Stellingen behorende bij het proefschrift

Chemical Aspects of Drinking Water Chlorination

van

Ruud Peters

1. Bromide komt in vele soorten water in lage hoeveelheden voor. Chlorering van dat water resulteert in de vorming van vele gebromeerde nevenproducten. Ondanks het feit dat deze verbindingen verantwoordelijk zijn voor een groot deel van de mutageniteit van gechloord drinkwater, is er slechts weinig onderzoek naar gedaan; verder onderzoek is dan ook gewenst.

   (Dit proefschrift, hoofdstuk 7; M. Fielding and H.Horth, Water Supply 103, 4, 1986.)

2. De bepaling van trichloorazijnzuur in drinkwater op basis van de thermische decarboxylatie tot trichloormethaan (chloroform) leidt tot systematisch te hoge uitkomsten, doordat ook vele andere verbindingen met -$COCCl_3$ groepen onder die omstandigheden chloroform zullen afsplitsen.

   (B.B. Hoogcarspel, Jaarboek 1989 Sectie Milieuchemie KNCV, 58, 1989.)

3. Aan de ene kant wordt het door de toenemende vervuiling van grondwater en oppervlaktewater steeds moeilijker om met de huidige middelen drinkwater van een onbesproken kwaliteit te produceren. Aan de andere kant wordt er in Nederland bij allerlei activiteiten veel te veel drinkwater verbruikt. Een verhoging van de prijs van drinkwater levert voor beide problemen de oplossing.

4. Het melden van overschrijdingen van normen in het milieu zonder verdere toelichting, zoals in de media regelmatig gebeurt (pesticiden in drinkwater, dioxines in melk), leidt bij het publiek slechts tot onbegrip en verontrusting.

5. Het is nog maar de vraag of het milieu gebaat is bij "groene" wasmiddelen, "groene" benzine en "groene" HPLC-kolommen.

6. Voor pesticiden is door de overheid een algemene norm gesteld van 0,1 μg/L. Door de toepassing van chloor bij de drinkwaterbereiding, maar ook bij de behandeling van afvalwater, koelwater en in industriële processen, zal voor een aantal stoffen (o.a. trichloorazijnzuur) niet aan deze norm kunnen worden voldaan.

   (Waterleidingbesluit 1984.)

7. Er bestaat op dit moment nog geen gevoelige en specifieke detectiemethode voor gehalogeneerde organische verbindingen. GC/MS met NCI/SIM (negative chemical ionisation/selected ion monitoring) en GC/MIP/MS (microwave induced plasma) zijn hiervoor twee interessante mogelijkheden.

8. Voor de verwijdering van zwavel- en stikstofoxyden in rookgassen wordt o.a. de injectie van poreuze siliciumdioxyde bolletjes, met daarop aangebracht koperoxyde, bestudeerd. De toepassing van dit proces bij vuilverbranders zou echter wel eens een toename van de productie van dioxines tot gevolg kunnen hebben.

   (J. Libbenga, Intermediair 51, 26, 1990.)

9. Er bestaat nog de nodige onduidelijkheid over de belangrijkste bijdragen aan het broeikaseffect. Dit blijkt onder meer uit het ontbreken van de CO_2-bijdrage van mens en dier in de diverse taartdiagrammen.

   (Chemisch Magazine, 22, 1, 1991.)

10. Wie met de auto in de file staat heeft tenminste nog een zitplaats !

Chemical Aspects of Drinking Water Chlorination

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PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Technische Universiteit Delft, op gezag van de Rector Magnificus, Prof. Drs. P.A. Schenck, in het openbaar te verdedigen ten overstaan van een commissie aangewezen door het College van Dekanen op donderdag 14 februari 1991 te 16.00 uur

door

Rudolphus Johannes Bernhardus Peters

geboren te Ootmarsum,

Doctorandus in de Chemie.

Dit proefschrift is goedgekeurd door de promotor

Prof. Dr. L. de Galan

Voor Hannie

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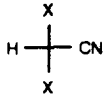
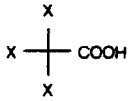
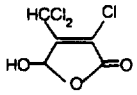
Chemical Aspects of Drinking Water Chlorination

1.1 Introduction

Although some crude water purification techniques were used as early as 2000 B.C., the recognition that drinking water needs to be treated and protected from bacterial contaminations did not occur until the nineteenth century. The first large scale treatment of drinking water was slow sand filtration, and was introduced in England in 1829. In 1902 another important practice in water treatment, drinking water chlorination, was introduced in Middelkerke in Belgium. Chlorine destroys most micro-organisms that are responsible for waterborne diseases, and the disinfection of drinking water with chlorine caused a dramatic decrease of those diseases. Due to the success of this technique, chlorination became the predominant method of drinking water disinfection (1). Besides in drinking water treatment, chlorine is also used in wastewater treatment, cooling water treatment and industrial processes as food treatment and pulp bleaching. Alternative disinfectants like ozone, chlorine dioxide and chloramine, are also used (2). However, chlorine has many advantages as it is effective against many micro-organisms, inexpensive, relatively convenient to use, and leaves a disinfection residual to the water (3).

In the Netherlands about two thirds of the drinking water originates from ground water and one third from surface water, mainly from the rivers Rhine and Meuse (4). While surface water is treated by a series of methods which include chlorine disinfection, groundwater treatment is simple, and the endproduct is chlorinated only exceptionally. Traditionally, chlorine was used in different stages of the production process, but since the early 1970's

Table 1. Some important chlorination by-products.

Chlorination By-Product	Typical Representative [1]	Concentration in Drinking Water in ug/L	Contribution to Total Mutagenicity in %	See also Chapter
Trihalomethanes		10 - 50 [2]	1 - 4	3
Dihaloacetonitriles		0.5 - 1.0 [2]	1 - 4	3
Haloacetic Acids		1 - 15 [2]	0 - 1	5
Halogenated Furanones	 [3]	0.01 - 0.05	20 - 50	6, 7

1; X = Cl or Br 2; Concentrations in Dutch drinking water 3; Brominated analogues may also be present and contribute significantly to the total mutagenicity of chlorinated drinking water.

its use has been gradually reduced. Nowadays chlorine is used by the water treatment plants generally for transport, and safety postchlorination.

In spite of the benefits of the use of chlorine as a disinfectant, problems are caused by its high reactivity. Chlorine reacts readily with organic materials present in water, resulting in the formation of many halogenated compounds (5-8). This problem was first reported in 1974 by Rook (9), and by Bellar and Lichtenberg (10) who independently found that chloroform and other trihalomethanes were produced during chlorine disinfection of drinking water. The presence of these compounds could be attributed to the reactions between chlorine and humic materials. Since then many other volatile and non-volatile chlorination by-products have been identified.

Chlorination also leads to an increased mutagenic activity of the finished drinking water (11). The major part of this mutagenic activity is found in the non-volatile fraction, showing that other potentially more harmful chlorination products are formed in addition to the trihalomethanes. Some important classes of chlorination products are listed in table I. Notice that the major part of the mutagenicity is associated with compounds present at very low concentrations. The detection of mutagenicity in drinking water merely indicates a potential health risk, and it is not possible to quantify the risks on the basis of such tests. To estimate the risks for the consumer, further toxicological and epidemiological studies are needed. However, such studies are difficult, and it would be of great help if the identity of the mutagenic compounds were known.

1.2 The Chemistry of Chlorination

Chlorine can be introduced into water in the form of chlorine gas or in the form of a sodium or calcium hypochlorite solution. Chlorine hydrolyses very rapidly in water according to the following reaction;

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^-$$

Under practical treatment plant conditions this hydrolysis is essentially complete. Hypochlorous acid is a weak acid and dissociates partly to hypochlorite ion;

$$HOCl \rightleftharpoons H^{+} + OCl^{-}$$

This equilibrium is controlled by the pH, and at pH 7.5 and 25^{0}C, the concentrations of hypochlorous acid and hypochlorite ion are about equal. However, since the specific reactivity of HOCl is about 10000 times higher than that of OCl-, HOCl is the most important species in oxidation and chlorination reactions of organic compounds (12,13).

The reactions of chlorine with organic materials in natural waters are extremely complex. In general, organic compounds of natural origin (loosely referred to as humic materials) are responsible for most of the observed reactions of chlorine with surface waters. Chlorine reacts with organic compounds in three different ways, namely through oxidation, addition and substitution (14). Oxidation is the predominant reaction and 50-80% of the added chlorine is consumed in this type of reactions (15). Chlorination of organic compounds takes place only through addition and substitution reactions.

The nature and extent of the reactions of organic substrates with chlorine in aqueous solutions is controlled by several factors, in particularly the type of precursor, the pH and the chlorine-to-carbon ratio. The chlorination products fall into two general categories: volatile hydrophobic and non-volatile hydrophilic compounds. Several studies have shown that the volatile halogenated organics, e.g. trihalomethanes, represent only a minor part of the total halo-organics (16,17). The non-volatile compounds, that contain the major part of the organically bound chlorine, are more difficult to identify then the volatile compounds since they are more polar and are present as a complex mixture of individual compounds in low concentrations.

The type and relative amounts of the chlorination products that are formed, varies not only with the organic content of the source water but also with the inorganic species present. Bromide is often present, either from natural or from anthropogenic sources, and in many surface waters the bromide concentration may be as high as 0.5 mg/L (18). During chlorination, bromide ion is oxidized by chlorine to bromine, which hydrolyses to hypobromous

acid (19).

$$HOCl + Br^- \longrightarrow HOBr + Cl^-$$

Subsequently, chlorination and bromination become competitive reactions. Bromine seems to be more effective as a halogen-substituting agent than chlorine, and if bromine acts as an oxidant, it will be reduced to bromide ion, which may then be reoxidized by chlorine (20). This results in a high bromine incorporation and the presence of brominated trihalomethanes in chlorinated water is well known.

If iodide is present in water this is also oxidized by chlorine, resulting in the production of iodinated trihalomethanes (21). However, since the iodide ion concentrations are generally below 0.05 mg/L, the concentrations of iodinated trihalomethanes in chlorinated water are low.

1.3 The Precursors of Chlorination By-Products

Many types of organic compounds are present in natural waters. The amount of organic material in the water is represented by the DOC, dissolved organic carbon, of the water. The DOC varies with the type of water

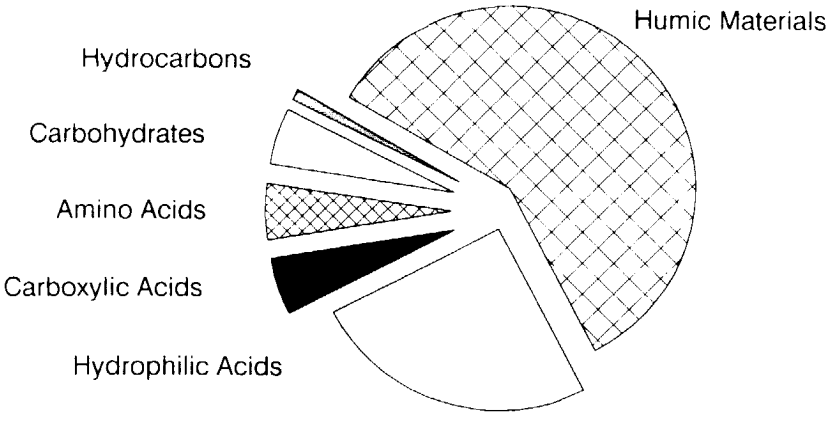


Fig. 1. The DOC of an average river water.

from approximately 0.5 mg/L for seawater to over 30 mg/L for colored water from swamps or wetlands. The DOC of rivers mostly lies in the range of 2 to 8 mg/L. The dominant natural organic compounds in water are aquatic humic and fulvic acids, which comprise 50 to 90% of the DOC (22). Other fractions of the DOC comprise hydrophilic acids (up to 30%), carboxylic acids (ca. 5%), amino acids (ca. 5%), carbohydrates (ca. 5%) and hydrocarbons (less than 1%). The composition of the DOC of an average river water is given in figure 1. Proteins are not mentioned but may form a part of the DOC, especially during summer months of high algae growth.
The reactivity of carbohydrates, carboxylic acids and hydrocarbons towards chlorine is low, and they are not expected to contribute to the production of organochlorine compounds. The important precursors of chlorination by-products will be discussed in more detail below.

Humic acids. Almost from the first report of trihalomethanes in finished drinking water, the presence of this group of compounds has been attributed to reactions between chlorine and humic materials (5). Humic materials are complex polymers of lignines, carbohydrates, proteins and fatty acids. They originate from plant and other organic material in soil systems, and are leached by water of soil into rivers and streams. Generally, humic materials have an elemental composition that is 45-55% carbon, 4-5% hydrogen, 35-40% oxygen and 1-3% nitrogen. They are operationally defined as colored, poly-electrolytic acids isolated from soil or water. Although the structure of humic substances is not clear, they are thought to comprise a mixture of large molecules containing phenolic components (23-25).

Christman and others studied the chlorination of humic and fulvic acid and found that the dominant chlorinated products were chloroform and chlorinated aliphatic acids, especially dichloro-, and trichloroacetic acid (6,8,26-28). Non-chlorinated products that were identified include a large number of monobasic fatty acids and aromatic acids. De Leer et al. chlorinated humic acid extracted from peat soil at different chlorine-to-carbon ratios, and identified more than 100 compounds (28). In general their results agreed well with earlier findings, but some new classes of compounds were also identified. These include cyanoalkanoic acids, chlorinated aromatic carboxylic acids and several chloroform precursors. The latter are structures with a trichloromethyl group which is easily hydrolysed to chloroform, and were found predominantly in the low chlorine dose experiments. The most important chlorination products of humic material are given in table II.

Table II. Important chlorination products of humic materials

Volatile products:	$CHCl_3$, CCl_3CHO, $CHCl_2CN$	
Non-volatile products:		
Chlorinated monobasic acids	$CHCl_2$-CO_2H, CCl_3-CO_2H, CH_3-CCl_2-CO_2H, CCl_2=CCl-CO_2H	
Chlorinated dibasic acids	HO_2C-CCl_2-CO_2H, HO_2C-$CHCl$-CH_2-CO_2H, HO_2C-CCl_2-CH_2-CO_2H, HO_2C-CCl=CH-CO_2H, HO_2C-CCl=CCl-CO_2H	
Chlorinated tribasic acids	HO_2C-CCl=$C(CO_2H)_2$	
Chloroform precursors	CCl_3-CO-CCl=$C(CO_2H)_2$, CCl_3-$CHOH$-CCl_2-$CHCl$-CO_2H	
Cyanoalkanoic acids	NC-(CH_2) -CO_2H	n = 1-3
Alkanoic acids	CH_3-(CH_2) -CO_2H	n = 7-25
Alkanedioic acids	HO_2C-(CH_2) -CO_2H	n = 0-8
Benzenecarboxylic acids	Phenyl-(CO_2H)	n = 1-6

Several studies show that phenolic structures, and in particular 1,3-dihydroxybenzenes, are good precursors for chloroform and other products (5,29-31). Based on a hypothesis of Moye (32), Rook proposed a ring rupture mechanism of resorcinol structures to explain the production of chloroform (5). This mechanism, that requires a decrease in the aromatic content of humic material after chlorination, was supported by several studies. Christman et al. demonstrated that phenolic structures are present in aquatic fulvic acid (33,34), and De Leer et al. showed a reduction of the total aromatic content of humic materials after chlorination (28). Furthermore, the chlorination of different phenolic model compounds showed that the ring rupture mechanism could explain the production of several chlorination products. Boyce and Hornig studied the chloroform production of several 1,3-dihydroxyaromatic compounds (31). With isotope labeling, they demonstrated that the C_2 position of resorcinol is responsible for chloroform generation, thus supporting the ring rupture mechanism. This mechanism, and the formation of some important chlorination products is given in figure 2.

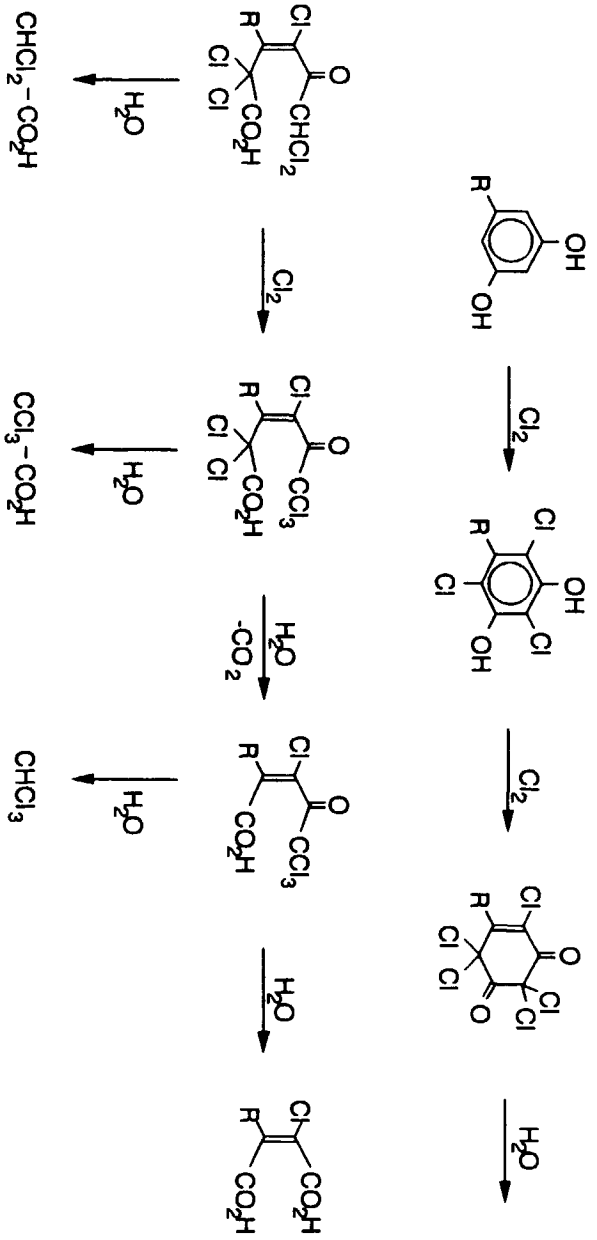
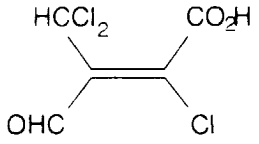
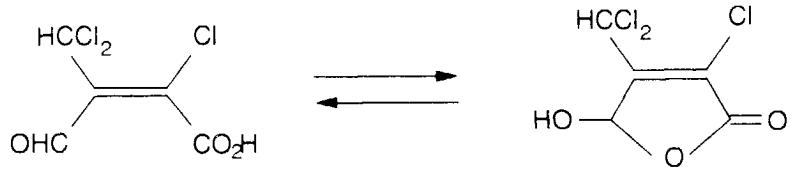


Fig. 2. The ring rupture mechanism and some well known chlorination products.

A highly potent mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, often referred to as MX, was identified by Holmbom et al. in chlorinated kraft pulp waste (35,36). Depending on the pH, MX exists in an open and a closed form, as shown in figure 3. MX, and its geometric isomer EMX, are also produced at low concentrations (typically 10 ng/L) during the chlorination of humic and drinking water (37,38). Kronberg and Meier identified this compound in finished drinking water and showed that it was a major contributer to the mutagenic activity of chlorinated drinking water (39-41). Recently we found that 3,5-dihydroxybenzaldehyde may explain the formation of MX and EMX, and this is described in more detail in chapter 6 (42). If bromide is present during chlorination then brominated analogs of MX and EMX are formed also (43), (see chapter 7).



EMX



MX, open and closed form

Fig. 3. The structures of MX and EMX.

Amino acids. Amino acids are common constituents of raw water (44). They may be free, proteinaceous or humic bound amino acids. The general reaction of amino acids with chlorine has been known for many years (45-47). The studies showed that an equimolar amount of chlorine resulted in the formation of an aldehyde. However, if an excess of chlorine was added, the corresponding nitrile could also be formed. The reaction involves a chlorination of the amino group, giving mono- and dichloroamine, which after decarboxylation reacts further to give the aldehyde or nitrile, respectively (48). The reactions are shown in figure 4. Although the intermediate imines

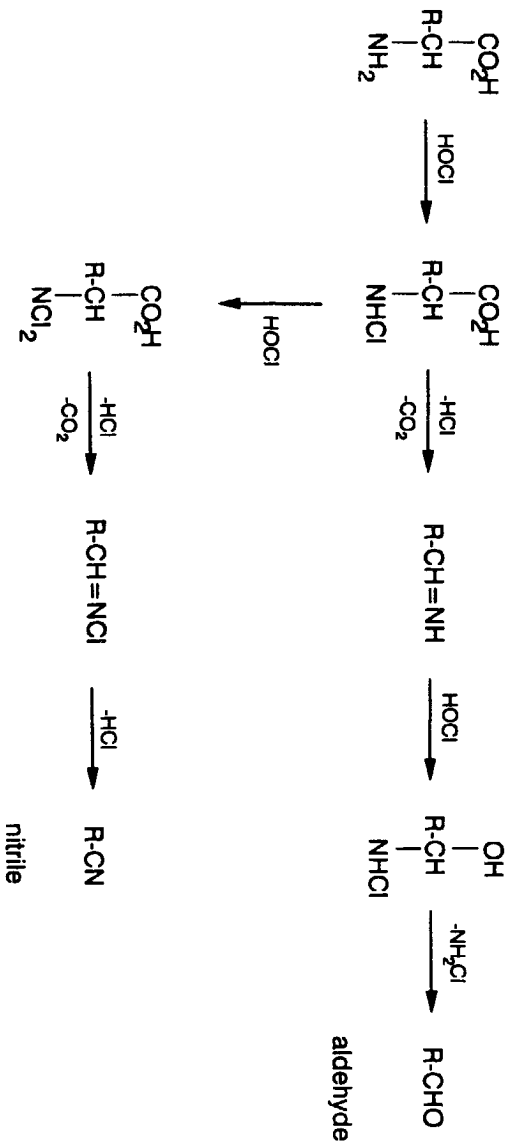


Fig. 4. The aqueous chlorination of amino acids.

are normally not isolated after the chlorination of amino acids, chloroimines were identified by Pereira et al, who studied the chlorination of alanyl-leucine, a dipeptide (49).

That humic acids may have amino acids associated with them, was indicated by the identification of 3-cyanopropanoic acid (CPA) and 4-cyanobutanoic acid (CBA) as chlorination products of humic acid (28). De Leer et al. suggested the amino acids glutamic acid and lysine as possible precursors, since CPA and CBA were found to be formed on chlorination of these amino acids (50). In a similar way cyanoacetic acid (CEA) may be formed by the chlorination of aspartic acid. However, CEA is very reactive towards chlorine and is therefore normally not isolated (51), (see chapter 2).

