectrum quantitative and qualitative analysis of compounds eluting at Kovát's indices between 700 and 1000 on a non-polar DB-1 column. It also limits the use of these extracts in toxicological testing. The data presented indicate:

- (1) Heat and refluxing does not, by itself, cause oxidation. The CLLE solvent blank does not produce the oxidation reaction sequence G, H<sub>1</sub>, H<sub>2</sub>.
- (2) The solvent alone has produced chlorocyclohexanol in all of the samples but the mechanism of how this occurs is not known.
- (3) Over ten cyclohexene derivatives were produced by substitution and addition reactions when free chlorine residual was present in the water sample.
- (4) Complete removal of the cyclohexene (b.p. 83°C) from dichloromethane (b.p. 39.75°C) cannot be done by spinning band distillation (30 plates), adsorption to alumina, oxidation by potassium permanganate or addition of bromine and iodine.
- (5) Chloramination inhibits the formation of cyclohexene artifacts to varying degrees depending on the contact time between the water sample and the dichloromethane. This appears to be the best approach to date.

The above results confirm that oxidation and chlorination products of cyclohexene are formed during liquid-liquid extraction of chlorinated water samples with dichloromethane. Interference problems with these reaction products are greatest when employing high sensitivity (ng/l) methods such as CLLE where 2000-fold concentration was utilized. The conditions for greatest sensitivity require large sample volume, extended sample-solvent contact times and large evaporative concentration steps. These conditions are most conducive to the formation of cyclohexene-related



artifacts. While chloramination minimizes the problem somewhat, it is clearly not a final solution. An optimum solution would be an identical product preserved with a compound that does not produce chlorination artifacts.

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