The major reaction products of amino acids with chlorine are oxidation by-products. However, when the amino acid contains an additional activating group near the amino acid functional group, then substitution reactions may occur (52). The presence of a second carboxylic group in aspartic acid ($R = CH_2CO_2H$, in figure 4) increases the reactivity of the beta hydrogens to chlorination, resulting in the production of dichloroacetonitrile and chloral. Especially the presence of dihaloacetonitriles in chlorinated drinking water strongly implies a significant role of amino acids or proteinaceous material in the formation of chlorination by-products (53-55), (see chapter 3). Amino acids also contribute to the production of chloroform, probably through chloral as an intermediate (56). The aqueous clorination of tyrosine produces not only chloroform, dichloroacetonitrile and chloral, but also mono- and dichloro-4-hydroxyphenylacetonitrile, indicating that chlorination of the aromatic ring occurs (49,56). It is also of interest that Horth et al., who studied the chlorination of several amino acids, identified MX and EMX, as chlorination products of tyrosine (57,58).

Proteins. The reactivity of proteins towards chlorine is considered low, since the amide nitrogen bond of dipeptides was found to be resistant to aqueous chlorination at room temperature (49). Dipeptides reacted only at the N-terminal amino group resulting in the corresponding N,N-dichlorodipeptides. Therefore the chlorination of proteins has received relatively little attention. However, Heltz et al. demonstrated that chlorination of natural waters resulted in the loss of proteinaceous material (52). Chlorination takes place at an amino group and may lead to chain fission or a stepwise degradation of the polypeptide, resulting in the production of

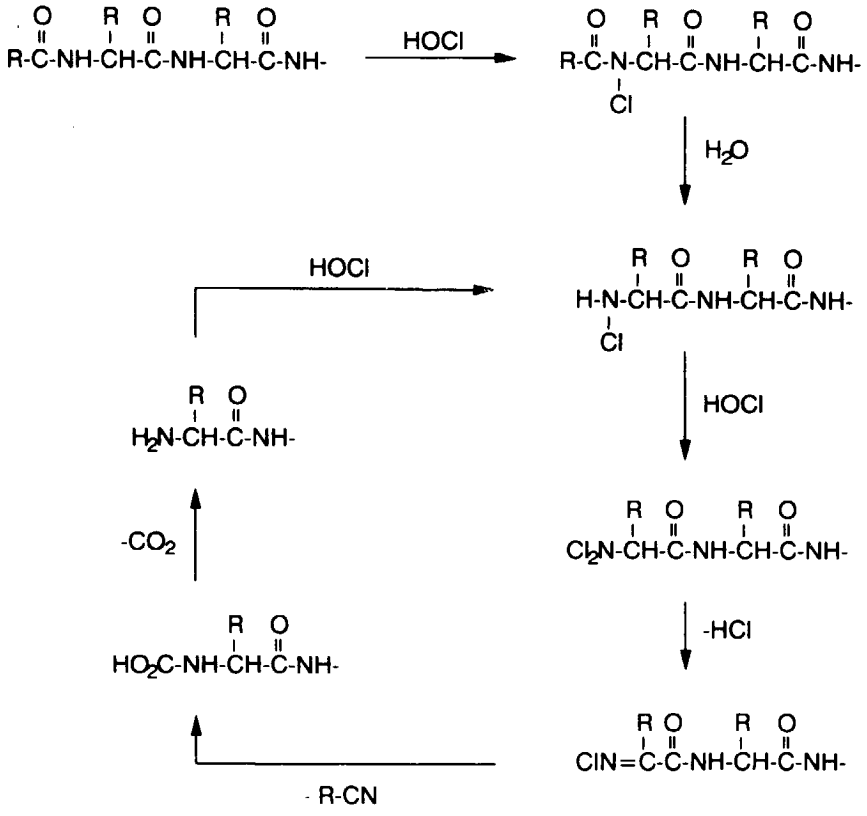


Fig. 5. The stepwise degradation of proteins during chlorination.

nitriles and smaller peptide fragments. The proposed mechanism of the degradation of proteins is shown in the scheme in figure 5. Scully et al. showed that chlorination of proteins in natural waters leads to the production of trihalomethanes (59,60). Seasonal variations in the trihalomethane concentrations in finished waters have been also linked to algal blooms (61,62), since a large part of algae consist of proteins. The studies also showed that chlorination of algae produced trihalomethanes, dihaloacetonitriles, and a total organic chloride (TOCl) comparable to a humic acid (61-64). In a recent study of the chlorination of proteins and proteinaceous matter in humic acid we demonstrated that in addition to the volatile products, many non-volatile organochlorine products are produced also (65), (see chapter 4).

1.4 Mutagenicity and Health Effects of Chlorination

Cancer may be initiated by changes, e.g. mutations, in the genetic material of a cell. Chemicals that are able to induce gene mutations, showing mutagenic activity, are therefore potential carcinogens. The bacterial mutagenicity assay, known as the Ames test, provides a rapid and relatively simple method for detecting mutagenic activity (66). The Ames test uses *Salmonella typhimurium* strains that contain well-characterized mutations in certain genes, which code for enzymes involved in the biosynthesis of histidine. Since histidine is needed for the growth of the bacteria these will grow only if this amino acid is supplied in the culture medium. Spontaneous reversions of the original gene do occur, but only very infrequently. However, if reverse mutation occurs, such cells regain their ability to produce histidine and can be detected by their ability to form visible colonies on histidine-free medium. If the cells are exposed to mutagenic agents then the frequency of reverse mutations, and hence the number of colonies growing on histidine-free medium, will increase. The *Salmonella typhimurium* strains TA100 and TA98 seem to be the most sensitive for the detection of mutagens in drinking water. TA100 is sensitive to base- pair substitution mutagens, while TA98 responds to frameshift mutagens. In order to simulate the metabolism in higher organisms, a rat liver homogenate (S9 mix) can be incorporated in the test. Compounds that are not mutagenic may be metabolically activated and vice versa. The observed mutagenicity of drinking water samples generally decreases upon incorporation of the S9 mix (11).

There are numerous studies of the mutagenic and carcinogenic properties of chlorinated and not-chlorinated drinking water (67-71). One finding common to most studies is that chlorination introduces mutagens that are not present in raw, untreated water. Since chlorine itself has not been found to be mutagenic, attention has focused on the reaction products formed by the chlorination of organic compounds present in untreated surface waters (11,72). Furthermore, no correlation was found between mutagenicity and the presence or absence of waste water in the raw water. Therefore it was concluded that the production of mutagenicity during chlorination is not related to contaminations of the raw water, but that naturally occuring precursors are involved (73). Meier et al. studied the mutagenic activity of drinking water samples and chlorinated humic materials and found that humic materials were the source of much, if not most, of the mutagenic activity of chlorinated drinking water (74). He also found that the mutagenicity was associated with the non-volatile fraction, as previously shown for extracts of drinking water (75), and that it was destroyed under alkaline conditions.

Table III. Mutagenic activity of chlorinated humic acid (11).

Humic Acid Sample	Mutagenic Activity (rev./mL) TA98	TA100
Chlorinated, pH 2.5	339 + 29	1696 + 148
Chlorinated, pH 7.0	62 + 10	367 + 34
Chlorinated, pH 11.5	Not significant	490 + 33
Non-Chlorinated	Not Significant	Not Significant

The observed mutagenicity and the presence of a wide variety of halogenated compounds has given rise to concern that their presence may be hazardous to the health of the consumer, especially if any potential carcinogenic effects may result from long-term consumption. However, the detection of mutagenicity in chlorinated drinking water indicates merely a potential health risk, and no quantitative risk assessment can be made on the basis of such tests. Epidemiological studies offer one way to evaluate the potential health risk and several of such studies have been undertaken in the USA and Eu-

rope (72,76). Cantor et al. were able to show a positive association between bladder cancer and the consumption of chlorinated drinking water (77). Among non-smokers, the relative risk increased with the level of tap water ingestion and the duration of exposure.

In general, however, the interpretation of the results of those studies is difficult, and the relations that are found between the consumption of chlorinated water and cancer (usually bladder and colon cancer) are weak. Beresford (76) reviewed these studies and indicated the need for further toxicological and analytical studies in order to establish whether any compound present in chlorinated water could account for the higher incidence of cancer.

Since the interpretation of epidemiological studies is difficult and does not always produce clear answers, one can try to evaluate the potential health risk by toxicological tests. Besides the Ames test, other tests, e.g. *in vitro* and *in vivo* tests that are more indicative of effects in man, are needed. Positive results have been obtained with *in vitro* tests (78), whereas *in vivo* studies have shown negative results (78-81). However, carrying out such tests with complex mixtures presents problems with toxicity, synergism and antagonism. If the identity of the mutagenic compounds were known, that task would be simplified.

1.5 The Identification of Mutagens

Many attempts have been made to identify the active mutagens in chlorinated drinking water. Detailed analyses of extracts by (GC/MS) have led to the identification of numerous chlorination by-products. However, the identification of the compounds responsible for the mutagenic activity of chlorinated drinking water has proven to be difficult. This is examplified by a study of Coleman et al., who analysed a drinking water extract and detected about 700 compounds (82). After analysis of the mass spectral data 460 compounds could be identified. Although many of these compounds were known mutagens, they only accounted for a few percent of the total mutagenic activity.

Meier et al. studied the mutagenic activity of drinking water samples and chlorinated humic materials and found certain similarities (73). Most of the mutagenicity (80%) of chlorinated humic acid was due to non-volatile

compounds, as previously shown for extracts of drinking water. They also concluded that humic materials were the source of much, if not most, of the mutagenic activity of chlorinated drinking water. Analysis of humic acid chlorination products by GC/MS, led to the identification of several known mutagens. However, when these compounds were tested individually or as a mixture, they accounted for only 7-8% of the total mutagenic activity of the sample (83).

Fielding and Horth analysed extracts of neutral drinking water samples by GC/MS and identified a number of halo-alkanes, halo-alkenes and halo-acetonitriles, many of which were known to be mutagenic (58). Again, the identified mutagens accounted for no more then 10% of the observed mutagenicity of the sample. All mutagenic compounds identified in chlorinated drinking water so far, were volatile compounds. As mentioned previously, most of the mutagenic activity was associated with the non-volatile fraction. Therefore it was concluded that mutagens in chlorinated drinking water are probably highly mutagenic, non-volatile compounds, present in very low concentrations.

Another approach to the identification of mutagens is a mutagenicity directed fractionation. Holmbom et al. used this method and identified several furanones in chlorinated kraft pulp waste (35,36). Hemming and others showed that low concentrations of these compounds were produced during chlorination of humic and drinking water (37). After chlorination, the fraction of the non-volatile compounds was concentrated and separated by high-pressure liquid chromatography (HPLC). Most of the mutagenic activity of the extract was found in a relativily small HPLC fraction. After analysis of this fraction by GC/MS, a highly potent mutagen, referred to as Mutant X or MX, was identified as 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone. Its structure and mutagenicity in the Ames test were confirmed by synthesis of the compound (84). Meier et al. identified the same compound in a similar procedure (85). He found that most of the mutagenic activity of chlorinated humic acid was recovered by extractions at low pH, and concluded that the active mutagens were acid compounds. Fractionation of the acids by C_{18}-column HPLC, and GC/MS analyses of the mutagenic fraction resulted in the identification of the same mutagen.

MX is often detected together with its structural isomer EMX. Although EMX is 10 times less mutagenic then MX, it cannot be neglected since EMX can rearrange to MX (39,42). MX was shown to be responsible for

most of the mutagenic activity of chlorinated humic acid, and for 15-50% of the mutagenicity of chlorinated drinking water (38-41). A similar study of Dutch drinking waters showed that MX could account for no more than 25% of the mutagenic activity of chlorinated surface water and partly purified drinking water (86).

A picture of the mutagens identified in drinking water, and the part of the total mutagenic activity they account for, is given in figure 6.

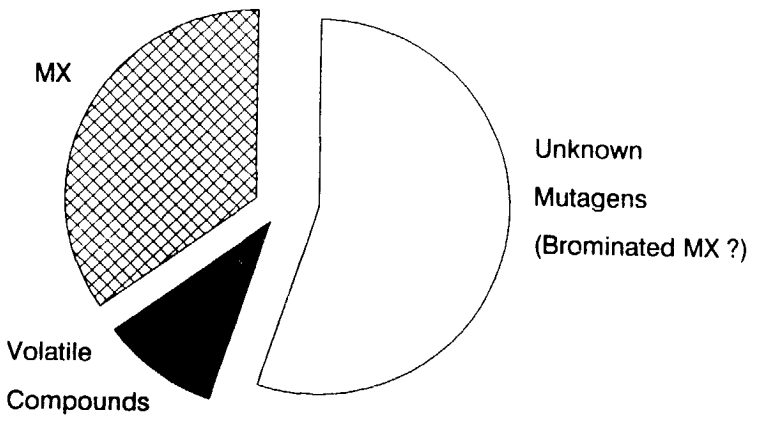


Fig. 6. Identified mutagens in drinking water and their contribution to the total mutagenic activity.

As mentioned previously, bromide is often present in raw waters. Treatment of such waters with chlorine results in the production of brominated and mixed chloro/bromo compounds. In fact many of the mutagens identified in drinking water were brominated compounds (58). Therefore, it may be considered likely that brominated analogs of MX are also formed. This was confirmed by our experiments with 3,5-dihydroxybenzaldehyde and humic acid. If bromide was present during chlorination, brominated MX analogs were formed in addition to MX (43). Horth, who identified MX in extracts of chlorinated tyrosine, found similar results (87). The mutagenic activity of brominated MX is comparable to MX. Together these compounds may explain the unknown part of the total mutagenic activity of chlorinated drinking water shown in figure 6. This will be discussed in more detail in chapter 7.

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2

The Chlorination of Cyanoacetic Acid in Aqueous Medium

Abstract

The reaction of cyanoacetic acid with chlorine in aqueous medium at different pH values produces dichloroacetic acid, dichloromalonic acid and trichloroacetic acid as the final products. At pH 4 and 7 dichloroacetonitrile was found as the only intermediate. At pH 10 dichloroacetonitrile together with N-chloro dichloroacetamide, N-chloro dichloromalonmonoamide and N-chloro trichloroacetamide were detected as the intermediates. The N-chloro amides gave an unexpected reaction with diazomethane producing N-chloro imidates which were previously erroneously identified as methylated hydroxamoyl chlorides. The identity of the intermediates and final products was confirmed by comparison with synthetic standards.

2.1 Introduction

The production of cyano compounds in the aqueous chlorination of amino acids was discovered early this century (1-3). The structure of a number of these compounds was confirmed much later by Burleson et al. (4) with gas chromatography-mass spectrometry (GC/MS). The presence of chlorinated cyano compounds in drinking water as the result of chlorine disinfection was reported by Trehy and Bieber (5,6), who identified dihaloacetonitriles (DIIAN) and demonstrated that dichloroacetonitrile (DCAN) was produced in the chlorination of aspartic acid in aqueous medium.

De Leer et al. (7) identified 3-cyanopropanoic acid (CPA) and 4-cyanobutanoic acid (CBA) after the aqueous chlorination of terrestrial humic acid (HA). Cyanoacetic acid (CEA) was not detected in this study, possibly as a result of a rapid conversion to DCAN and dichloroacetic acid (DCA). In a second study (8) the production of CPA and CBA was standard confirmed and the amino acids glutamic acid and lysine respectively, were shown to be potential precursors.

In further chlorination reactions in aqueous medium at different pH values it was shown that CPA and CBA were quite stable. CEA reacted rapidly with chlorine at all pH values producing DCA, dichloromalonic acid (DCMA) and a small amount of trichloroacetic acid (TCA) as the final products. DCAN was detected as the only intermediate at pH 4 and 7. However at pH 10 several intermediates were detected, which were tentatively identified from their EI and CI mass spectra as the corresponding amides and hydroxamoyl chlorides of DCA, DCMA and TCA.

The reaction mixture which resulted after chlorination of CEA showed to be mutagenic. Since the hydroxamoyl chlorides are interesting compounds in this respect, we planned to synthesize all the proposed intermediates in the mixture to find out which of them are responsible for the observed mutagenicity. Doing so we would also be able to confirm the identity of the compounds by comparison with the synthetic compounds. During this study we noticed however that some of the intermediates had not been correctly identified, partly as the result of an unexpected reaction during the methylation of the reaction products with diazomethane. We report here on a re-investigation of the reaction of CEA with chlorine in aqueous medium, in which the identification of the intermediates is standard confirmed.

2.2 Experimental Section

Chlorination Procedure. The chlorination of CEA was carried out at pH 4.5, 7.2 and 10.0. For pH 4.5 and 7.2, 0.3 M phosphate buffers were used, and for pH 10.0, a 0.3 M carbonate buffer was used.

For the identification studies, a solution (160 mL) of CEA (6.25 mM) and sodium hypochlorite (62.5 mM) was allowed to react in the dark without head space. After a selected reaction time, the excess of chlorine was destroyed by addition of solid sodium arsenite, 50 g of sodium chloride was added, and the solution was acidified to pH 0.5 with concentrated sulfuric acid. The chlorination products were extracted with 3 portions of 25 mL of distilled diethyl ether. The extracts were dried with sodium sulfate and methylated by passing a stream of diazomethane gas through the solution. The extracts were analyzed initially on a Varian 3700 gas chromatograph equipped with a flame ionization detector (FID) and a ^{63}Ni electron capture detector (ECD) which were operated simultaneously with an effluent splitter. The GC-column used was a 25 m fused-silica capillary CP-SIL-5 column, i.d. 0.22 mm and film thickness 0.12 um (Chrompack, Middelburg), while nitrogen was used as the carrier gas. The oven temperature was held at 50^{0}C for 3 min. and then programmed to 280^{0}C at 8^0/min. The extracts were further concentrated with a stream of N_2 gas and analyzed by GC/MS with a Hewlett Packard 5890 gas chromatograph equipped with a 12-m fused-silica capillary HP-1 column, i.d. 0.2 mm and 0.33 um filmthickness, coupled with a Hewlett Packard 5970B mass selective detector. Temperature was programmed as above, while helium was used as the carrier gas.

For the quantitation of the chlorination products at pH 10 the experiments were conducted on the same scale as above. After reaction times of 2, 5, 15, 30 and 60 min., aliquots (10 mL) were taken for analysis. The excess of chlorine was destroyed, the samples acidified to pH 0.5, extracted with diethyl ether and methylated with diazomethane. The ether extracts were analyzed by GC/ECD/FID with nonane as the internal standard and GC conditions as before. Retention times and response factors of the chlorination products were determined by injection of standards prepared as described below.

Chemical Standards. DCA and TCA were obtained commercialy from J.T.Baker Chemicals B.V..

DCMA was synthesized by addition of 33 mL sulfuryl chloride to 21 g of

malonic acid in 200 mL of ether (9). After all the malonic acid had dissolved refluxing was continued for 30 min.. The solvent was removed at room temperature, first at the rotary evaporator and then at the oil pump, giving a crystalline material which was recrystallized twice from thionyl chloride yielding 18 g of DCMA, m.p. 111-112^{0}C.

Dichloroacetamide (DCAA) was synthesized from chloral hydrate in 65% yield, m.p. 98-99^{0}C, described as in Org. Synthesis (10).

Trichloroacetamide (TCAA) was prepared by treating the acid chloride (11) with concentrated ammonia in 72% yield, m.p. 141-142^{0}C (12).

Dichloroacetohydroxamoyl chloride (DAHC) (13) was prepared by slowly adding 30 g of trans-dichloroethylene to a solution of 7 g of $AlCl_3$ in 30 g of nitrosyl chloride, at -30^{0}C. After 2 h at -30^{0}C the mixture was stirred an additional hour at room temperature. Then 50 mL of methylene chloride were added and the insoluble parts removed by filtration. The solvent was removed at the rotary evaporator and the residue distilled under reduced pressure yielding 14 g of the hydroxamoyl chloride, b.p. 90^{0}C at 20 mmHg.

Trichloroacetohydroxamoyl chloride (TAHC) (14) was synthesized by adding 83 g of chloral hydrate to a cold solution of 35 g of hydroxylamine-HCl and 220 g of calcium chloride hexahydrate in 100 mL water. After 15 min. at 60^{0}C, the mixture was allowed to cool to room temperature. The oily layer was separated, and the water layer extracted twice with ether. The combined organic layers were dried ($MgSO_4$) and the solvent removed at the rotary evaporator. Distillation under reduced pressure gave 22 g of trichloroacetaldoxime, b.p. 85^{0}C at 20 mmHg, m.p. 56^{0}C. These 22 g of trichloroacetaldoxime were heated to 80^{0}C and a gentle stream of chlorine gas was passed through the liquid during 3 h. After the mixture cooled to room temperature, 50 mL of methylene chloride was added and the insoluble parts removed by filtration. The solvent was evaporated at the rotary evaporator and the residue distilled under reduced pressure giving 9 g of trichloroacetohydroxamoyl chloride, b.p. 96^{0}C at 15 mmHg, m.p. 60-62^{0}C.

Methyl N-chloro dichloroacetimidate. 55 g of dichloroacetonitile were added to a stirred solution of 0.5 g potassium carbonate in 25 mL of abs. methanol at 0^{0}C. Water was excluded carefully from the reaction mixture. After 30 min. the mixture was distilled directly yielding 52 g of methyl dichloroacetimidate, b.p. 138^{0}C. 14.1 g of methyl dichloroacetimidate in 50 mL of benzene was cooled in an ice bath and 12.3 mL of t-butyl hypochlorite in 12 mL benzene was added slowly. After stirring for 3 h at room temperature, the solvent was evaporated at room temperature and the residue

distilled carefully under reduced pressure giving 12 g of methyl N- chloro dichloroacetimidate, b.p. 67^0C at 1 mmHg.

Methyl N-chloro trichloroacetimidate was synthesized in the same way as the dichloro compound, 72 g of trichloroacetonitrile yielded 68 g of methyl trichloroacetimidate, b.p. 150^0C. 17.5 g of methyl trichloroacetimidate gave 14.4 g of methyl N-chloro trichloroacetimidate, b.p. 78^0C at 1 mmHg. DCAN and trichloroacetonitrile (TCAN) were synthesized from their amides as Described by Fieser (15).

N-chloro dichloroacetamide and N-chloro trichloroacetamide were prepared as described by Lessard (16).

2.3 Results and Discussion

The chlorination of humic materials in aqueous medium produces a number of cyano-substituted compounds. CEA is very interesting in this respect, since it is converted rapidly into DCAN and DCA. At high pH the conversion of DCAN into DCA has been suggested (8) to proceed through dichloroacetohydroxamoyl chloride (DAHC) (eq 1). This intermediate in the the reaction of CEA to DCA is interesting from a mutagenicity point of view since the reaction mixture was shown to be mutagenic in the Klebsiella pneumoniae fluctuation test (17).

eq. 1.

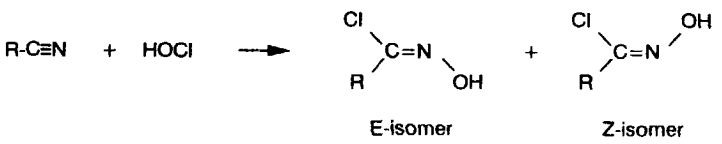
Our purpose in this study was to synthesize DAHC together with the hydroxamoyl chlorides of TCA and DCMA, and the corresponding amides, to confirm the earlier structural assignments and to prepare sufficient material for mutagenic testing.

Compounds 5/7, 9 and 10/11 (5/7 and 10/11 are pairs of E/Z isomers) were previously identified as DAHC and the hydroxamoyl chlorides of TCA and DCMA after methylation with diazomethane and interpretation of their EI mass spectra. The mass spectra showed similar fragmentation patterns with an intens fragment at m/z 92/94 (fig 1a).

DAHC and the corresponding trichloro compound were synthesized and compared to compounds 5/7 and 9 respectively. Trichloroacetohydroxamoyl chloride (TAHC) was synthesized by an addition of hydroxylamine to chloral and subsequent chlorination with chlorine gas (14), while DAHC was prepared by an addition of nitrosyl chloride to trans- dichloroethylene (13). The synthetic compounds behaved different from the compounds in the reaction mixture since they were only partly methylated by diazomethane. The methylated hydroxamoyl chlorides showed mass spectra (fig 1b), in which loss of chlorine was the major fragmentation and in which the m/z 92/94 fragments were almost absent. The retention times of the methylated synthetic hydroxamoyl chlorides also differed from compounds 5/7 and 9. So, we conclude that the compounds 5/7, 9 and 10/11 have been erroneously identified as hydroxamoyl chlorides.

The hydroxamoyl chlorides were thought to result from the addition of HOCl to the cyano group of the nitrile (eq 2).

eq. 2.



Alternatively, addition of HOCl to the cyano group may also result in bonding of chlorine to the nitrogen producing an imidic acid. Free imidic acids however are very instable and have never been detected before (18), since they rearrange immediately to the isomeric and more stable N-chloro amides (eq 3). On methylation, the hypothetical N-chloro imidic acid intermediate would give a N-chloro methyl imidate (eq 4), which is more stable than the imidic acid itself.

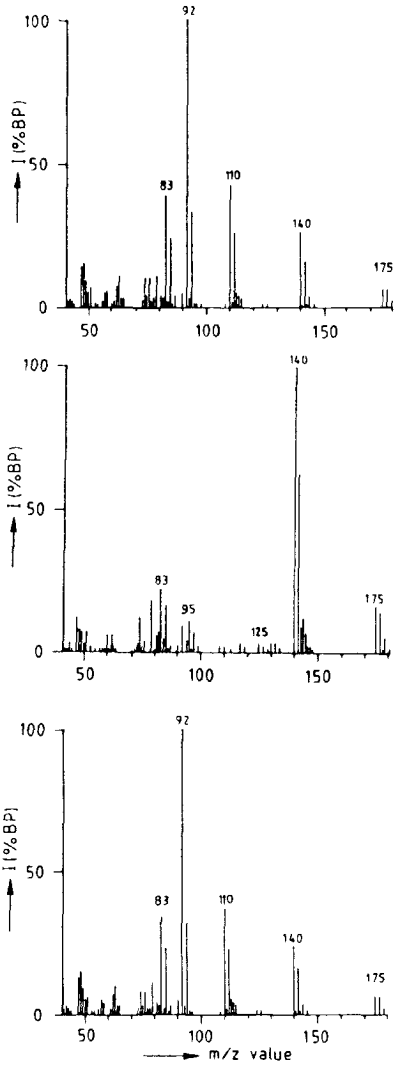
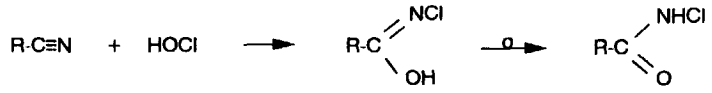
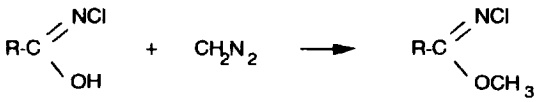


Fig. 1. Mass spectra of: (a) compound 5/7, (from (8), both isomers showed identical mass spectra): (b) synthetic methylated dichloroacetohydroxamoyl chloride (m/z: 175, 177, 179 (M); 140, 142, 144 (M-Cl); 125, 127, 129 (M-Cl-CH_3); 95, 97, 99 (M-Cl-CH_3-NO); 83, 85, 87 ($CHCl_2$)): (c) synthetic methyl N- chloro dichloroacetimidate (m/z 175, 177, 179 (M); 140, 142, 144 (M-Cl); 110, 112, 114 (M-Cl-CH_2O); 92, 94 (M-$CHCl_2$); 83, 85, 87 ($CHCl_2$)).

eq. 3



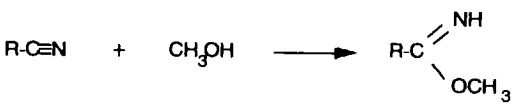
eq. 4

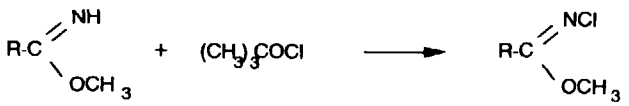


We synthesized methyl N-chloro dichloroacetimidate and the corresponding trichloro compound to compare them with the compounds 5/7 and 9 in the reaction mixture. The retention times and mass spectra of these synthetic imidates (fig 1c) were in excellent agreement with those of the compounds 5/7 and 9, previously identified as hydroxamoyl chlorides. Since 10 and 11 have mass spectra with a similar fragmentation pattern as compounds 5/7 and 9 we expect them to be N-chloro imidates as well and therefore 10 and 11 are tentatively identified as the E/Z isomers of methyl N-chloro dichloromonomalonimidate, although this is not confirmed by synthesis.

The synthesis of the methyl N-chloro imidates consists of two steps. In the first step the N-unsubstituted imidates are prepared by a base-catalyzed addition of methanol to the nitriles DCAN and TCAN. In the second step the N-unsubstituted imidates are subsequentley chlorinated at the nitrogen by a reaction with t-butyl hypochlorite (eq 5).

eq. 5





Comparison of the N-unsubstituted imidates, methyl dichloroacetimidate and methyl trichloroacetimidate, with compounds 2 and 4 showed that their retention times and mass spectra were completely identical. Compounds 2 and 4 were previously identified after methylation with diazomethane and were thought to be N-methylated amides (8). However, the synthetic amides DCAA and TCAA could not be methylated at all, indicating that 2 and 4 were not amides. Compounds 2 and 4 are now identified as imidates and they are the corresponding N-unsubstituted imidates of compounds 5/7 and 9 respectively.

When we repeated the chlorination experiment at pH 10, we noticed a few differences. Peak 6 in the chromatogram of de Leer (8), identified as an amide, was most of the time absent in our chromatogram while peak 9 appeared to consist of two peaks, 9a and 9b (fig 2).

Comparison of the mass spectra showed that 9a was the methyl N-chloro trichloroacetimidate mentioned before, while 9b showed a fragmentation

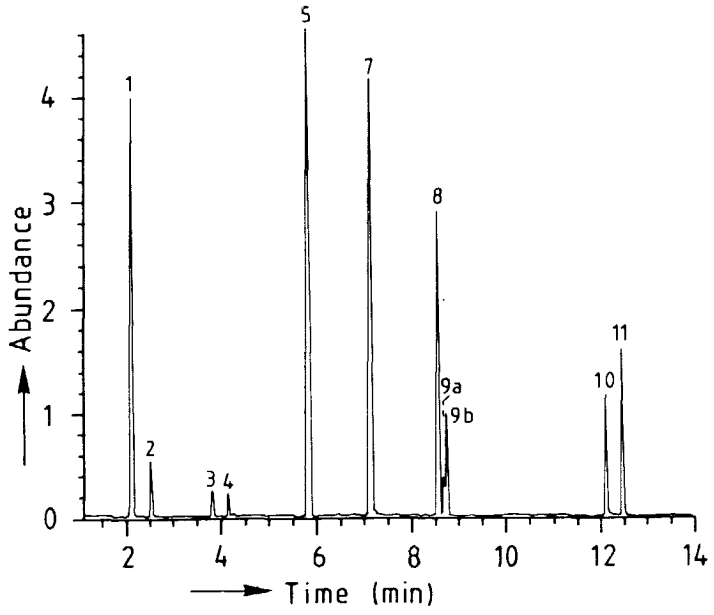


Fig. 2. Gas chromatogram of methylated chlorination products of CEA after a reaction time of 1 h at pH 10. Peak numbers refer to numbers in the text and numbers in table I.

Table I. Compounds in the chlorination mixture after 1 h reaction time at pH 10 before and after methylation with diazomethane. The previous identifications (8) are included also.

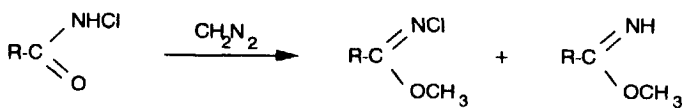
before methylation	compound number[1]	after methylation	previous identification[2]
$CHCl_2CO_2H$	1	$CHCl_2CO_2Me$	$CHCl_2CO_2H$
$CHCl_2CONHCl$	2	$CHCl_2COMe{=}NH$	$CHCl_2CONH_2$
	5/7	$CHCl_2COMe{=}NCl$	$CHCl_2CCl{=}NOH$
CCl_3CO_2H	3	CCl_3CO_2Me	CCl_3CO_2H
$CCl_3CONHCl$	4	$CCl_3COMe{=}NH$	CCl_3CONH_2
	9a	$CCl_3COMe{=}NCl$	$CCl_3CCl{=}NOH$
$HO_2CCCl_2CO_2H$	8	$MeO_2CCCl_2CO_2Me$	$HO_2CCCl_2CO_2H$
$HO_2CCCl_2CONHCl$	9b	$MeO_2CCCl_2COMe{=}NH$	co-eluted with 9a
	10/11	$MeO_2CCCl_2COMe{=}NCl$	$HO_2CCCl_2CCl{=}NOH$

[1] Compound numbers refer to peak numbers and numbers in the text.
[2] Identified after methylation with diazomethane.

pattern very similar to compounds 2 and 4. Therefore we concluded that 9b probably is the corresponding N- unsubstituted imidate of compound 10/11.

Although the methylated compounds 2, 4, 5/7, 9a, 9b, and 10/11 are now identified as imidates it is unlikely that free imidic acids exist in the reaction mixture and we asked ourselves if the isomeric N-chloro amides could be their precursors. To find out we synthesized N-chloro dichloroacetamide and the corresponding trichloro compound and methylated them with diazomethane. Both N-chloro amides gave a mixture of the N-chloro imidate and the N-unsubstituted imidate (eq 6).

eq. 6



This type of reaction has been reported before by Stieglitz (19) for N- chloro benzamide which was converted into methyl N-chloro benzimidate by the action of diazomethane. A later report (20) showed that two geometrical isomers were formed and that apart from O-methylation some N-methylation occured, while no reaction took place at the N-Cl bond. In our case we found that N-chloro dichloroacetamide gave methyl N-chloro dichloroacetimidate (two peaks, E and Z isomers) and also the corresponding N-unsubstituted imidate, methyl dichloroacetimidate. N-chloro trichloroacetamide gave methyl N-chloro trichloroacetimidate (one peak only) and the corresponding N- unsubstituted imidate. The ratio between the N-chloro imidate and the N- unsubstituted imidate depended on the excess of diazomethane used and varied between 10:1 to 2:1. All methylation products of both N-chloro amides showed the same retention times and mass spectra as the compounds in the reaction mixture. This indicated that N-chloro dichloroacetamide was responsible for the production of peaks 2 and 5/7, and the corresponding trichloro compound for peaks 4 and 9a. In the same way we expect that the N-chloro imidates 10/11 and the corresponding N-unsubstituted imidate 9b are the methylation products of N-chloro dichloromalonmonoamide. Table I gives a list of the compounds in the reaction mixture, and of the compounds found after methylation of the reaction mixture with diazomethane. The previous identifications are also included in this table. All the compounds, except 10/11 and 9b, are identified by comparison with synthetic standard compounds.

To find out more about the mechanism of the conversion of CEA in DCA and the other products, we stopped the chlorination experiment after selected reaction times and analyzed the products with GC. The results are given in figure 3.

The first reaction step is a rapid chlorination of CEA due to the electron-withdrawing effect of the substituents (21), producing dichlorocyanoacetic acid (DCEA), followed by a decarboxylation to DCAN. DCEA was detected only once when the reaction was stopped after 5 min. and the tentatively identified mass spectrum is given in figure 4. Trichloroacetonitrile is likely to be produced through a chlorine induced decarboxylation of DCEA since chlorination of DCAN under the same conditions as CEA did not give trichloro compounds. After chlorination and decarboxylation the next step is an addition of HOCl to the cyano group leading to the N-chloro amides. This may be a direct addition of HOCl but also a hypochlorite-catalyzed

hydrolysis of the cyano group, producing an amide which reacts rapidly with HOCl to give the N-chloro amide. Both routes are shown in scheme I.

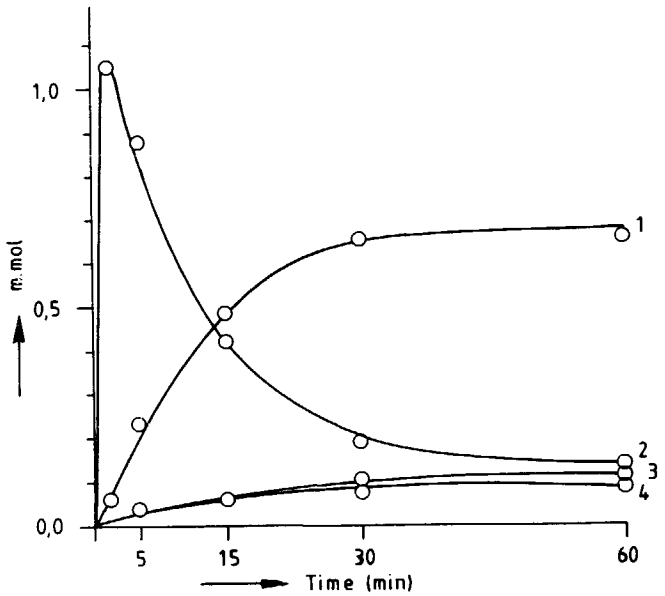
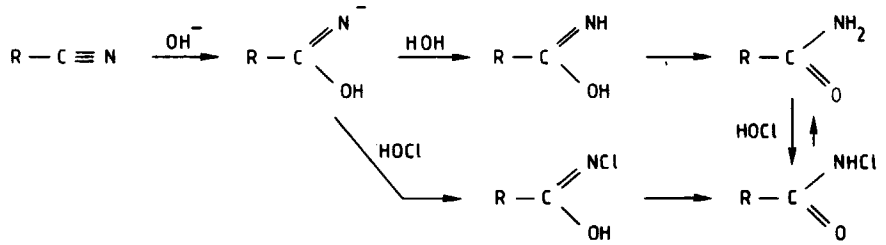


Fig. 3. Profiles giving the amounts of intermediates and final products in the reaction mixture during the chlorination of CEA at pH 10 after different reaction times (1; N-chloro dichloroacetamide, 2; dichloroacetonitrile, 3; dichloroacetic acid, 4; other chlorination products).



Scheme I

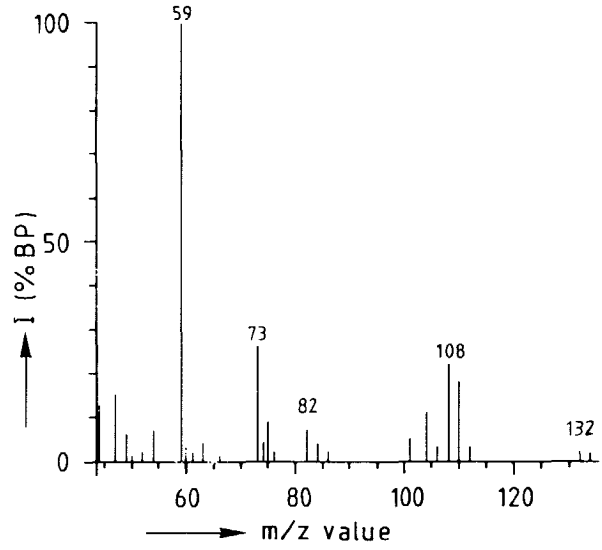


Fig. 4. Mass spectrum of methylated dichlorocyanoacetic acid (m/z: 132, 134 (M-Cl); 108, 110, 112 (M-CO_2Me); 82, 84, 86 (CCl_2); 73, 75 (M-Cl-CO_2Me); 59 (CO_2Me)).

Hypochlorite-catalyzed hydrolysis has been shown before in the chloroform formation from trichloroacetone (22). Oliver reported that DCAN is quite stable in water for several days, but the presence of chlorine induced a rapid disappearance of DCAN (23). We measured the hydrolysis rate of DCAN and found that it was much higher when chlorine was present, as shown in figures 5a and b.

When DCAA was chlorinated under the conditions used before it reacted rapidly to the N-chloro amide. This may explain peak 6 in the gas chromatogram of de Leer, since peak 6 had the same retention time as DCAA. The mass spectrum of 6 however can be explained in more than one way.

The last step in the reaction sequence is the hydrolysis of the N-chloro amides. In most cases N-halo amides will yield amines, which are the products of the Hofmann rearrangement.

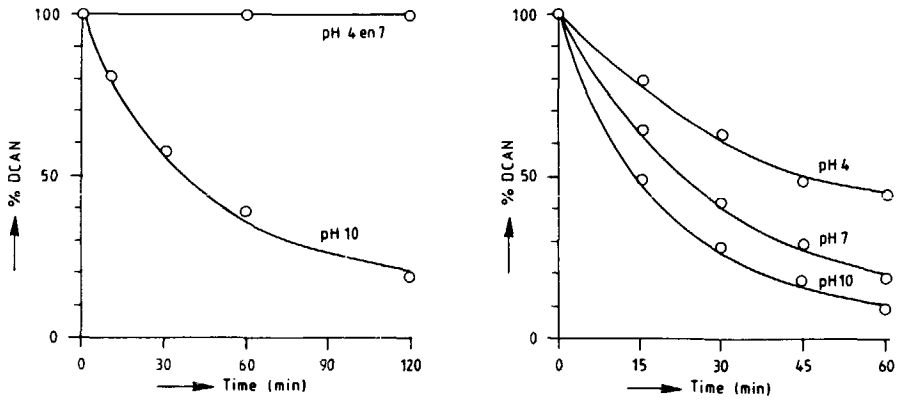
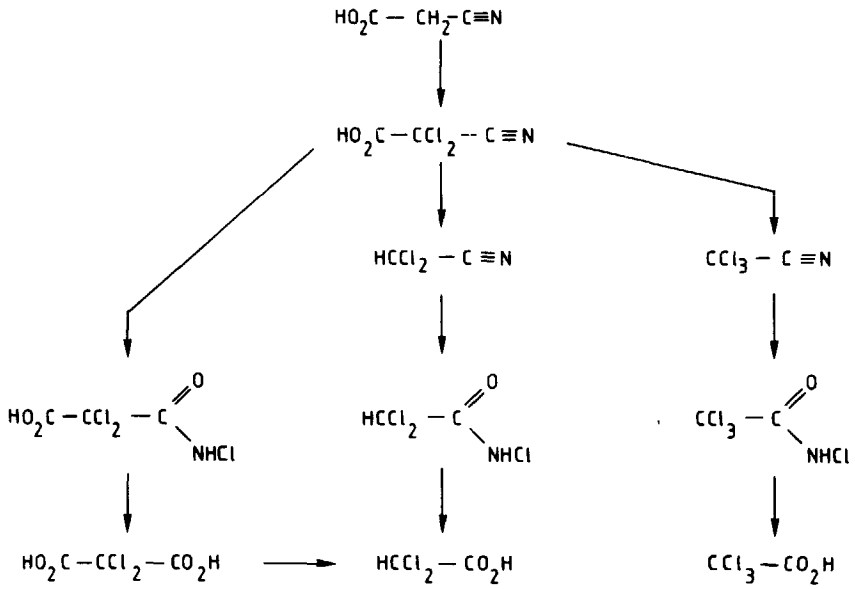


Fig. 5. The hydrolysis of DCAN at different pH values in buffered water solutions without (a) and with (b) the presence of chlorine.



Scheme II

However, hydrolysis of N-chloro amides containing strong electron-withdrawing groups, as an alternative to the Hofmann rearrangement, has been observed (24). The N-chloro amides showed to be quite stable in water for several days at different pH values. However when chlorine was present the N-chloro amides were easily hydrolyzed. Especially at lower pH values the hydrolysis was complete within a few minutes, while at pH 10 50% of the material was hydrolyzed after 8 hours. The reaction of CEA with chlorine, is visualized in scheme II. The N-chloro amides were not detected as intermediates in the reaction at pH 4 and pH 7 and after 1 h reaction time only the acids DCA (1), TCA (3) and DCMA (8), which were also standard confirmed, were detected. DCMA showed to be unstable in aqueous medium and decarboxylated almost completely in 12 hours at room temperature (fig 6) and may thus contribute to the amount of DCA found.

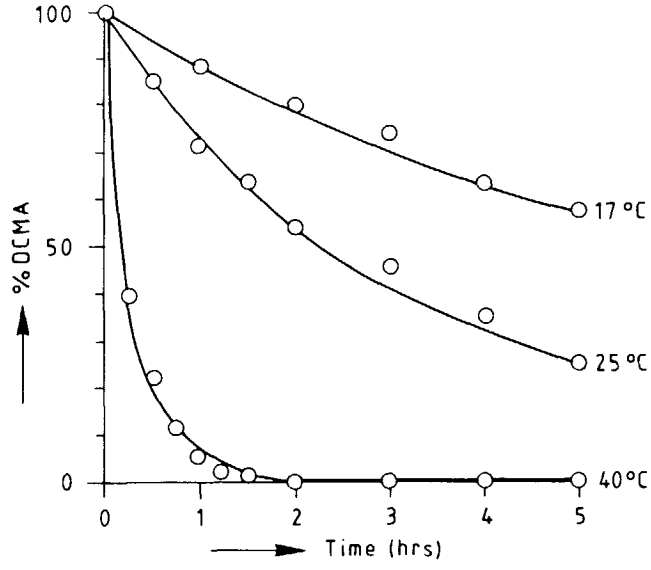


Fig. 6. The decarboxylation of dichloromalonic acid (DCMA) at different temperatures.

2.4 Conclusions

CEA showed to be very reactive under chlorination conditions in aqueous medium producing DCA, TCA and DCMA as the final products. At pH 10, N-chloro amides were detected as the intermediates which were previously reported as N-hydroxamoyl chlorides. The first step in the reaction sequence is a rapid chlorination followed by a decarboxylation producing mainly DCAN. DCAN is then converted into a N-chloro amide by an addition of HOCl. Depending on the pH the N-chloro amides will hydrolyze more or less rapidly to the final products DCA, DCMA and, to a lesser extent, TCA. The hydrolysis of the N- chloro amides was hypochlorite-catalyzed. On methylation with diazomethane these N-chloro amides gave an unexpected reaction producing N-chloro imidates and N-unsubstituted imidates by O-methylation and an unexplained reaction at the N-Cl bond.

2.5 Acknowledgements

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3

Dihaloacetonitriles in Dutch Drinking Waters

Abstract

Dihaloacetonitriles (DHAN) are formed during chlorine disinfection of drinking water, together with the better known and more frequently measured trihalomethanes (THM). Nine Dutch drinking waters were analyzed for DHANs and THMs. For this analysis we developed a method, using a thick-film capillary column, that gave a good separation and detection limits lower than 0.04 μg/L and 0.1 μg/L for DHANs and THMs respectively. All chlorinated drinking waters have been found to contain DHANs (range 0.04-1.05 μg/L) and THMs (range 3.1-49.5 μg/L). In most cases the brominated DHANs and THMs were higher in concentration then the chlorinated ones. The average DHAN concentration was about 5% of the average THM concentration and there was a reasonable correlation between the DHAN and THM concentrations.

Key Words

Chlorination products, drinking water, dihaloacetonitriles, trihalomethanes.

3.1 Introduction

Since the beginning of this century chlorine is used for the disinfection of drinking water. However, chlorine is also very reactive towards natural organic compounds present in water. This problem was first reported by Rook (1974) who found that chloroform and other trihalomethanes (THMs) are formed during chlorine disinfection of drinking water prepared from river Rhine water. Since then many other products of the reaction of chlorine with humic materials have been identified. Some of these chlorination products, such as dichloroacetonitrile, 1,3-dichloroacetone, chloral, and especially 3-chloro- 4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), are mutagenic and their presence in drinking waters is therefore undesirable.

Dichloroacetonitrile (DCAN) was first found in drinking water by Coleman (1975). Later, Trehy and Bieber (1979) identified not only DCAN but also the brominated analogs, bromochloroacetonitrile
(BCAN) and dibromoacetonitrile (DBAN). Since the dihaloacetonitriles (DHANs) were found only in chlorinated waters they concluded that these compounds were formed during chlorine disinfection. Trehy and Bieber also showed that amino acids, in particular aspartic acid, are good precursors for DHANs. These amino acids may be free, proteinaceous or humic-bound. Unlike the THMs, the DHANs are not stable and are therefore more difficult to detect. Trehy and Bieber (1979) reported that DHANs hydrolyze in aqueous solution at elevated temperatures and pH values, while during GC-analysis they may decompose on commonly used gas chromatographic stationary phases such as OV 101. Furthermore, purge and trap techniques, which are often used as the isolation step in the analysis of volatile organohalogen compounds, gave very poor results for DHANs. Trehy and Bieber (1979, 1986) used liquid- liquid extractions with pentane and diethylether and analyzed the extracts with gas chromatography on a column packed with 10% squalane coated on 60/80 mesh Chromosorb W/AW. This method was adopted by others like Oliver (1983), who measured DHAN concentrations in several Ontario water supplies, and Keith (1982) who used two-dimensional chromatography to detect not only DHANs but also trihaloacetonitriles.

In the Netherlands the concentration of THMs is frequently measured in finished drinking waters which are subjected to chlorination, but information about the concentration of DHANs is missing. In this paper we report

the concentration of DHANs and the correlation between DHAN concentrations and THM concentrations in a number of Dutch drinking waters. In the USA and Canada DHAN concentrations of 1-10 μg/L were found by Trehy (1986) and Oliver (1983). Since the amount of chlorine applied by the water treatment plants in the Netherlands is 5-10 times lower, typically 0.2-1.0 μg/L, than the amounts used in the USA we expected to find DHAN concentrations in the range of 0.1-1 μg/L. We therefore developed a method for the analysis of DHANs and THMs, capable to detect concentrations as low as 0.04 μg/L.

3.2 Materials and Methods

Chemical standards. All four trihalomethanes were obtained commercially from Fluka AG. DCAN was prepared from dichloroacetamide as described by Fieser (1967) and redistilled from P_2O_5, bp 112-113^{0}C. BCAN and DBAN were synthesized by adding 40 mL of a sodium hypochlorite solution (0.5M) to a solution of 2 g of cyanoacetic acid and 1 g of sodium bromide in 200 mL of water. After 5 min. the reaction mixture was extracted three times with 50 mL of pentane, the extracts were combined, the solvent was removed, and the residue carefully distilled from P_2O_5 giving BCAN, bp 128-132^{0}C and DBAN, bp 168-170^{0}C.

Sampling. Drinking water samples were collected at the treatment plants and from the taps of houses several miles from the plants. In the laboratory the samples were stored at 5^{0}C. Directly after collection the pH of the water samples was measured, while the DOC was measured later.

Extraction procedure. For the extraction of DHAN's and THM's a 1:10 (vol:vol) liquid-liquid extraction with pentane was used. All glassware used in this method was baked in an oven at 150^{0}C for at least 1 hour prior to use. To aqueous samples of 50 mL in a 100 mL erlenmeyer was added 17 g of NaCl and a magnetic stirring bar. The samples were then extracted by rapid stirring for 30 min. with 5 mL of pentane. The pentane layer was separated from the water layer and 2 μL of the extract was injected on the gas chromatograph.

Gaschromatography. All standards and extracts were analyzed on a Varian 3700 gas chromatograph equipped with a ^{63}Ni-electron capture detector and a 25 m, 0.32 mm i.d., CP-Sil-5CB (film thickness 5 μm) capillary column. The carrier gas was nitrogen and the splitter was set at a split ratio of

1:9. The temperatures were as follows; column, 120^{0}C isothermic; injector, 150^{0}C; detector, 300^{0}C. Under these conditions the DHANs are well separated from the THMs. The retention times of standards and extracts were in good agreement while the reproducibility of the gas chromatographic determinations with a 2 μL injection volume was within $\pm$ 3%. The detector response was linear for standards dissolved in pentane with DHAN concentrations ranging from 0.3-100 μg/L and THM concentrations ranging from 1-100 μg/L.

3.3 Results and Discussion

Method Development. The DHAN concentrations in drinking water were expected to be in the range of 0-2 μg/L and we therefore developed a new method for their analysis. A thick-film capillary column was used, thus combining the advantages of a packed column (larger injection volumes and greater retention for volatile compounds) and capillary columns (better separation and peak shape). Such a column gave a good separation of the DHANs and THMs as is shown in figure 1, which gives the gas chromatogram of one of the samples. To increase the recoveries of DHANs a 1:10 extraction with pentane was used. Pentane was preferred as the extraction solvent over diethylether since the chromatogram showed less interference. Furthermore diethylether extracts often need to be dried prior to analysis which probably results in a loss of DHANs. Extraction efficiences for DHANs in the 0.05-1 μg/L range from distilled water were found to be 69 $\pm$ 2%, 70 $\pm$ 4% and 73 $\pm$ 3% for DCAN, BCAN and DBAN respectively. For the THMs in the range of 0.1-10 μg/L the extraction efficiences were 85 $\pm$ 2%, 89 $\pm$ 2%, 91 $\pm$ 2% and 95 $\pm$ 2% for $CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$ and $CHBr_3$ respectively. The detection limit for the DHANs was lower than 0.04 μg/L and for the THMs it was 0.1 μg/L.

Drinking Water Analyses. In order to find out the range of DHAN concentrations in Dutch drinking waters, we collected water samples from nine treatment plants, lettered A to I. To minimize losses of DHANs by hydrolysis the samples were stored at 5^{0}C. Measurements with spiked samples buffered at pH 7.2 showed that storage for a week at 5^{0}C gave little or no change while at room temperature appreciable hydrolysis took place resulting in a loss of 20% of DHANs. Samples were collected at the treatment plant and from the taps of houses several miles from the plant, to see

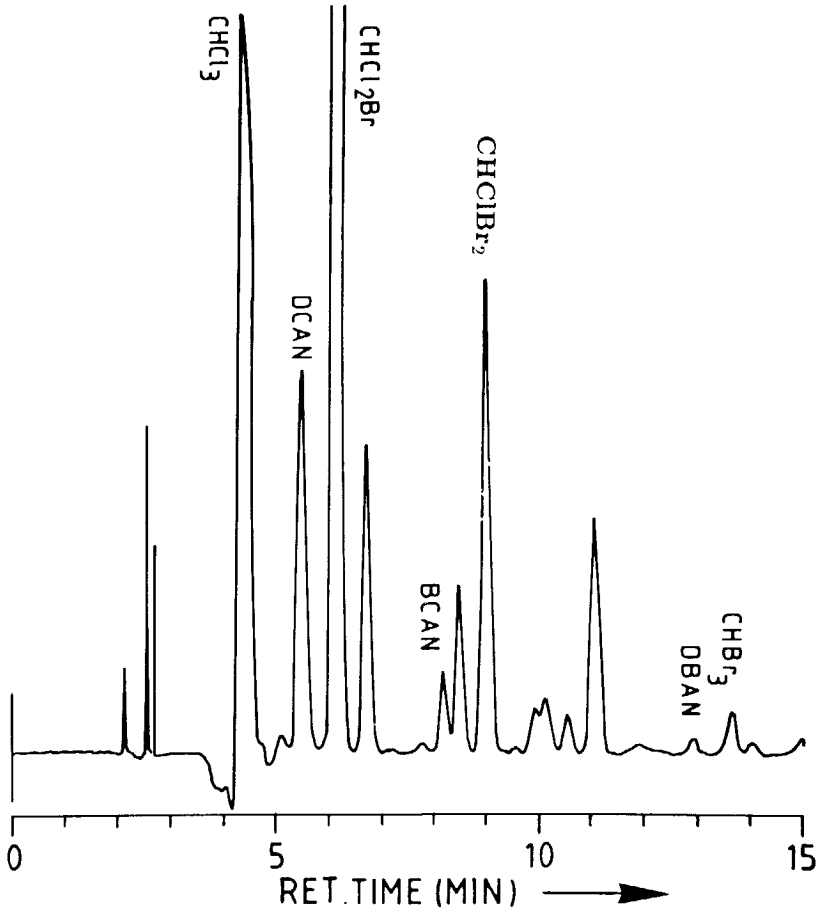

Fig. 1. Gas chromatogram of a sample of treatmentplant H showing the seperation of DHANs and THMs. The concentrations are $CHCl_3$, 9.4 μg/L; $CHCl_2CN$, 0.34 μg/L; $CHCl_2Br$, 3.6 μg/L; $CHClBrCN$, 0.11 μg/L; $CHClBr_2$, 0.9 μg/L; $CHBr_2CN$, 0.04 μg/L; $CHBr_3$, 0.3 μg/L.

Table I. Dihaloacetonitriles in nine Dutch drinking waters

water work	DOC ppm	Cl_2 ppm	DCAN μg/L	BCAN μg/L	DBAN μg/L	Total μg/L
A1	2.2	n.c.	-	-	-	-
A2			-	-	-	-
B1	1.8	5.50	0.06	-	-	0.06
B2			0.04	-	-	0.04
C1	2.8	n.c.	-	-	-	-
C2			-	-	-	-
D1	1.7	0.25	0.08	0.16	0.81	1.05
D2			0.06	0.15	0.70	0.91
E1	2.3	0.20	0.13	0.26	0.46	0.85
E2			0.12	0.21	0.36	0.69
F1	5.6	0.03	0.04	-	-	0.04
F2			-	-	-	-
G1$\star$	2.4	0.43	0.10	0.16	0.25	0.51
G2			0.24	0.30	0.40	0.94
H1	5.3	0.45	0.34	0.11	0.04	0.49
H2			0.20	0.04	-	0.24
I1	3.0	n.c.	-	-	-	-
I2		n.c.	-	-	-	-

1 = treatment plant; 2 = distribution system; n.c. = not chlorinated
- = $< 0.04\mu$g/L; $\star$ = to this sample dehalogenation reagent was added.

if any changes in concentration occur during transport. No dehalogenation reagents were added to the samples (except for the sample of treatment plant G, to which some sodium thiosulfate was added) as such reagents have been demonstrated to cause hydrolysis of DHANs (Trehy and Bieber, 1979). The results of the DHAN and THM analysis for these samples are given in tables I and II respectively. Three of the nine plants, A, C and I, do not use chlorine at all and this is reflected by the fact that no DHANs or THMs are detected in samples from these plants. In all cases where chlorine is used, DHANs and/or THMs were detected. The THM concentrations in tapwater are equal to, or 5-10% lower than those found at the treatment plant, except for treatment plant G. This can be explained by the addition of a small amount of dehalogenation reagent to the sample of treatment plant G. In this sample, the residual free chlorine was destroyed

Table II. Trihalomethanes in nine Dutch drinking waters

water work	DOC ppm	Cl_2 ppm	$CHCl_3$ $\mu g/L$	$CHBrCl_2$ $\mu g/L$	$CHBr_2Cl$ $\mu g/L$	$CHBr_3$ $\mu g/L$	Total $\mu g/L$
A1	2.2	n.c.	-	-	-	-	-
A2			-	-	-	-	-
B1	1.8	5.50	22.1	19.3	7.1	0.8	49.3
B2			22.3	19.3	7.1	0.8	49.5
C1	2.8	n.c.	-	-	-	-	-
C2			-	-	-	-	-
D1	1.7	0.25	1.8	2.1	7.1	11.9	22.9
D2			2.7	1.7	6.1	9.5	20.0
E1	2.3	0.20	3.4	5.8	9.2	5.1	23.5
E2			3.2	5.4	8.3	4.7	21.6
F1	5.6	0.03	1.7	0.8	0.7	0.3	3.5
F2			1.4	0.7	0.7	0.3	3.1
G1$\star$	2.4	0.43	9.5	4.4	4.1	2.1	20.1
G2			12.6	9.4	9.7	1.3	33.0
H1	5.3	0.45	9.4	3.6	0.9	0.3	14.2
H2			8.3	3.8	1.2	0.6	13.9
I1	3.0	n.c.	-	-	-	-	-
I2			-	-	-	-	-

1 = treatment plant; 2 = distribution system; n.c. = not chlorinated
- = $< 0.1 \mu g/L$; $\star$ = to this sample dehalogenation reagent was added.

immediately at the sampling site, while in the other samples chlorination continued during storage, resulting in a higher production of THMs. The concentrations of DHANs in tapwater were 20-50lower then those found at the treatment plants indicating that some hydrolysis takes place during transport. This hydrolysis leads to dichloroacetic acid as shown by De Leer et al. (1986), while Oliver (1983) demonstrated that the hydrolysis is faster if free chlorine is present. An explanation for these phenomena was given by Peters et al. (1989) who showed that the hydrolysis of DCAN is catalyzed by hypochlorite. DCAN was found to be present in all chlorinated water samples, except in one. The brominated DHANs were found in higher concentrations than the chlorinated ones and accounted for 60many samples. A reasonable positive correlation was found between the concentrations of brominated THMs and brominated DHANs. The formation of brominated

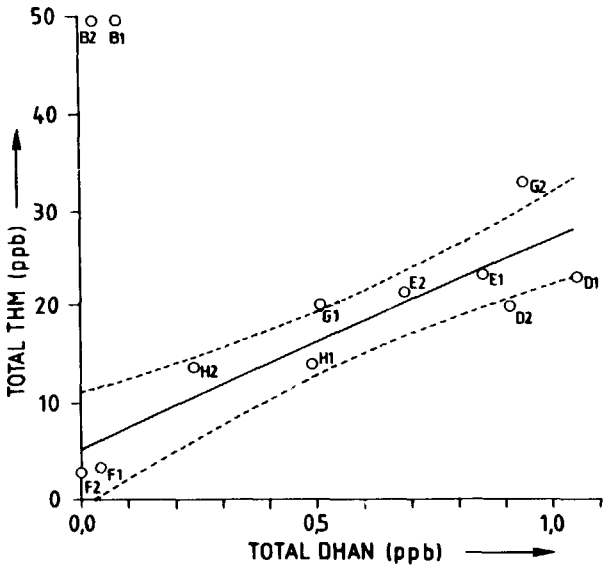

Fig. 2. The correlation between the total THM concentration and the total DHAN concentration. The dotted lines give the 95% confidence interval.

compounds during water chlorination is due to the presence of inorganic bromide in the water. In many surface waters this concentration may be as high as 0.5 mg/L. During chlorination, the bromide is oxydized by chlorine to bromine as shown by Farkas (1949) and chlorination and bromination become competitive reactions.

For the samples where DHANs and THMs were both detected, the total DHAN concentration averages about 5% of the total THM concentration. It is shown in figure 2 that there is a positive correlation between the total DHAN concentration and the total THM concentration of the samples of D, E, F, G and H. The samples of B are different from the others in that they are low in DHAN and high in THM concentration. This can be explained however by the treatment of the raw waters at the treatment plants. In all plants coagulation, flocculation, filtration and adsorbtion on activated carbon is used. In B a high prechlorination is used and after passage through

the activated carbon filters, which probably removes the formed DHANs, there is no postchlorination. In the cases of D, E, F, G and H however no prechlorination and only postchlorination was used after passage through the activated carbon filters. This also indicates that the activated carbon filters, which remove the DHANs produced in early chlorination stages, are not capable to remove the substrates responsible for DHAN formation, i.e. free and bound amino acids, and as a result DHANs are formed during postchlorination.

3.4 Conclusions

The analytical method used in this study showed good results for the analyses of DHANs and THMs in drinking water samples. Base line separation was achieved for all compounds and the detection limits for DHANs and THMs were below 0.04 μg/L and 0.1 μg/L, respectively. DHANs and THMs were found exclusively in chlorinated drinking waters, indicating that these compounds are present as a consequence of the use of chlorine. The DHAN concentrations in chlorinated Dutch drinking waters are in the range we expected and varied between 0.04 and 1.05 μg/L. The average total DHAN concentration is about 5% of the total THM concentration. Furthermore there seems to be a relation between the total DHAN concentration and total THM concentration in the water of treatment plants which use activated carbon filtration followed by postchlorination. This also shows that activated carbon filters are not capable of removing all precursor materials for DHANs.

3.5 Acknowledgements

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4

The Chlorination of Proteins and their Contribution to Chlorinated Humic Materials

Abstract

The aqueous chlorination of proteins was investigated. Dichloroacetic acid, trichloroacetic acid, 2,2-dichlorobutanedioic acid and 2,2-dichloro-3-methylbutanoic acid were detected as major chlorinated products, while 3-methylbutanoic acid, benzoic acid and phenylacetic acid were found as the major non-chlorinated products. A stepwise degradation of proteins, in which α-ketocarboxylic acids play a role as intermediates, is proposed to explain the formation of these compounds. Humic material was hydrolyzed and fractionated to separate the non-hydrolyzable, e.g. lignin, part from the protein part in humic material. Protein material seems to contribute significantly to the chlorination products from humic material and may explain the formation of compounds like 2,2-dichlorobutanedioic acid, which was produced for at least 69% from the hydrolyzable, e.g. protein, part of humic material. Chlorinated proteins also show a TA-100 activity (without metabolic activation) in the Ames test. The strong Ames-mutagen MX was not detected and the mutagens remain unknown.

4.1 Introduction

During the chlorine disinfection of drinking water, trihalomethanes (THMs) and many other chlorination by-products are formed by the reaction of chlorine with organic compounds, mostly humic materials (HM), present in water (1,2). Rook proposed a ring rupture mechanism of resorcinol structures to explain the production of organohalogen compounds (3). This mechanism, that requires a decrease in the aromatic content of HM after chlorination, was supported by several studies. Christman et al. demonstrated that phenolic structures are present in aquatic fulvic acid (4,5), and De Leer et al. showed a reduction of the total aromatic content of HM after chlorination (6). Furthermore, the chlorination of different phenolic model compounds showed that the ring rupture mechanism could explain the production of several chlorination products (3,6-8). However, the production of some compounds, like 2,2-dichlorobutanedioic acid cannot be explained by the chlorination of phenolic structures. De Leer et al. showed that the formation of 2,2-dichloroalkanoic acids can be explained by the chlorination of amino acids (9).

The reaction of chlorine with amino acids in aqueous solution has been known for many years and results in the formation of aldehydes, when an equimolar amount of chlorine is used, or aldehydes and nitriles when an excess of chlorine is used (10-12). More recent studies demonstrated that the reaction results in the production of many chlorinated and non-chlorinated carboxylic acids and nitriles (13-18). Especially the presence of dihaloacetonitriles in chlorinated water strongly suggests a significant role of amino acids or proteinaceous material in the formation of chlorination by-products (19). Amino acids may also contribute to the production of chloroform, probably through chloral as an intermediate (20).

The reactivity of chlorine towards proteins is considered to be low, since the amide nitrogen bond of dipeptides was found to be resistant to aqueous HOCl at room temperature (15). Dipeptides reacted only at the N-terminal amino group resulting in the corresponding N,N-dichlorodipeptides. However, Heltz et al. (21) showed that chlorination of natural waters resulted in a loss of proteinaceous material while Scully et al. (22,23) demonstrated that the chlorination of proteins in natural waters leads to the production of THMs in the range of 0.2-0.5% (M $CHCl_3$/M C). Although this is lower

than the chloroform production of HM, being 1-2% (M $CHCl_3$/M C) (6), the contribution of proteins to the total THM production of natural waters cannot be neglected, especially during summer months of high algae growth.

In this work we examined the chlorination of some model proteins and proteinaceous material in HM. Especially the production of non-volatile organochlorine compounds was investigated and a possible mechanism for their formation is given. We also investigated the potential contribution of proteins to the observed mutagenicity of chlorinated drinking water.

4.2 Experimental section

Materials. All chemicals were of reagent grade or better. Three proteins, bovine serum albumin, trypsinogen and ovalbumin were obtained commercially from Sigma Chemical Co, St. Louis, USA, and used as such. Humic material was obtained commercially from Carl Roth KG, Karlsruhe, West Germany. Chlorine- demand-free water was prepared from Milli-Q purified water by adding one drop of a hypochlorite-solution and leaving the water in a glass bottle in the sunlight until all chlorine had disappeared. All buffers and other aqueous solutions were prepared in chlorine-demand-free water.

Chlorination of Proteins. The proteins (24.2 mg of carbon) were dissolved in 100 mL of a 0.2M phosphate buffer with a pH of 7.2, and aqueous hypochlorite was added to obtain Cl_2/C molar ratios of 5.0 and 0.3. The volume was ajusted to 160 mL and the bottles were capped head-space free. After a reaction time of 16 hours in the dark at room temperature, any excess of chlorine was destroyed by the addition of solid sodium arsenite, the reaction mixture was saturated with NaCl and acidified to pH 0.5 by the addition of concentrated H_2SO_4. This solution was extracted three times with 25 mL of freshly distilled diethyl ether. The combined extracts were stored overnight at -18^{0}C to freeze out the residual water, and concentrated to 25 mL in a Kuderna Danish apparatus. Finally, the extract was methylated with diazomethane and further concentrated to a volume of approximately 1 mL.

Humic Material Fractionation. A solution of 80 mg of HM was dissolved in 50 mL of 6M HCl and refluxed at 110^{0}C for 24 hours. After cooling to

room temperature, the resulting solution was fractionated on a XAD-8 column (0.25 m, 12 mm i.d.). The non-adsorbable fraction was eluted with 25 mL of distilled water, neutralized to pH 7.2, and diluted to 100 mL with chlorine- demand-free water. The XAD-adsorbable fraction was eluted with a 0.1M NaOH solution and, after neutralization to pH 7.2, diluted to 100 mL with chlorine-demand-free water. The total organic carbon (TOC) of both solutions was determined. The solutions were chlorinated with a Cl_2/C molar ratio of 5.0 and the chlorination products extracted as described before.

Analysis of Chlorination Products. The qualitative analyses of the chlorination products was done with GC/MS on a Hewlett Packard 5890 gas chromatograph coupled to a VG Analytical 70-250 SE mass spectrometer, using both electron impact (EI) and chemical ionization (CI). The GC was equipped with a 25 m, 0.22 mm i.d., CP-Sil-5CB (film thickness 0.12 μm) fused silica capillary column and helium was used as the carrier gas. The temperature settings were as follows; column oven programmed from 50^0C (5 min.) to 300^0C (5 min.) at 8^0/min; injector and MS-source temperatures were 270^0C and 200^0C, respectively. To increase the confidence level of the stuctural assignments, the extracts were also analyzed with a GC coupled to a microwave emission detector (GC/MED). The GC/MED signals of carbon, hydrogen and chlorine were monitored to determine the elemental composition of the compounds.

For the quantitative analyses of the chlorination products an internal standard, tetradecane, was added to the ether extracts. Gas chromatography was performed on a United Technologies Packard model 439 gas chromatograph equipped with the same column as mentioned before and operated under the same conditions. A flame ionisation detector (FID) and a ^{63}Ni electron capture detector (ECD) were used simultaneously by means of an effluent splitter and were set at a temperature of 300^0C. The relative response factors for tetradecane and all standard confirmed compounds were determined experimentally and used for the calculation of the production yield. For compounds which were not standard confirmed, the relative response factor of a similar compound was used.

Chloroform Determination. The yield of chloroform was determined gas chromatographically after extraction of a chlorine free sample with an exactly known volume of n-pentane. The GC was a United Technologies

Packard model 438 S, equipped with a 10 m, 0.53 mm i.d., CP-SIL-5CB (filmthickness 5.3 μm) fused silica capillary column and a ^{63}Ni-ECD detector. The temperature settings were as follows; column oven 50^{0}C isothermal; injector and detector, 280^{0}C and 300^{0}C, respectively. Calibration of the ECD-response was done with external standards.

Chlorine Consumption. The chlorine consumption after a reaction time of 16 hours was determined iodometrically by adding 10 mL of the sample to a solution of 5 mL of acetic acid and 2 g of potassium iodide in 75 mL of water, and titrating the resulting solution with a standardized sodium thiosulphate solution and starch as the indicator.

Carbon Dioxide Determination. The carbon dioxide produced during the chlorination reactions was determined by injecting 100 μL of the sample into a bottle with a 1% sulfuric acid solution. Free carbon dioxide was stripped from the bottle by a stream of nitrogen gas and passed through a washing bottle with concentrated sulfuric acid and a magnesium perchlorate drying tube. Quantification was done with an infrared analyzer. All measurements were corrected for the blanc from the reagent solutions.

Determination of Mutagenic Activity. The mutagenicity of the protein chlorination products was determined using the mutagenicity tests according to Ames et al. (23) using *Salmonella typhimurium* strain TA-100 with, and without, metabolic activation (S9-mix). A fresh sample of 300 mg of ovalbumin was chlorinated at pH 7.2 with Cl_2/C molar ratios of 5.0 and 0.3, and the products were isolated as described before. The extracts were evaporated to dryness and the residue was redissolved in DMSO.

Mutant X (MX) Determination. A proportion of the ether extract was methylated using sulphuric acid and methanol, followed by neutralisation and extraction with hexane. MX was determined by GC/MS according to the method described by Hemming et al. (25).

4.3 Results and Discussion

Chlorination of Proteins. The chlorination of proteins in aqueous medium was investigated with bovine serum albumin, trypsinogen and ovalbumin as the model compounds. Chlorination of these three proteins at pH 7.2 with

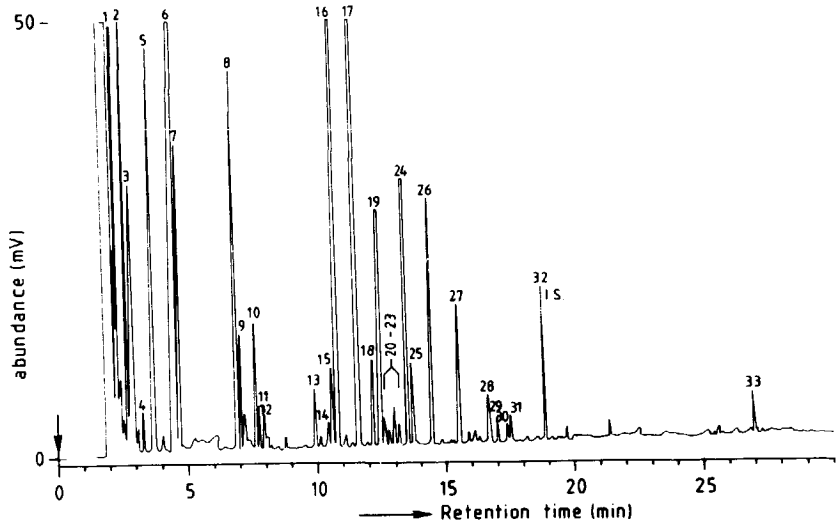


Fig. 1. Gas chromatogram of the methylated ether extract of chlorinated ovalbumin in the high chlorine dose experiment. Peaknumbers correspond with the numbers in table I.

a high chlorine dose produced a great number of products (figure 1), which are summarized in table I. The products were identified by GC/MS with electron impact (EI) and chemical ionization (CI). Additional information concerning the elemental composition of the chlorination products could be obtained by the use of a microwave emission detector (MED). In GC/MED the GC-effluent enters a high-temperature microwave induced helium plasma. The organic compounds are first decomposed into their elemental constituents, and then the atoms are exited to a higher energy level. Once a sufficient number of atoms is excited, atomic emission occurs, which is recorded at element specific wavelengths. In our case the emissions of hydrogen, carbon and chlorine were recorded simultaneously. The peak heights of dichloro-, and trichloroacetic acid and the internal standard tetradecane, were used to calculate the relative elemental response factors

Table I. Major chlorination products of proteins in the high chlorine dose experiments. Peak numbers correlate with the numbers in figure 1 and 2. The assignments are confirmed (SC; comparison of mass spectra with an authentic sample or a literature reference spectrum, confirmation by GC/MED) or tentative (T; interpretaton of EI and CI mass spectra). The molar yield based on carbon is indicated; $+++ => 2.0\%, ++ = 0.5 - 2.0\%, + =< 0.5\%$.

Compound	peak number	assign-ment	abundancy		
			album.	tryps.	ovalb.
Aliphatic monobasic acids					
3-methylbutanoic acid	5	SC	+++	+++	+++
dichloroacetic acid	6	SC	+++	+++	+++
2-chloro-2-methylpropanoic acid	7	T	+++	+++	+++
trichloroacetic acid	8	SC	+++	+++	++
2-chloro-2-methylbutanoic acid	9	T	+	++	++
2-chloro-3-methylbutanoic acid	10	T	++	++	++
3-methyl-2-oxobutanoic acid	14	T	+	+	+
2,2-dichloro-3-methylbutanoic acid	16	T	+++	+++	+++
Aliphatic dibasic acids					
butanedioic acid	13	SC	+	+	+
monochlorobutanedioic acid	19	SC	++	++	++
dichloropropanedioic acid	20	SC	+	++	+
2,2-dichlorobutanedioic acid	26	SC	+++	+++	+++
2,2-dichloropentanedioic acid	31	T	++	++	+
Aromatic carboxylic acids					
benzoic acid	17	SC	+++	+++	+++
phenylacetic acid	24	SC	++	++	+++
phenylchloroacetic acid	28	SC	+	+	+
2-chlorophenylacetic acid	29	SC	+	+	
4-chlorophenylacetic acid	30	SC	+	+	+
Cyano-substituted monobasic acids					
3-cyanopropanoic acid	12	SC	+	+	+
4-cyanobutanoic acid	15	SC	++	++	+
2-chloro-4-cyanobutanoic acid	25	T	++	++	+
2,2-dichloro-4-cyanobutanoic acid	27	T	++	++	++
Nitriles					
3-methylbutanenitrile	3	SC	++	++	++
benzylcyanide	18	SC	+	+	+
Other compounds					
chloroform	1	SC	+	+	+
chloral	2	SC	++	++	++
2,2-dichloropropanoic acid, N-chloro amide	22	T	+	+	+
unknown #1	21	-	++	+	+
unknown #2	23	-	+	+	+
unknown #3	33	-	+	++	+

and thus the C:H:Cl atomic ratios of the compounds. The C:H:Cl atomic ratios confirmed the MS- interpretations. The EI-mass spectrum of 2,2-dichloro-3-methylbutanoic acid methyl ester together with the data from the CI and GC/MED is given in figure 2.

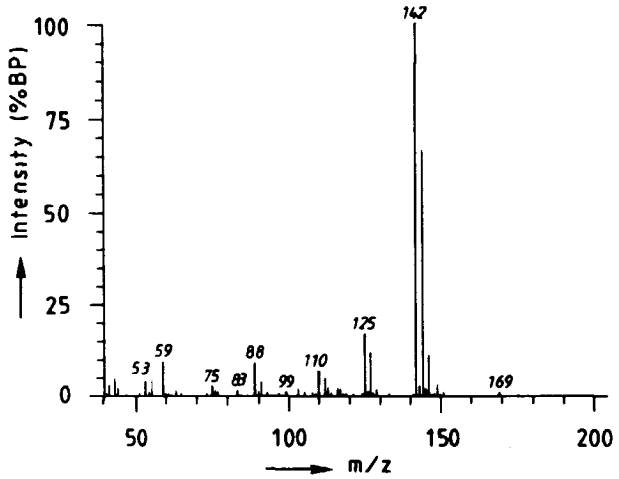


Fig. 2. EI-mass spectrum of 2,2-dichloro-3-methylbutanoic acid methyl ester ($C_6H_{10}Cl_2O_2$). The CI-mass spectrum indicated a molecular mass of 184, and the isotopic ratio showed the presence of 2 chlorine atoms. The GC/MED gave an atomic ratio of C:H:Cl = 5.8:9.6:2.0 Which is in good agreement with the elemental formula.

Many chlorination products of proteins identified in the high chlorine dose experiments, have also been detected as chlorination products of HM (6,26). Identical products, that were also the major chlorination products of the three proteins, include di-, and trichloroacetic acid, the mono-, and dichlorinated butanoic acids and diacids, 3-cyanopropanoic acid and 4-cyanobutanoic acid. Cyanoacetic acid was not detected as it is rapidly chlorinated and further oxidised, producing dichloropropanedioic acid and dichloroacetic

acid (27). Products that were identified exclusively after the chlorination of proteins are benzyl cyanide, 3-methylbutanenitrile and 3-methyl-2-oxobutanoic acid. The cyanides are well known chlorination products of the amino acids phenylalanine and leucine, respectively. The oxo-acid will be mentioned later, and may be an intermediate in the formation of the carboxylic acids.

The molar yield of chloroform, based on carbon, was found to be 0.3-0.4%, which is lower than the 1-3% yield of chloroform from HM, but in good agreement with the results of Scully et al. (22,23) who found a 0.2-0.5% for model proteins. The high molar yield of 25-42% of carbon dioxide indicates a full breakdown of the protein structure and a complete decarboxylation of the amino acids. Since the abundance of carbon as C=O bonds in proteins is lower than the yield of carbon dioxide, considerable oxidation must occur. The chlorine consumption varied from 29% in the chlorination of ovalbumin to 42% in the chlorination of trypsinogen, which means that about two moles of chlorine are consumed for each mole of protein carbon. The yields of chloroform, carbon dioxide and the chlorine consumption of the proteins are given in table II.

Table II. The chlorine consumption and the carbon dioxide, and chloroform production (molar yield based on carbon) of the three different proteins in the high and low chlorine dose experiments.

protein	chlorine dose molar ratio Cl−2/C	chlorine consumption in %	carbon dioxide production in %	chloroform production in %
BSA	5.0	35	29	0.28
Trypsinogen	5.0	42	42	0.42
Ovalbumin	5.0	29	25	0.27
BSA	0.3	83	4	0.10
Trypsinogen	0.3	90	3	0.07
Ovalbumin	0.3	89	5	0.16

In the low chlorine dose experiments the yields of all products was about 10 times lower then in the high chlorine dose experiments (see figure 3 and table II). Compounds as 2,2-dichloro-3-methylbutanoic acid and 4-cyano-2,2-dichlorobutanoic acid, which were major chlorination products in the high chlorine dose experiment, are almost absent. The carbon dioxide production and the chlorine consumption were also lower by a factor 10. On the other hand, the molar yield of chloroform of 0.1% is relatively high. Apparently a large part of the chloroform production is already achieved at a low chlorine dose. This can be explained by the smooth chloroform production of some amino acid fragments of the protein, like tryptophan and proline (20). Typical chloroform precursors, which were detected after the chlorination of HM at a low chlorine dose (9), were not found. This is not surprising since 1,3-dihydroxybenzenes are not present in proteins.

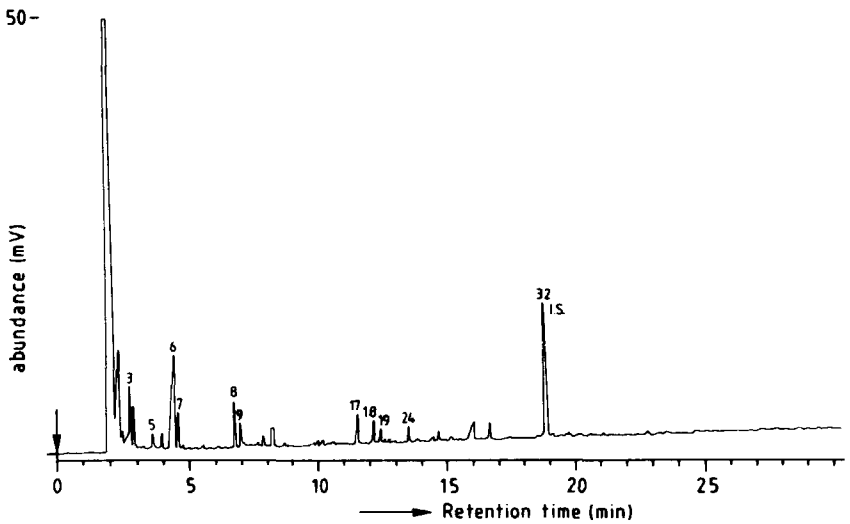
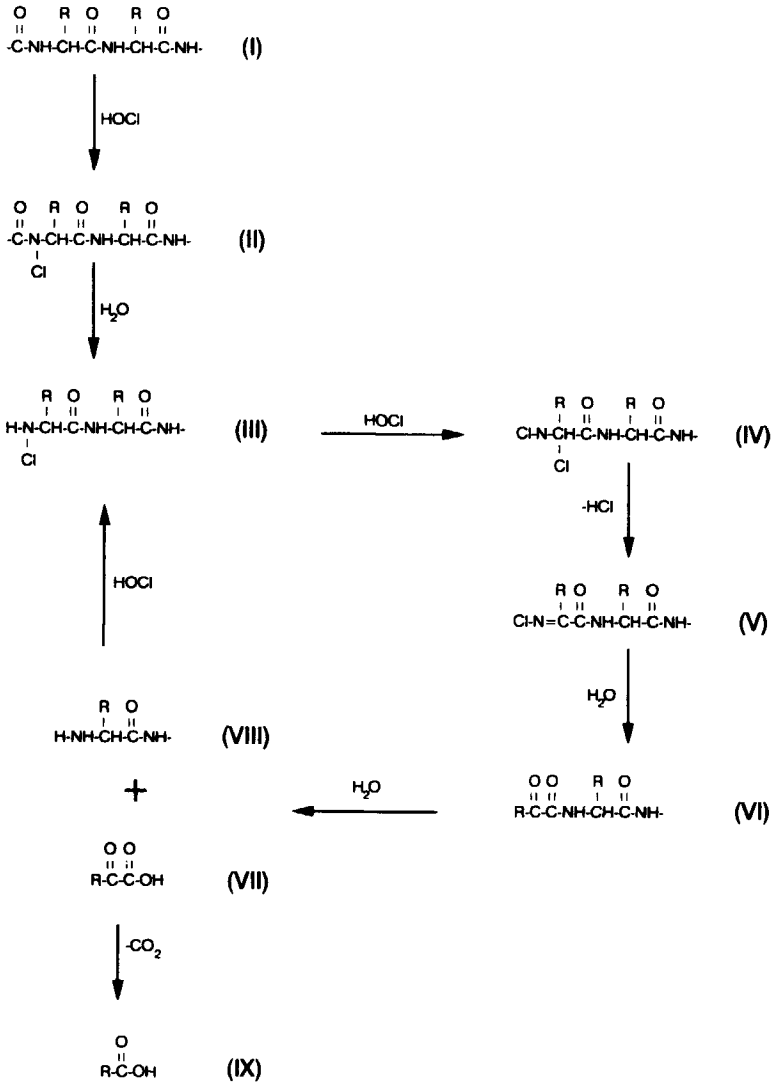


Fig. 3. Gas chromatogram of the methylated ether extract of chlorinated ovalbumin in the low chlorine dose experiment. Peaknumbers correspond with the numbers in table I.

Chlorination of proteins with a low chlorine dose also resulted in the production of some compounds that were not found in the high chlorine dose experiment. The most important one is probably dichloroacetonitrile (DCAN), which was not found in the high chlorine dose experiment as a result of the fast conversion of DCAN to dichloroacetic acid in the presence of an excess of chlorine (27). Two other products found here are chlorobutenedioic acid and phenyloxoacetic acid. The latter may be an intermediate in the production of benzoic acid which was found in the high chlorine dose experiments.

A possible pathway for the production of most carboxylic acids in the chlorination of proteins is given in scheme 1. Chlorination of a protein can take place at a terminal amino group, or at an amino group in the chain, resulting in a N-chlorinated compound (II) (21). Hydrolysis then leads to chain fission and the monochloroamine (III), which on further N-chlorination produces a dichloroamine (IV). Elimination of HCl from (IV) will produce the N-chloro-imine (V), which is hydrolyzed (VI). Alternatively, (VI) may also be produced by an elimination of HCl and subsequent hydrolysis, from the monochloroamine (III). Hydrolysis of (VI) produces a α-ketocarboxylic acid (VII), which in general oxidises to R-COOH and CO_2. This is supported by the identification of phenyloxoacetic acid in the low chlorine dose experiments and 3-methyl-2-oxobutanoic acid in both the high, and the low chlorine dose experiments. With the production of the polypeptide (VIII) the process can be repeated, resulting in a complete breakdown of the protein.

Humic Material Fractionation. HM contains between 1-3 weight percent of nitrogen (28), which is equivalent with 5-15 weight percent of protein material or amino acids. Since proteins and amino acids both produce chlorinated products under aqueous chlorination, they may explain some of the chlorination products that cannot be understood on the basis of resorcinol structures. To study the role of protein material, humic acid was hydrolyzed in 6M HCl for 24 hours at 110^0C. The protein material in HM will be hydrolyzed to amino acids, and the non-hydrolyzable part of HM can then be separated from the amino acids by fractionation over XAD-8 resin. The lignin derived aromatic part of HM will be adsorbed, while the polar hydrolysis products will elute from the column. The dark brown adsorbable fraction could be eluted with 0.1M NaOH, and the TOC of both fractions



Scheme I. The stepwise degradation of proteins that explains the formation of carboxylic acids during chlorination reactions. α-Ketocarboxylic acids are probably intermediates in this reaction pathway.

was determined. Chlorination of both fractions resulted in a large number of products. As expected, aromatic carboxylic acids were produced exclusively from the XAD-8 adsorbable fraction. This confirms the lignin derived aromatic structure of HM as their precursor (3,6-8). The non-adsorbable fraction produced 2,2-dichlorobutanedioic acid (DBA), dichloroacetic acid (DCA) and trichloroacetic acid (TCA) as the major products. These compounds are also produced in the chlorination of the adsorbable fraction, however, with the exception of TCA, in lower amounts. Based on the TOC values of both fractions, the production of DBA, DCA, and TCA from the protein fraction in HM was found to be 67, 60 and 19%, respectively.

Mutagenicity of chlorinated proteins. Ovalbumin was chlorinated with a low and high chlorine dose and the mutagenicity (TA-100 activity) of the low pH extracts was determined. Figure 4 shows the number of revertants per plate without metabolic activation in the low and the high chlorine dose experiments. The mutagenic activity was determined from the lineair part of the graphs, and was found to be 139 and 252 revt/mg C for the low and

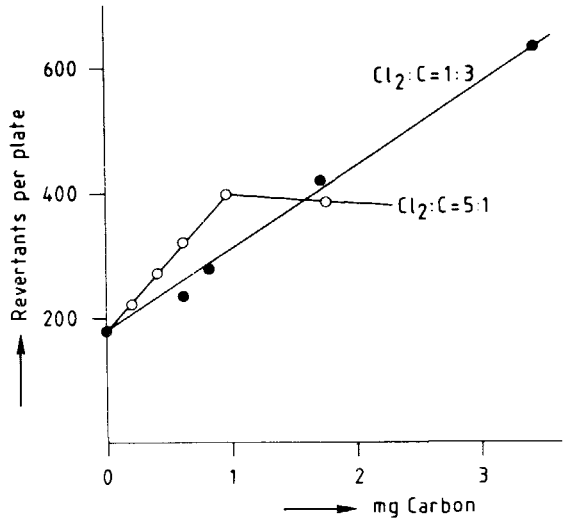


Fig. 4. TA-100 mutagenicity (without metabolic activation) of chlorinated ovalbumin in the high and low chlorine dose experiments.

high chlorine dose experiments, which is about 30% of the mutagenicity found after the chlorination of HM (29). With metabolic activation no mutagenicity was observed. The highly potent mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, often referred to as MX, also shows a strong decrease in mutagenic activity in the Ames test with metabolic activation. Horth et al. identified MX after the chlorination of the amino acid tyrosine (30). We recently identified 3,5-dihydroxybenzaldehyde as an important precursor of MX (31). Although it is difficult to see how the latter may be present in ovalbumin, tyrosine is one of the amino acids present in ovalbumin. A stepwise degradation of the protein then may lead to the production of MX and therefore a part of the extract was used to determine whether MX was present. However, no MX could be detected in the chlorination mixtures and therefore the mutagens remain unknown.

4.4 Conclusions

Chlorination of proteins results in a large number of chlorinated and non-chlorinated products, mostly carboxylic acids and cyano-substituted compounds. Many products are also found after the chlorination of humic materials and their production can be explained by a stepwise degradation of the protein chain. The contribution of protein type material in HM to the production of 2,2-dichlorobutanedioic acid was found to be at least 67%. Trichloroacetic acid is produced mainly from the non-hydrolyzable lignin derived part of HM. Protein material in HM may also contribute to the observed mutagenicity of chlorinated HM. The chlorination of ovalbumin showed a mutagenic activity of 139 revt/mg C (Cl_2/C=0.3) and 252 revt/mg C (Cl_2/C=5.0) which is about 30% of the mutagenicity found after the chlorination of HM. MX was not found in the chlorination extract of ovalbumin and the identity of the mutagens remains unknown.

4.5 Acknowledgements

We thank A. de Wit of the Royal Shell Laboratory in Amsterdam, for his the GC/MED measurements, and C. Voogd and J. van der Stel of the RIVM in Utrecht, for the determination of the mutagenicity of the chlorinated ovalbumin.

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5

The Analysis of Halogenated Acids in Drinking Water

Abstract

Halo-acetic acids are produced during chlorine disinfection of drinking water. Besides the well-known dichloro-, and trichloroacetic acid, brominated and mixed chloro/bromo acetic acids are also produced. A method was developed to determine all halo-acetic acids. This method was applied for the analysis of 20 drinking waters prepared from different source waters. Halo-acetic acids were found in all drinking waters prepared from surface water while they could not be detected in drinking waters prepared from ground water. The acid concentrations were in the range of 0-14.7 mg/L and dibromoacetic acid was found to be the most prominent halo-acetic acid in chlorinated waters. Brominated acetic acids accounted for 65% of the total acid concentration showing that brominated compounds form a large part of the chlorination products. The total haloacetic acid concentration correlated positively with the chlorine-to-carbon ratio and with the adsorbable organic halogen, to which it accounted for 15%.

Key Words

Chlorination products, drinking water, halo-acetic acid, chlorinated acids, brominated acids, AOX.

5.1 Introduction

Chlorine is the most widely applied disinfectant in drinking water treatment, and in many countries disinfection is synonymous to chlorination. Due to its high reactivity, chlorine reacts very fast with many natural organic compounds, e.g. humic materials, present in water. This results in the formation of chloroform and other trihalomethanes (THMs) as reported by Rook (1974). The dominant chlorination products are chloroform and chlorinated aliphatic acids, especially dichloroacetic acid (DCA) and trichloroacetic acid (TCA). Several studies have shown that the volatile halogenated organics, e.g. THMs, represent only a minor part of the total halo-organics while the larger part exists as non-volatile polar compounds. Rook (1980) found that during water chlorination under practical treatment plant conditions, the formation of non-volatile halo-organics exceeded the volatile fraction by three to five times. Christman et al. (1982, 1983) identified DCA and TCA as the major non- volatile chlorination products of humic materials.

However, the type and relative amounts of these halo-acetic acids (HAAs), varies not only with the organic content of the source water but also with the inorganic species present. If bromide is present in the chlorinated water then brominated compounds, like bromoform (and the other bromochloro THMs), are also produced. In a study of the concentrations of dihaloacetonitriles (DHANs) in drinking water by Peters et al. (1990), it was shown that the major part of the DHANs and THMs found in Dutch drinking water were brominated.

HAAs are rarely determined in drinking waters. TCA is the best known and most often determined representative of these compounds. For the determination of low levels of TCA an acid extraction followed by methylation and gaschromatographic detection is often used. Uden and Miller (1983) used a microwave emission detector, GC/MED, and found TCA and DCA in US tap water in concentrations up to 160 μg/L. Lahl et al. (1984) reported much lower TCA concentrations of 0-3 μg/L for several German drinking waters, using a more common GC/ECD. Norwood et al. (1986), who used a novel isotope dilution GC/MS method for the analysis, reported TCA concentrations in the range of 4-54 μg/L for several drinking waters in North Carolina. In a previous investigation we found TCA concentrations in the range of 0-2 μg/L for several Dutch drinking waters.

Brominated acetic acids are not determined in drinking water. In the

present study we determined not only the chlorinated, but also the brominated and mixed chloro/bromo halides of acetic acid. In total nine different HAAs can be formed and the concentrations of eight of them have been determined in several Dutch drinking waters.

5.2 Materials and Methods

Chemical standards. Monochloroacetic acid (MCA), dichloroacetic acid (DCA), trichloroacetic acid (TCA), monobromoacetic acid (MBA), dibromoacetic acid (DBA), and tribromoacetic acid (TBA) were obtained commercially from JT. Baker Chemicals BV. and Janssen Chimica. Methyl esters of these compounds were prepared by heating the acid with an excess of methanol in the presence of concentrated sulfuric acid. After refluxing for several hours the reaction mixture was washed with a $NaHCO_3$-solution till the CO_2-evolution ceased. The resulting mixture was extracted twice with ether, and the combined ether fractions were dried. After removal of the solvent the residue was distilled to give the product; MCA methyl ester, bp 128^0C; DCA methyl ester, bp 142^0C; TCA methyl ester, bp 153^0C; MBA methyl ester, bp 142^0C; DBA methyl ester, bp 182^0C. TBA decomposed to bromoform during refluxing. The mixed halides of acetic acid are little more than chemical curiosities, and there is not much information about their preparation in the literature. Bromochloroacetic acid (BCA) and its methyl ester were prepared from dichlorovinyl ethyl ether as described by Crompton (1921). Chlorodibromoacetic acid (CDBA), and bromodichloroacetic acid (BDCA) were prepared from monochloro-, and dichloroacetaldehyde diethyl acetal as described by Neumeister (1882). Although these two acids were the main products of the synthesis (confirmed with mass spectrometry), it was not possible to purify them to the desired level. TCA isopropyl ester (used as internal standard), bp 172^0C, was prepared in the same way as the methyl esters, except that isopropyl alcohol was used instead of methanol.

Sampling. Drinking water samples were collected at the treatment plants and analyzed within two days. All samples were stored at 5^0C till their analyses. A second sample was taken at the same time to determine the DOC and the AOX of the samples.

Extraction procedure. For the extraction of the HAAs a 1:10 (vol:vol) liquid-liquid extraction with diethylether was used. All glassware was baked in an oven prior to use. Aqueous samples of 100 mL in a 150 mL separa-

tion funnel were saturated with NaCl to reduce the mutual solubility of the solvent/water mixture. The water samples were acidified to pH 0.5 by the addition of concentrated sulfuric acid, and 10.0 mL of freshly distilled diethylether was added as the extraction solvent. The internal standard, TCA isopropyl ester, was already added to the extraction solvent in a known concentration (10 μg/L). The HAAs were then extracted by vigorous shaking for 3 min. The organic layer was separated and collected in a 50 mL erlenmeyer flask. The aqueous layer was extracted once more for 3 min. with 10 mL of the extraction solvent. The combined ether extracts were stored at -20^{0}C for at least 2 hours to freeze out residual water. After filtration over glasswool, to remove the water crystals, the dry extracts were methylated with diazomethane. Diazomethane was generated by adding 10 drops of a methanolic KOH-solution to 0.5 g of diazald (N-methyl-N-nitroso-p-toluenesulfonamide, Merck) in a washbottle. A stream of nitrogen, saturated with ether, was passed through the washbottle and carried the diazomethane to the extract. The methylation was stopped when the color of the extract started to turn yellow. 2 μL of the extract was then injected on the gas chromatograph.

Gaschromatography. All standards and extracts were analyzed on a Varian 3700 gas chromatograph equipped with a ^{63}Ni-electron capture detector and a 25 m, 0.25 mm i.d., CP-Sil-5CB (film thickness 0.12 μm) capillary column. The carrier gas was nitrogen and the splitter was set at a split ratio of 1:15. The temperatures were as follows; injector, 250^{0}C; column, 40^{0}C (5 min.) programmed at a rate of 2^0/min to 80^{0}C (2 min.); detector, 300^{0}C. Under these conditions the HAA methyl esters are well separated with the exception of MCA methyl ester which co-elutes with the solvent-, and THM peaks. The retention times of standards and extracts were in good agreement while the detector response was linear for standards dissolved in diethylether with concentrations ranging from 0-100 μg/L.

5.3 Results and Discussion

Method Development. HAAs generally are difficult to quantify at low levels. One reason for this is their strong acidic character and high water solubility. Miller et al. (1982) reported that water samples have to be acidified to pH 0.5 to prevent losses of TCA and that a concentration of the extract, especially after methylation, led to considerable losses. This is

in agreement with our own results that showed losses of 20% or more and poor reproducibility. To avoid these concentration problems we developed a simple and sensitive method for the analysis of HAAs. After acidification of the water samples to pH 0.5, the HAAs were extracted by two successive 1:10 extractions with diethylether. TCA isopropyl ester was used as the internal standard since this compound has a suitable retention time, a comparable response on the ECD, and is not likely to be present in water.

Diazomethane was used for the methylation of the samples since it is fast, easy to use (in this modification), and gives quantitative results. However, samples that were methylated with diazomethane gave rise to artefact peaks after storage for several hours and therefore should be analyzed within two hours. Methylation with methanol/sulfuric acid resulted in the decarboxylation of the higher brominated acids. As a result of the increased recovery of the 1:10 extraction and the sensitivity of the ECD, used for the detection of the HAAs, a further concentration of the extracts was not necessary. Standards in the range of 1-20 μg/L were used to determine the retention times and reponse factors of the individual compounds. MCA eluted in the same region as the solvent-, and THM-peaks and could not be quantified. BDCA and CDBA could not be sufficiently purified after synthesis and their relative response factors were set to 1.00. The recovery of the total procedure was found to be 100 $\pm$ 6%, while the reproducibility was within 10% (for TCA- solutions ranging from 0.2-20 μg/L). The detection limit of this method was 0.1 μg/L. A chromatogram of one of the samples is given in figure 1.

Water Analysis. In order to study the range of HAA concentrations in Dutch drinking waters, samples from 20 treatment plants, numbered A to T, were collected. In the Netherlands two third of the drinking water originates from ground water and one third from surface water. Surface water is used mainly in the western part of the country, where ground water is scarce, and is taken mostly from the rivers Meuse and Rhine after bank filtration, dune filtration, or storage in reservoirs. While surface water is treated by a series of methods which may include disinfection with chlorine, groundwater is only exceptionally chlorinated. In a previous investigation we found that TCA was present at low levels in Dutch drinking waters prepared from surface water, even if no chlorine was applied. Therefore we also analyzed several drinking waters prepared from ground water since these were expected to be free of these acids. The results of the HAA analysis of all samples are given in tables I and II. The source of the water, the amount

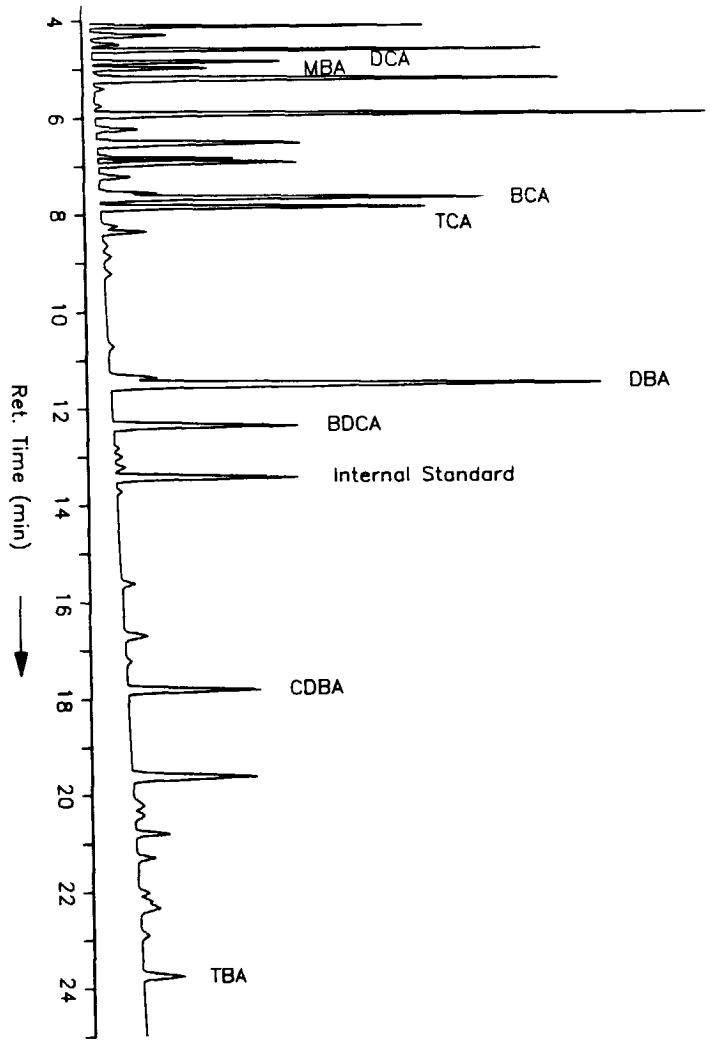

Fig. 1. Gas chromatogram of the drinking water sample of treatment plant G in which the peaks of the individual HAAs are indicated. The concentrations are; DCA, 1.2 μg/L; MBA, 0.5 μg/L; BCA, 2.1 μg/L; TCA, 1.4 μg/L; DBA, 2.6 μg/L; BDCA, 1.7 μg/L; CDBA, 1.4 μg/L; TBA, 0.7 μg/L.

Table I. Parameters of the water samples of the 20 treatment plants.

Water work	Source	DOC mg/L	Cl_2 mg/L	AOX μg/L	Total HAAs μg/L
A.	surface	5.2	0.3	66.4	6.8
B.	surface	1.9	0.06	49.3	4.2
C.	surface	4.6	0.03	47.6	3.8
D.	surface	2.1	0.2	41.9	7.2
E.	surface	3.5	0.5	104.7	14.7
F.	surface	1.9	0.2	62.8	9.3
G.	surface	2.7	0.4	81.3	10.4
H.	surface	1.8	nc	3.2	2.0
I.	surface	2.9	nc	3.9	0.8
J.	surface	2.3	nc	11.6	0.7
K.	surface	1.6	nc	6.7	0.9
L.	surface	1.5	nc	11.7	0.9
M.	surface	2.1	nc	10.3	0.9
N.	surface	3.9	nc	17.0	1.4
O.	surface	1.4	nc	2.5	0.5
P.	ground	5.2	nc	7.1	< 0.1
Q.	ground	1.9	nc	4.3	< 0.1
R.	ground	2.1	nc	3.9	< 0.1
S.	ground	1.3	nc	3.6	< 0.1
T.	ground	2.1	nc	13.5	< 0.1

nc = not chlorinated.

of chlorine used, the DOC and AOX, and the total HAA concentrations are given in table I.

Table II lists the concentrations of the individual HAAs. Table I shows that the total HAA concentration in the samples of the treatment plants that use surface water but do not use chlorine, is in the range of 0.5-2.0 μg/L. For samples of treatment plants were chlorine is applied this value is increased sevenfold to a range of 3.8-14.7 μg/L. The AOX values of the chlorinated waters are also higher by a factor of 7-10. Figure 2a shows a positive correlation between the total HAA concentration and the AOX of the drinking water samples.

The treatment plants that use chlorine are indicated in this figure and the dotted lines give the 95% confidence interval. Non-volatile polar compounds

Table II. Concentrations of the individual HAAs in the 20 drinking water samples.

Water work	DCA µg/L	MBA µg/L	BCA µg/L	TCA µg/L	DBA µg/L	BDCA µg/L	CDBA µg/L	TBA µg/L
A.	3,0	0,4	1,6	1,1	0,6	0,1	-	-
B.	0,8	0,1	0,5	1,0	1,6	-	0,2	-
C.	1,0	0,1	1,3	0,5	0,7	-	0,2	-
D.	0,8	0,1	1,3	0,5	2,9	0,4	0,4	0,8
E.	0,9	0,2	2,5	0,3	6,5	0,6	1,6	2,1
F.	1,9	0,2	2,0	0,8	3,2	0,5	0,4	0,3
G.	1,2	0,5	2,1	1,4	2,6	1,7	1,4	0,7
H.	0,6	-	0,6	0,6	0,2	-	-	-
I.	0,3	-	0,1	0,2	0,2	-	-	-
J.	0,2	-	0,2	0,2	0,1	-	-	-
K.	0,4	-	0,2	0,2	0,1	-	-	-
L.	0,3	-	0,3	0,1	0,2	-	-	-
M.	0,2	-	0,2	0,3	0,2	-	-	-
N.	0,3	-	0,5	0,3	0,3	-	-	-
O.	-	-	0,2	0,2	0,1	-	-	-
P.	-	-	-	-	-	-	-	-
Q.	-	-	-	-	-	-	-	-
R.	-	-	-	-	-	-	-	-
S.	-	-	-	-	-	-	-	-
T.	-	-	-	-	-	-	-	-

$- =< 0.1 \mu g/L$.

are believed to make up a large part of the halo-organics in chlorinated waters. In this case the HAAs accounted for about 5% of the AOX. However, since the HAAs are considered to be reaction end products, this percentage may be higher when a higher chlorine-to-carbon ratio is used. This assumption is supported by figure 2b that shows that there is a reasonable correlation between the total HAA concentration and the chlorine-to-carbon ratio.

Table 2 shows that varying amounts of HAAs were found in drinking waters prepared from surface water, and that BCA, TCA and DBA were always present. By contrast, these acids could not be detected in any of the drinking waters prepared from ground water. Therefore, these acids are probably

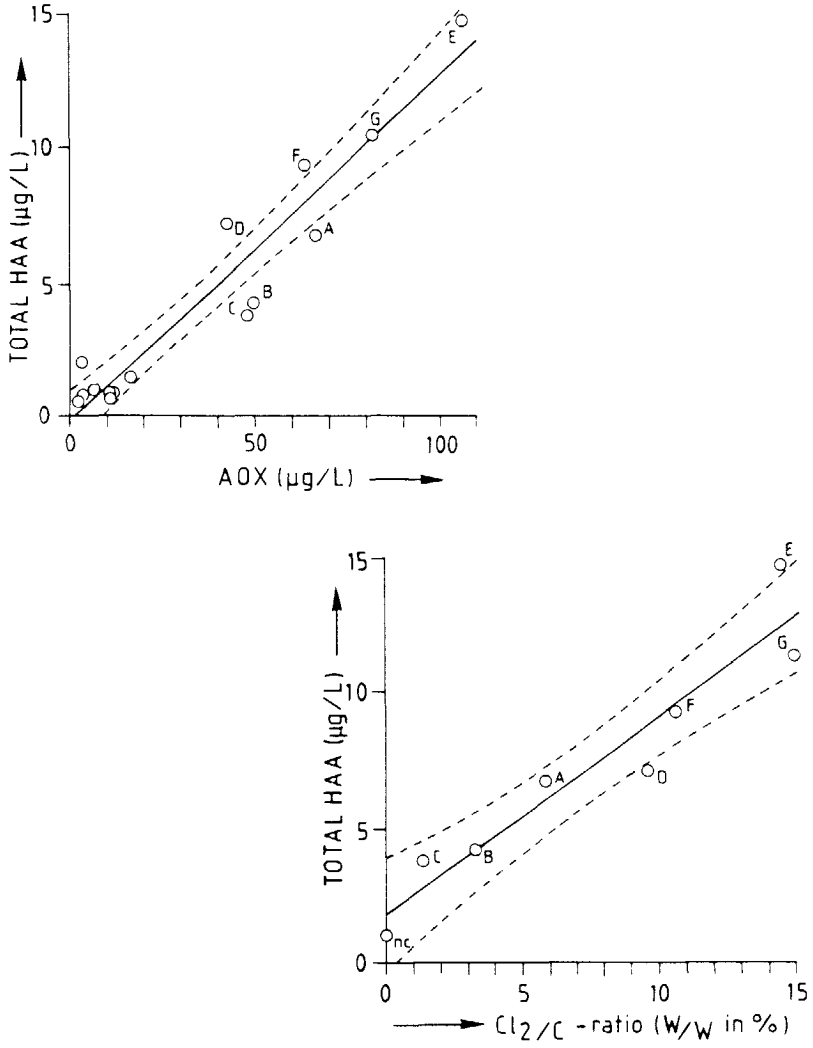

Fig. 2. (a) The correlation between the total HAA concentration and the AOX. Treatment plants that use chlorine are indicated. (b) The correlation between the total HAA concentration and the chlorine-to-carbon ratio. Treatment plants that use chlorine are indicated, while "nc" is an average value for the treatment plants that do not use chlorine. The dotted lines represent the 95% confidence interval.

present at low levels in the surface waters. This is in agreement with our earlier findings and TCA was also detected in river Rhine and river Meuse water at levels around 1-2 μg/L (Mulder, 1987; Faaij, 1989).

While in not-chlorinated waters DCA and TCA are the major HAAs, DBA and BCA predominate in chlorinated waters. In general brominated acetic acids are found in higher concentrations than chlorinated ones and account for about 65% of the total HAA concentration. This percentage is in good agreement with earlier findings for dihaloacetonitriles and THMs (Peters et al. 1990). The formation of brominated compounds during water chlorination is due to the presence of inorganic bromide in the water. Bromide is present in many Dutch surface waters and Rook (1975) found bromide concentrations of 0.1-0.5 mg/L for river Rhine and river Meuse water. During chlorination, the bromide is oxydized by chlorine to bromine and chlorination and bromination become competitive reactions. Since the amount of chlorine applied by the Dutch treatment plants is low and almost equals the bromide concentration of the surface waters, all chlorine may be converted in to bromine resulting in bromination as the predominant process. Our findings agree with this, and show that brominated HAAs are more prominent then chlorinated ones.

5.4 Conclusions

A method was developed for the determination of HAAs in drinking water. This method was used to analyse 20 drinking water samples. HAAs were found in all Dutch drinking waters prepared from surface water while no HAAs could be detected in drinking waters prepared from ground water. The HAA concentrations were in the range of 0-6.5 μg/L for the individual acids, and 0.5-14.7 μg/L for the total concentrations. BCA, TCA and DBA could be detected in all drinking water samples prepared from surface water and DBA was found to be the most prominent HAA. Brominated acetic acids accounted for 65% of the total HAA concentration showing that brominated compounds may form a large part of the chlorination products. A positive relationship was found for the chlorine-to-carbon ratio and the total HAA concentration, and for the AOX and the total HAA concentration, with the latter accounting for 15% of the AOX.

5.5 Acknowledgements

This study was carried out under project number 718629 on behalf of the Directorate for Drinking Water Supply at the Ministery of Public Housing, Physical Planning and Environmental Protection.

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6

3,5-Dihydroxybenzaldehyde, The MX Precursor ?

Summary

MX is a strong Ames mutagen that is responsible for a substantial part of the mutagenic activity found in chlorinated drinking water. The formation of MX, EMX and related compounds from 3,5-dihydroxyaromatic compounds during chlorination in aqueous solution has been studied. 3,5-Dihydroxybenzaldehyde is shown to be a potential precursor for MX and EMX, while the corresponding 3,5-dihydroxybenzoic acid and 3,5-dihydroxybenzyl alcohol are precursors for the oxidized and reduced forms of MX and EMX. The mechanism involves a chlorination of the resorcinol structure, a ring opening and subsequent elimination of chloroform, followed by a decarboxylation. The resulting EMX then isomerizes to MX. The optimal Cl_2-dose for the production of MX was determined. The maximum yield of MX and EMX was found to be 0.125 and 0.475 % respectively at a Cl_2/3,5-dihydroxybenzaldehyde ratio of 6.5. If 3,5-dihydroxybenzaldehyde constitutes 0.2% of humic acid, the formation of MX from humic acid is completely explained by this precursor.

6.1 Introduction

Chlorine is used for the disinfection of drinking water since the beginning of this century. However, chlorine is also very reactive towards natural organic compounds, e.g. humic materials, present in water. This results in the formation of numerous chlorination products and an increased mutagenic activity of chlorinated water (1-3). The presence of mutagenic compounds in drinking water is undesirable and therefore much research has been devoted to find the active mutagens. A number of chlorinated mutagens have been identified but these compounds accounted for only a small part of the total mutagenic activity (4,5).

The major part of the mutagenicity is found in the fraction of the non-volatile polar compounds. Especially 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, often referred to as MX, is responsible for a large part (15-50%) of the observed mutagenicity in chlorinated drinking water (6). This compound was first tentatively identified in pulp bleaching liquors by Holmbom et al. (7,8) in 1980. Later, Padmapriya et al. (9) succeeded in synthesizing the compound and confirmed its identity and mutagenic activity. Depending on the pH, MX exists in an open and a closed form which are shown in figure 1. While MX is stable at pH 2, it undergoes ring opening around pH 6-8 and gradually degrades above pH 4. MX is often found together with the geometric isomer of the open form of MX, E-2-chloro-3-(dichloromethyl)-4-oxo-butenoic acid (EMX). Although EMX exhibits only one tenth of the mutagenic activity of MX the compound is of particular interest since it has the ability to isomerize to MX (6).

It is still unknown how MX and EMX are formed during the drinking water chlorination and which organic compounds are responsible for their formation. Horth et al. studied the chlorination of amino acids and identified MX and EMX as chlorination products of tyrosine (10). However, the formation of these compounds, which represented only a minor proportion of the total products, could not be explained. Moreover, the levels of amino acids in water are generally too low to explain the mutagenicity produced during drinking water chlorination. In the present study we investigated the aqeuous chlorination of some 3,5-dihydroxybenzene structures. We will show that 3,5-dihydroxybenzaldehyde is a potential precursor of MX and EMX, and a mechanism for their formation will be given.

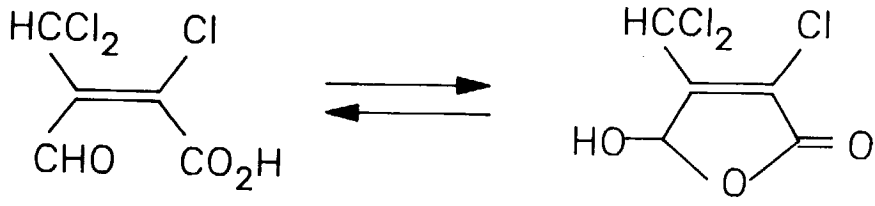

Fig. 1. Structure of the open and closed forms of MX.

6.2 Experimental Section

Chemicals. 3,5-Dihydroxybenzoic acid was obtained commercially from Aldrich and used as such. 3,5-Dihydroxybenzaldehyde was synthesized by trimethylsilylation and reduction of commercial methyl 3,5-dihydroxybenzoate (Aldrich) to 3,5-dihydroxybenzyl alcohol followed by a Jones oxidation to the resulting aldehyde (11). A mixture of 20 g methyl 3,5-dihydroxybenzoate and 2 mL trimethylchlorosilane in 40 mL hexamethyldisilazaan was refluxed for 5 hours and stirred overnight at room temperature. 100 mL of ether was added and the precipitate removed by filtration. The solvent was removed in a rotary evaporator and the residual oil was dissolved in 75 mL of THF. This solution was added slowly to a stirred suspension of 7 g $LiAlH_4$ in 300 mL of THF and the resulting mixture was refluxed for 5 hours and left overnight at room temperature. The mixture was hydrolyzed by the slow addition of aqueous ammonium chloride followed by concentrated hydrochloric acid. The mixture was filtered, and the residue washed with THF. The solvent was removed giving 17 g of 3,5-dihydroxybenzyl alcohol as a gray-white solid, mp 182^{0}C. 75 mL of 1.0 M Jones reagent was added dropwise to a solution of 10 g 3,5-dihydroxybenzyl alcohol in 100 mL of acetone. After 1 hour, 400 mL of ether was added and the resulting solution washed with aqueous sodium hydrogencarbonate. The aqueous phase was extracted twice with 100 mL of ether, and the combined ether fractions were dried. The solvent was removed giving 5 g of 3,5-dihydroxybenzaldehyde, a tan-colored solid, mp 146^{0}C.

Chlorination. Reagent grade chemicals were used and all solutions were prepared in Milli-Q water. Chlorinations were performed in 100 mL glass

stoppered bottles in the dark at room temperature. 100 mg of the 3,5-dihydroxy-substrate was dissolved in 90 mL of a 0.2M phosphate buffer with a pH of 7. A calculated amount of chlorine, in the form of a 0.65M NaOCl-solution, was added to establish a specific Cl_2-substrate ratio and the volume was ajusted to 100 mL with deionized water. After storage for 16 hours in the dark, the residual chlorine was destroyed by the addition of an excess of solid sodium arsenite. 15 g of sodium chloride was added and the reaction mixture was acidified to pH 1 with concentrated sulfuric acid. The mixture was extracted 3 times with 25 mL of glass-distilled diethyl ether. The combined ether extracts were stored at -20^{0}C for 4 hours to freeze out residual water and then concentrated in a Kuderna-Danish apparatus to about 5 mL.

Analyses. All determinations of MX and EMX were performed as described previously by Kronberg et al. (12) using a HP 5890 gas chromatograph interfaced with a HP 5970B mass selective detector. The extract was concentrated in a gentle stream of nitrogen to 2 mL and then transferred to a 5 mL reaction vial. 500 μL of a mucobromic acid solution (MBA, 500 mg/L in ethylacetate) was added as the internal standard and the solvent was removed by careful purging with nitrogen. The residue was methylated using 300 μL of 2% (v/v) sulphuric acid in methanol and by heating the mixture for 1 hour at 70^{0}C . After cooling and neutralizing with 600 μL of 2% sodium hydrogencarbonate the mixture was extracted twice with 600 μL of hexane. The combined hexane extracts were concentrated under nitrogen to about half of the original volume and 2 μL was injected in the GC. The GC was equipped with a 25 m, 0.2 mm i.d., HP-1 (film thickness 0.33 μm) fused silica capillary column and helium was used as carrier gas. The temperature settings were as follows; column oven programmed from 80^{0}C (0 min.) to 200^{0}C (10 min.) at 6^0/min; injector and transfer line, 280^{0}C. For the identification of the chlorination products the MSD was operated in the SCAN-mode (m/z=40 to 400). For the quantitative determination of MX and EMX the MSD was operated in the SIM-mode. Ion peaks monitored were; m/z=241 for MBA (internal standard); m/z=199, 201 and 203 for MX; m/z=241, 243, 245 and 247 for EMX. The dwell time was set at 60 ms and was equal for all masses. The identification of MX and EMX was based on positive matching of retention times and relative ion peak ratios.

Mutagenicity testing. Mutagenicity tests were performed according to Ames et al. (13) using *Salmonella typhimurium* strain TA 100 without

metabolic activation (S9 mix). The mutagenic response was calculated from the slope of the first part of the dose response curves.

6.3 Results and Discussion

Although MX and EMX are frequently measured in chlorinated waters and their mutagenic and chemical behavior is being studied, there is not much known about the mechanism of their formation. Kronberg suggested that MX could be an intermediate in the oxidation reactions in water (14) since it contained an aldehyde-group. The reduced form of MX, (RED-MX) in which the aldehyde-group is reduced to an alcohol, could then be the direct precursor of MX, while MX itself could be oxidized to a final product (OX-MX) in which the aldehyde-group is replaced by an acid-group. Kronberg also identified RED-MX and OX-MX in chlorinated drinking water. In the same way EMX can be an intermediate with RED-EMX as the precursor and OX-EMX as the final product. The structure of all the different MX and EMX forms and their mutual relations are given in figure 2.

From measurements of MX in chlorinated drinking water it is clear that there is a relationship between the amount of humic material in the water and the amount of MX formed. Unfortunately, little is known of the chemical structural properties of humic material. Chemical degradation studies of aquatic and soil humic material using extensive alkaline hydrolysis or oxidation with $KMnO_4$ or alkaline $CuSO_4$ have shown that the structure includes various phenols and phenolic acids such as resorcinol and 3,5-dihydroxybenzoic acid (15-17). Therefore these compounds were often selected as representative structures for the phenolic polymer core of humic materials as they are very efficient precursors of chloroform. There have been a few detailed studies of the reaction of chlorine with dihydroxybenzoic compounds. In 1890 Zincke (18) studied the chlorination of resorcinol in organic solvent and found that a pentachlororesorcinol was produced, which was subsequently hydrolyzed to a keto carboxylic acid upon addition to water. In a more recent investigation, Moye (19) proposed a somewhat modified degradation mechanism which is shown by the first two steps in figure 3. The production of chloroform from several resorcinolic compounds, including 3,5-dihydroxybenzoic acid, was described by Boyce and Hornig (20). In chlorination experiments with carbon-13 labelled resorcinol (indicated by an asterisk in fig. 3) they showed that the ring-carbon positioned between the two hydroxyl-groups is converted into chloroform. Further-

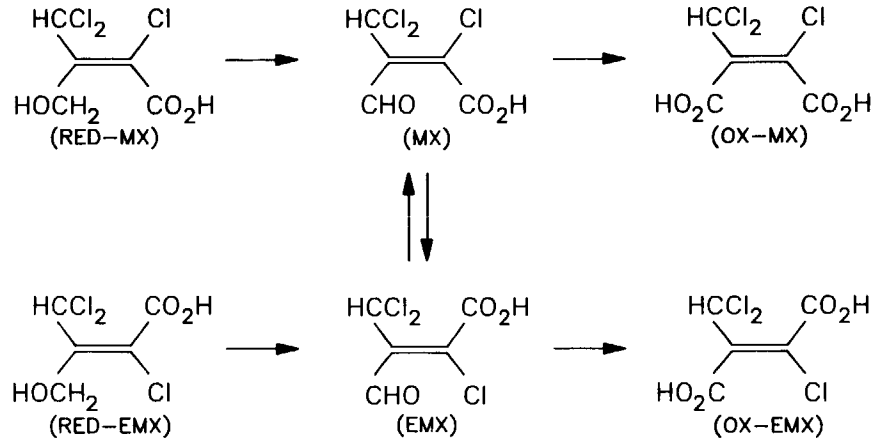

Fig. 2. MX and EMX, their oxidized and reduced forms, and their mutual relations.

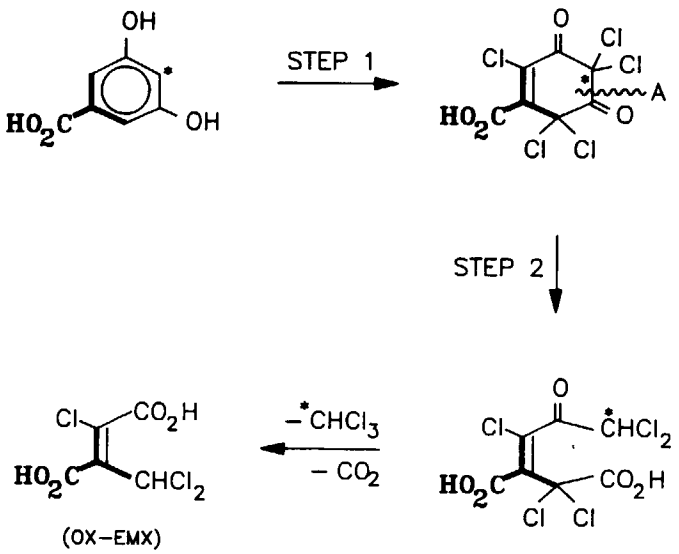

Fig. 3. The formation of OX-EMX from 3,5-dihydroxybenzoic acid. The chlorination of the resorcinol structure was already described by Moye (17). The part of the starting compound that remains intact during chlorination is indicated.

more, they showed that ring cleavage took place in position A.

With this information we envisaged a reaction pathway that explains the formation of OX-EMX from 3,5-dihydroxybenzoic acid (fig. 3). After the initial chlorination (step 1) and ring opening (step 2), an elimination of chloroform and a decarboxylation could result in the formation of OX-EMX. The elimination of chloroform is expected according to the findings of Boyce and Hornig (20). A decarboxylation is also reasonable since the acid-group is adjacent to an electron withdrawing CCl_2-group which promotes the decarboxylation (5). GC/MS analyses of the reaction products of the aqueous chlorination of 3,5-dihydroxybenzoic acid showed that OX-EMX was one of the major chlorination products. OX-MX was also identified but found in lower concentrations than OX-EMX. In the reaction pathway we assume that the acid group of 3,5-dihydroxybenzoic acid remains intact as is indicated in figure 3. This is an interesting assumption, for if the acid-group in 3,5-dihydroxybenzoic acid would be replaced by an aldehyde-group, then that compound could be a precursor for EMX and MX.

3,5-Dihydroxybenzaldehyde was chlorinated as before and the products analyzed. MX and EMX were both identified in the reaction mixture and are indicated in the gas chromatogram (fig. 4). While EMX is a major chlorination product, MX represents one of the smaller peaks in the chromatogram. The complete reaction pathway for the formation of MX is given in figure 5. After the initial chlorination, ring opening and elimination of chloroform, a decarboxylation takes place resulting in EMX. Finally, EMX slowly isomerizes to MX. This isomerisation, which is often reported in other studies, is in fact the last step in the formation of MX. Besides MX and EMX, OX-EMX was also identified as one of the minor chlorination products. This product probably originates from the oxidation of precursor material or EMX itself as was suggested by Kronberg (14).

According to the reaction scheme in figure 5, aqueous chlorination of 3,5-dihydroxybenzyl alcohol should result in the formation of the reduced forms of EMX and MX. Indeed, RED-EMX and RED-MX could be identified as two of the chlorination products, but MX, EMX, and OX-EMX could also be identified in the reaction mixture. An oxidation of the alcohol to an aldehyde, and finally to an acid, explains the production of these compounds. It is not clear however, during what stage of the chlorination this oxidation takes place.

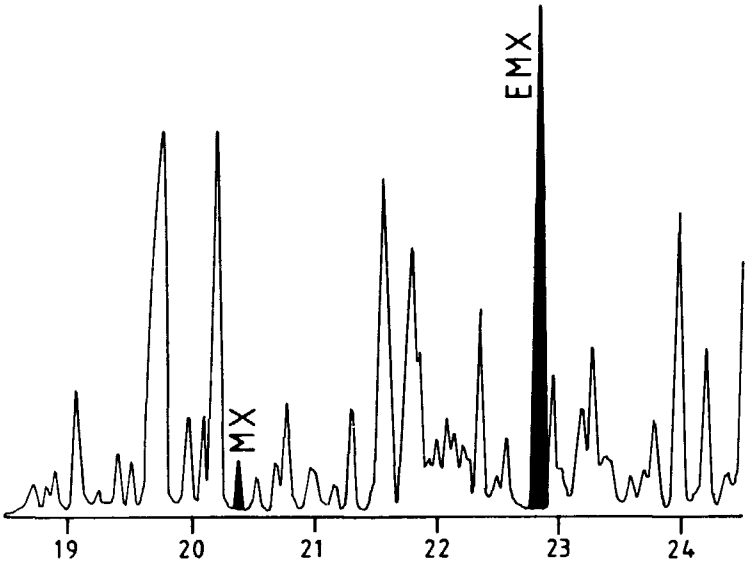

Fig. 4. The interesting part of the gas chromatogram of the methylated chlorination products of 3,5-dihydroxybenzaldehyde. The peaks of MX and EMX are indicated.

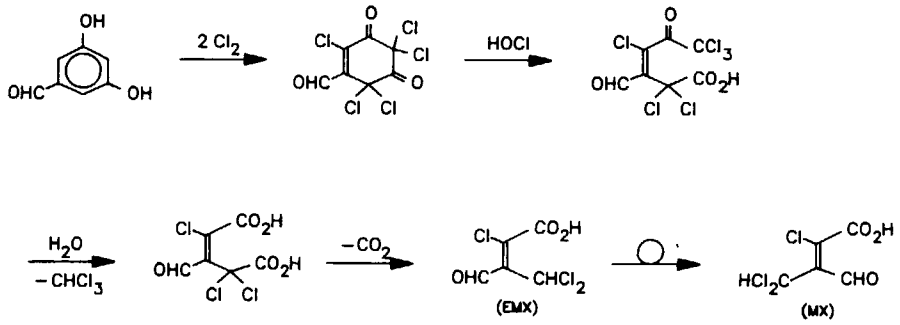

Fig. 5. The mechanism for the formation of EMX and MX from 3,5-dihydroxybenzaldehyde. The often reported isomerisation of EMX to MX is in fact the last step in the formation of MX.

The optimum Cl_2-dose for the production of MX was determined by aqueous chlorination of 3,5-dihydroxybenzaldehyde at Cl_2-substrate molar ratio ranging from 5 to 9. The results of these experiments are given in table I and figure 6. The production of MX and EMX was optimal at a Cl_2-substrate molar ratio of 6.5 with a yield of 0.125 and 0.475% respectively. At other ratios the production was lower and especially at higher Cl_2-substrate ratios the amount of MX and EMX decreased rapidly. The EMX/MX ratio was about 4 in all cases. Data of Kronberg (6), who analyzed chlorinated humic water samples (lake water with a TOC of 25 mg/L, reaction time 60 hours) show an EMX/MX ratio of about 2. These findings support the assumption that EMX is formed first and then isomerizes to MX.

The isomerisation of EMX to MX is an important step in the mechanism. To see if this isomerisation takes place in the chlorination mixture 3,5-dihydroxybenzaldehyde was chlorinated as before. After a reaction time of 16 hours any residual chlorine was destroyed, and the chlorination mixture acidified to pH 2. This was done to avoid the degradation of EMX at higher pH values as reported by Kronberg (6). One sample was analyzed directly and the mixture was then stored in the dark. Two more samples were analyzed after a period of several days and weeks. The results of the analysis are given in table I and figure 7 and show an almost quantitative isomerisation of EMX to MX.

MX is a strong Ames mutagen and is one of the most active mutagens ever tested in the Ames assay. Holmbom et al. (7,8) reported that the mutagenic activity of MX was between 2800 and 10000 revertants/nmole. Later Kronberg reported a TA100 activity of 5600 revertants/nmole (5), while Padmapriya et al. (9) gave a number of 6000 revertants/nmole. We also determined the mutagenicity of two chlorination mixtures of 3,5-dihydroxybenzaldehyde and compared that with the amount of MX in these mixtures. We found 6500 and 4800 revertants/nmole for the TA100 activity of MX, a number which is in good agreement with the activities reported earlier.

3,5-Dihydroxybenzaldehyde showed to be a potential precursor for MX, but can it also account for MX found in chlorinated drinking-, and humic water? To answer that question, we chlorinated a humic acid solution with a DOC of 200 mg/L, and found an MX concentration of 1 μg/L. Calculation of the yield of MX based on the DOC gives 5×10^{-6} mg MX/mg C, or 5×10^{-4}%. Calculation of the MX production from data of Kronberg

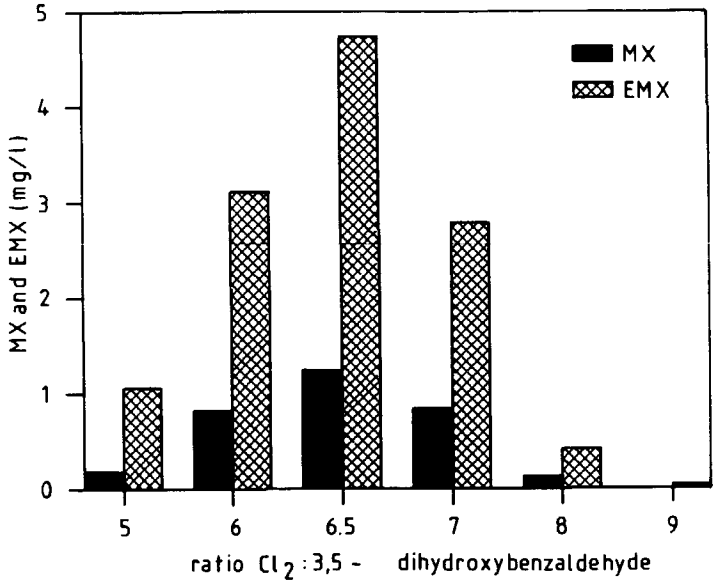

Fig. 6. The amounts of MX and EMX that are formed during chlorination of 3,5-dihydroxybenzaldehyde with several Cl_2-substrate molar ratio. The ratio EMX/MX is about 4.

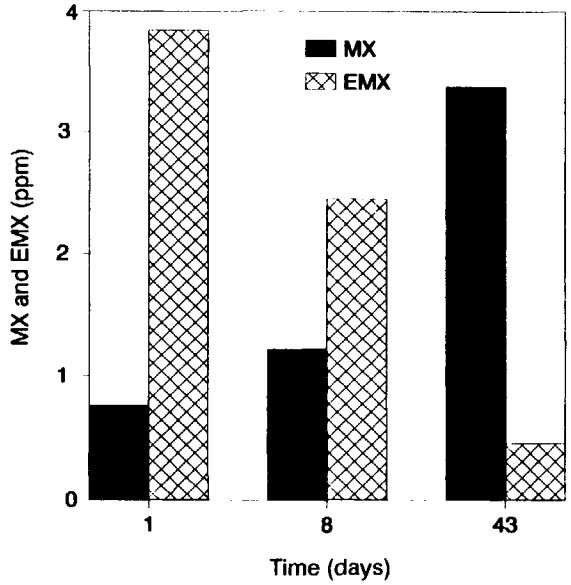

Fig. 7. The isomerisation of EMX to MX at pH 2 in the chlorination mixture during several days.

Table I. The concentrations of MX and EMX in the reaction mixture produced during aqueous chlorination of 3,5-dihydroxybenzaldehyde. Chlorination were performed at pH 7, in the dark and at roomtemperature. The reaction time was 16 hours.

Cl_2-substrate molar ratio	storage time (days)	MX (mg/L)	EMX (mg/L)	ratio EMX/MX
5.0	0	0.19	1.08	5.6
6.0	0	0.83	3.13	3.8
6.5	0	1.25	4.75	3.8
7.0	0	0.85	2.80	3.3
8.0	0	0.13	0.43	3.3
9.0	0	0.01	0.03	3.0
7.0	0	0.74	3.71	4.3
7.0	8	1.14	2.43	2.1
7.0	43	3.38	0.42	0.1

(6), who measured MX in several tap-, and humic waters in Finland, gave yields that were almost equal. Now, for pure 3,5-dihydroxybenzaldehyde 0.125 mg MX per 100 mg of the aldehyde was produced, giving a yield of 2.5×10^{-3} mg MX/mg C, or 2.5×10^{-1}%, so that the MX production from 3,5-dihydroxybenzaldehyde is 500 times higher then from humic acid. Consequently, if 3,5-dihydroxybenzaldehyde would be present in humic acid for only 0.2%, all MX formed during chlorination of drinking-, and humic water could be explained by this precursor. Unfortunately we do not know how much, if any, 3,5-dihydroxybenzaldehyde is present in humic acid. However, Morrison (21) found similar compounds like syringaldehyde, vanillin and p-hydroxybenzaldehyde in low yields (less than 2%) in peat humic acid. Also, 3,5-dihydroxybenzoic acid is a very common structure in humic acid. These observations make it reasonable to assume that 3,5-dihydroxybenzaldehyde constitutes a part of the humic structure or is formed as an intermediate in the oxidation of humic material, although the amounts may be low (less than 1%).

6.4 Conclusions

3,5-Dihydroxybenzaldehyde is an efficient precursor of MX and EMX and a reaction pathway for their formation is suggested. After the initial chlorination of the resorcinol derivative a ring opening followed by an elimination of chloroform and decarboxylation, results in EMX. A slow isomerisation of EMX then results in the formation of MX. Although 3,5-dihydroxybenzaldehyde is the logical MX precursor, 3,5-dihydroxybenzyl alcohol can also contribute to the amount of MX and EMX through oxidation of the alcohol, or the reduced MX and EMX forms that are produced upon chlorination. 3,5-Dihydroxybenzoic acid was a precursor for OX-MX and OX-EMX.

The isomerisation of EMX to MX was shown to be quantitative at pH 2 in the chlorination mixture. The TA100 activity of MX was determined to be 5500 rev./nmole.

A maximum MX production was found at Cl_2/3,5-dihydroxybenzaldehyde molar ratios of 6.5. A comparison with the MX production from chlorinated drinking-, and humic water showed that the production from 3,5-dihydroxybenzaldehyde was higher by a factor of 500. If 3,5-dihydroxybenzaldehyde is present in humic acid for 0.2% then this precursor can explain all MX found in chlorinated drinking-, and humic water.

6.5 Acknowledgements

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7

The Identification of Organobromine Compounds of Aqueous Chlorinated Humic Acid

Summary

The chlorination products and the mutagenic activity of humic acid chlorinated with and without bromide present have been compared. The presence of bromide ion results in the production of many brominated and mixed bromo/chloro compounds. Trihalomethanes, halogenated aliphatic acids and diacids, and trihalomethane precursors are major products. The presence of brominated compounds was also confirmed by the use of an atomic emission detector. The mutagenic activity was determined and is shown to be 2-3 times hihger when bromide is present during chlorination. Brominated analogues of MX and EMX were identified when an extract of 3,5-dihydroxybenzaldehyde, an efficient MX precursor, was chlorinated in the presence of bromide. Subsequently, the samples of chlorinated/brominated humic acid, were analysed for MX and its brominated analogues (BMX). When no bromide was present during chlorination MX accounted for the total mutagenic activity of the samples. However, if bromide was present during chlorination, BMX compounds were detected in addition to MX, and MX accounted for less than 50% of the total mutagenic activity. It was estimated that MX and the BMX compounds together accounted for 70 to 110% of the total mutagenicity of the samples, indicating that brominated MX analogues may be important mutagens in chlorinated drinking water if the raw water contains bromide.

7.1 Introduction

Chlorine is often used for the disinfection of drinking water. Due to its high reactivity, chlorine reacts very fast with many natural organic compounds, mainly humic materials, present in raw water. This results in the formation of numerous chlorination by-products and an increased mutagenic activity of chlorinated drinking water (1-3). The type and relative amounts of the chlorination by-products, varies not only with the organic content of the source water but also with the inorganic species present. If bromide is present, then brominated compounds like bromoform (and the other bromochloro trihalomethanes (THMs)), are also produced. During chlorination, bromide is oxidised by chlorine to bromine and chlorination and bromination become competitive reactions (4). In a study of the concentrations of THMs and dihaloacetonitriles (DHANs) in drinking water by Peters et al., it was shown that the major part of the THMs and DHANs found in Dutch drinking water were brominated (5). Furthermore, many of the volatile mutagens identified in chlorinated drinking water are brominated or mixed chloro/bromo compounds (6).

However the volatile halogenated compounds represent only a minor part of the total halogenated products, and these compounds account for only a small part (5- 10%) of the total mutagenic activity (6,7). The major part of the halogenated products is found in the non-volatile polar fraction, which is also responsible for most of the mutagenic activity. Studies of this fraction resulted in the identification of an extremely strong mutagen, 3- chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, often referred to as MX, that explains a large part (15-50%) of the mutagenic activity of chlorinated waters (8).

Untill now most studies of the non-volatile polar fraction have focused on chlorinated products and little is known about brominated and mixed chloro/bromo products. Recently, we reported that the major part of the halo-acetic acids (HAAs), typical representatives of the non-volatile polar fraction, found in Dutch drinking waters, are brominated (9). Aqueous chlorination of phenol in the presence of bromide also resulted in the formation of brominated and chloro/bromo acetic acids (10). These findings suggests that brominated compounds may form a substantial part of the chlorination products if bromide is present in the source water.

Brominated compounds may also contribute to the mutagenic activity of

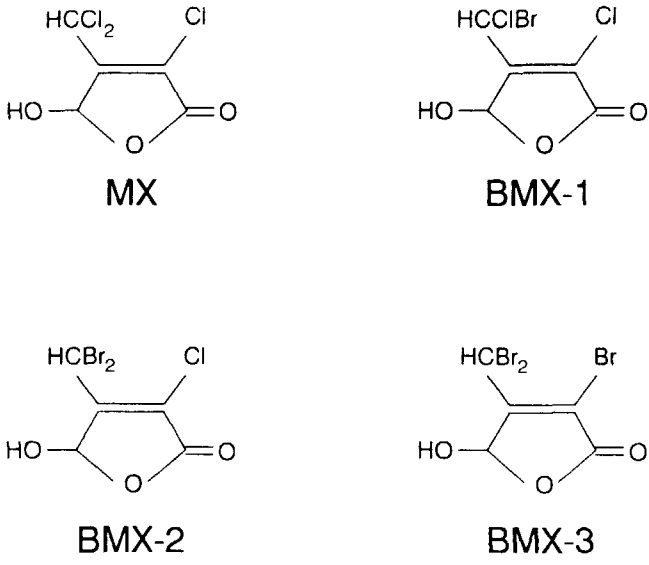

Fig. 1. The structures of MX and its brominated analogues BMX-1, BMX-2 and BMX-3.

chlorinated waters, as was already shown for the volatile fraction. Horth identified MX after the aqueous chlorination of tyrosine. However, if bromide was present during chlorination, three brominated analogues of MX, referred to as BMX, and a higher mutagenicity were found (11). The structures of these compounds, BMX-1, BMX-2, BMX-3 and MX are given in figure 1. These compounds were also synthesised, and were shown to be strong mutagens, similar to MX (11).

The aim of this study is to report the results of the aqueous chlorination of humic acid with and without bromide present. The chlorination products are tentatively identified and the mutagenic activity of the extracts of the reaction mixtures was determined. Furthermore, 3,5-dihydroxybenzaldehyde, a strong MX-precursor (12), was also chlorinated in the presence of bromide to identify brominated analogues of MX and EMX. The chlorinated/brominated humic acid extracts were analyzed for MX and BMX, and their contribution to the total mutagenic activity was estimated.

7.2 Experimental Section

Materials. Reagent grade chemicals were used and all solutions were prepared in Milli-Q water. Humic acid was obtained commercially from Fluka AG. 3,5-Dihydroxybenzaldehyde was synthesised as described before (12).

Chlorination. 400 mg of humic acid was dissolved in 500 mL of a 0.02M NaOH- solution and stirred overnight. After neutralizing the mixture with hydrochloric acid it was filtered through glass fiber filters. The resulting humic solution was mixed with 200 mL of a 1.0M phosphate buffer of pH 7. A 0.05M NaBr-solution was added to achieve bromide to chlorine molar ratios of 0, 0.05 and 0.10, and the total volume was adjusted to about 900 mL with deionized water. Then a calculated amount of chlorine, in the form of a 0.5M NaOCl-solution, was added to establish Cl_2/C molar ratios of 0.5 and 2.0, respectively. Finally the volume was ajusted to 1000 mL with deionized water, and the bottles stored in the dark. After a reaction time of 16 hours any excess of chlorine was destroyed by the addition of solid sodium arsenite. The reaction mixture was saturated with sodium chloride and acidified to pH 0.5 with concentrated sulfuric acid. The mixture was extracted 3 times with 100 mL of glass-distilled diethyl ether. The combined ether extracts were stored overnight at -20^{0}C to freeze out residual water and concentrated in a Kuderna-Danish apparatus to 5 mL. The extracts were then carefully purged with nitrogen untill dryness. The residue was redissolved in 10.0 mL of ethyl acetate and divided into two portions to be used for the mutagenicity testing and GC/MS analysis, respectively. The samples were stored at -20^{0}C. For the identification of brominated MX and EMX analogues, 100 mg of 3,5-dihydroxybenzaldehyde was dissolved in 80 mL of a 0.2M phosphate buffer of pH 7. Calculated amounts of a 0.05M NaBr-solution and a 0.5M NaOCl-solution were added in that order to obtain a Cl_2/C molar ratio of 1.0 and a bromide to chlorine molar ratio of 0.2. The volume was ajusted to 100 mL with deionized water and the reaction mixture stored in the dark. After a reaction time of 16 hours, residual chlorine was destroyed by the addition of solid sodium arsenite. 15 g of sodium chloride was added and the mixture acidified to pH 1 with concentrated sulfuric acid. The mixture was extracted 3 times with 25 mL of glass-distilled diethyl ether. The combined ether extracts were stored at -20^{0}C for 4 hours to freeze out residual water and then concentrated in a Kuderna-Danish apparatus to about 2 mL.

GC/MS analysis of chlorination/bromination products. Prior to analysis the chlorinated humic acid extracts were methylated with diazomethane. To 2 mL of the extracts 2 mL ethyl acetate and 0.2 mL of methanol was added to aid methylation. 1-Chlorododecane was added as an internal standard and the samples were methylated with diazomethane. Diazomethane gas was generated fresh from N-methyl-N-nitroso-p-toluenesulfonamide (Merck) and was stripped from the generation vessel by nitrogen gas into the sample vials. The samples were analyzed by GC/MS with a HP 5890 gas chromatograph interfaced with a HP 5970B mass selective detector. The GC was equipped with a 25 m, 0.2 mm i.d., HP-1 (film thickness 0.33 μm) fused silica capillary column and helium was used as the carrier gas. The temperature settings were as follows; column oven programmed from 50^{0}C (5 min.) to 300^{0}C (10 min.) at 8^0/min; injector and transfer line, 280^{0}C. For the identification of the chlorination products the MSD was operated in the SCAN-mode (m/z=40 to 400). The extract of chlorinated 3,5-dihydroxybenzaldehyde was methylated with methanol/sulfuric acid since methylation using diazomethane results in the loss of MX. The extract was transferred to a 5 mL reaction vial and the solvent was removed by careful purging with nitrogen. The residue was methylated using 300 μL of 2% (v/v) sulphuric acid in methanol and by heating the mixture for 1 hour at 70^{0}C. After cooling and neutralizing with 600 μL of 3% sodium bicarbonate the mixture was extracted twice with 600 μL hexane. The combined hexane extracts were concentrated under nitrogen to about half of the original volume. The sample was analyzed with GC/MS under the same conditions as before.

GC/AED analysis of the chlorination/bromination products of humic acid. The methylated extracts of chlorinated humic acid were also analyzed with an atomic emission detector (AED) (13). The samples were analyzed with a HP 5890 gas chromatograph interfaced with a HP 5921A atomic emission detector. The GC was equipped with a 50 m, 0.2 mm i.d., HP-1 (film thickness 0.33 μm) fused silica capillary column and helium was used as the carrier gas. The temperature settings were the same as with the GC/MS analyses. With the AED four elements, carbon, hydrogen, chlorine and bromine were monitored simultaneously at 495.7, 486.1, 479.5 and 478.6 nm, respectively. The plasma power was 50 W and the additional makeup flow 40 mL/min of helium.

Analysis of MX and BMX in chlorinated/brominated humic acid. MX and three BMX compounds, BMX-1, BMX-2 and BMX-3 were analyzed in the extracts of chlorinated humic acid. The method described by Hemming et al. (14) was used with some modifications for the determination of MX and BMX analogues. 1 mL of the extract was transferred to a 5 mL reaction vial. 500 μL of a mucobromic acid solution (MBA 50 mg/L in ethyl acetate) was added as the internal standard and the solvent was removed with a gentle stream of nitrogen. The residue was methylated in the same way as the chlorination extracts of 3,5-dihydroxybenzaldehyde. The combined ether extracts were concentrated under nitrogen to a volume of about 100 μL, and 2 μL was injected in the GC. The GC was equipped with a 25 m, 0.2 mm i.d., HP-1 (film thickness 0.33 μm) fused silica capillary column with helium as carrier gas. The temperature settings were as follows; column oven programmed from 50^{0}C (1 min.) to 300^{0}C (5 min.) at 8^0/min; injector and transferline, 280^{0}C. For the quantitative determination of MX and BMX the MSD was operated in the SIM-mode. Ion peaks monitored were; m/z=241 for MBA (internal standard); m/z=199, 201, 203 for MX; m/z=195, 197, 199 for BMX-1; m/z=239, 241, 243 for BMX-2; m/z=283, 285, 287 for BMX-3. The dwell time was set at 100 ms and was equal for all masses. The identification of MX and BMX was based on positive matching of retention times and relative ion peak ratios.

Mutagenicity testing. Mutagenicity tests were performed according to Ames et al. (15) using *Salmonella typhimurium* strain TA 100 without metabolic activation (S9 mix). The mutagenic response was calculated as the slope of the first linear part of the dose response curves.

7.3 Results and Discussion

Bromide is often present in raw water, either from natural or anthropogenic sources. In Dutch waters bromide concentrations vary from 0.1 to 0.5 mg/L. During chlorination of the raw water bromide ion is oxidised to bromine. Bromine seems to be more effective as a halogen-substituting agent (16) and if bromine acts as an oxidant, it will be reduced to bromide ion, which may then be reoxidized by chlorine. This results in a high bromine incorporation into the well known THMs and other halogenated species. In contrast with the THMs there is only very little information about the formation of non-volatile brominated compounds, and there has been no systematic study of

these products. Therefore, we studied the aqeuous chlorination of humic acid in the presence of bromide and tried to identify the major products and mutagens of the reaction.

The chlorination/bromination of humic acid at pH 7 was performed with chlorine to carbon molar ratios of 0.5 and 2.0, and bromide to chlorine molar ratios of 0.00, 0.05 and 0.10, respectively. Chromatograms of two of the high chlorine dose experiments are given in figure 2a (Br /Cl_2 molar ratio = 0) and 2b (Br /Cl_2 molar ratio = 0.10), and show that many products were formed. Dominant products identified after chlorination of humic acid without bromide, were chlorinated aliphatic acids and diacids, especially dichloro-, and trichloroacetic acid, chloro-, and dichloromaleic acid, and dichlorosuccinic acid. Several chloroform precursors, previously identified by De Leer et al. (2), were also found. Non-chlorinated products include aliphatic diacids and aromatic acids, as well as small quantities of methyl-substituted aromatic acids and aromatic glyoxylic acids. Generally, these results agree well with those of earlier studies (1-3). If bromide was present during chlorination the chlorinated products were present in lower amounts, and in addition many brominated products with similar structures were identified. Although dichloro-, and trichloroacetic acid are still major products, brominated and mixed bromo/chloro acetic acids are also important products. The same is true for the halogenated diacids. A noticeable difference is the presence of several halogenated alkanes. These include not only the THMs, but also dibromoethane and chlorobromo-, and dibromopropane. The latter compounds have also been identified in mutagenic extracts of chlorinated drinking water and were shown to be active mutagens (6). Interesting also is the tentative identification of several THM precursors. The structures of these compounds are similar to the chloroform precursors that were identified by De Leer et al. (3), and they are major products in the reaction mixture. The non- chlorinated products that were identified were the same as in the chlorination experiment without bromide. All products that were identified are given in table I. The compound numbers correspond with those in the chromatograms of figures 2 and 3.

The reaction products were identified with GC/MS, however, additional information concerning the elemental composition of the chlorination products, was obtained by the use of an atomic emission detector (AED). The AED is an element-specific detector, and thus is able to discriminate be-

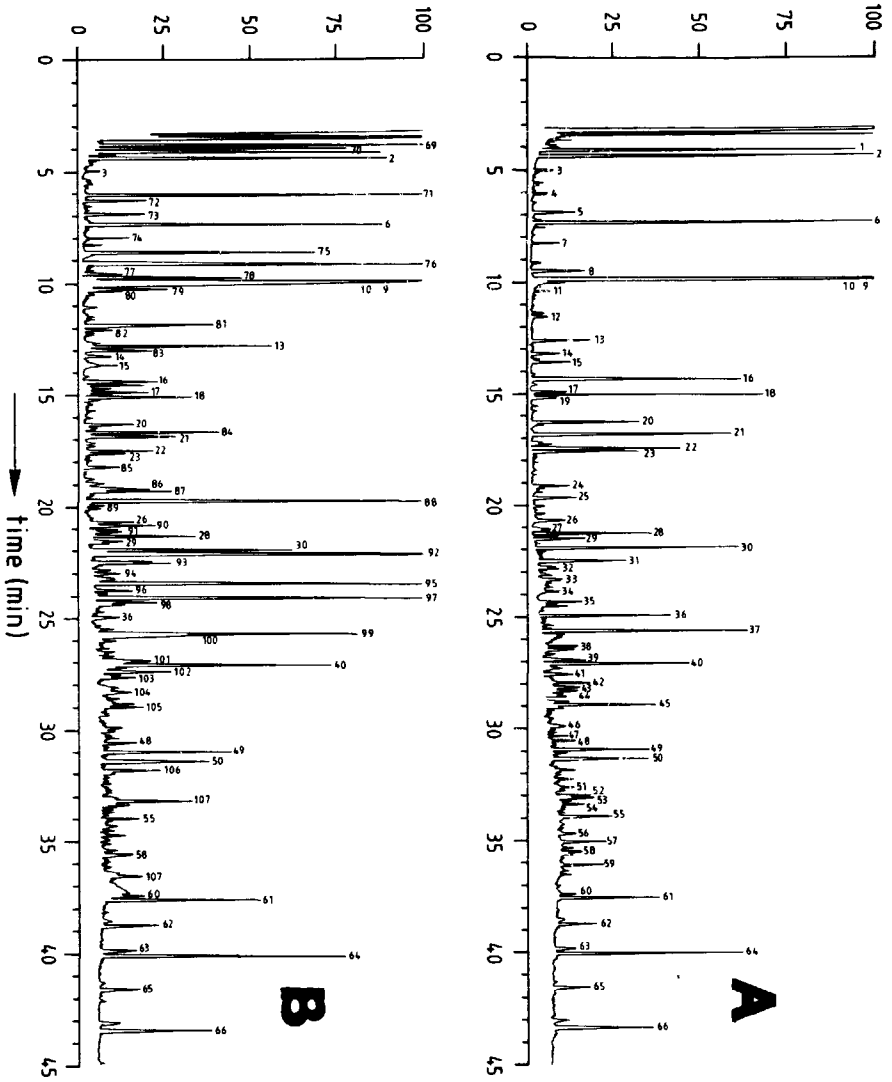

Fig. 2. Chromatograms of two of the high chlorine dose experiments without (a), and with (b), bromide present during chlorination of humic acid. The peak numbers refer to the numbers in table I.

Table I. Compounds identified after chlorination/bromination of humic acid. Peak numbers refer to peak numbers in the chromatograms of figures 2 and 3.

Peak	Compound	ID[1]	RRT[2]
1	ethyl propionate	SC	0.128
2	l-butanol	SC	0.200
3	chloroethanoic acid	SC	0.227
4	2-chloropropenoic acid	C	0.277
5	proanedioic acid	SC	0.314
6	dichloroethanoic acid	SC*	0.336
7	trichloroethanal	SC	0.378
8	1,1,1-trichloro-2,3-epoxypropane	C	0.434
9	trichloroethanoic acid	SC*	0.449
10	trimethylphosphate	SC	0.458
11	3,3-dichloropropenoic acid	C	0.476
12	2-hydroxypropanoic acid	C	0.529
13	butenedioic acid	SC	0.576
14	chloropropanedioic acid	SC	0.604
15	3,3,2-trichloropropenoic acid	T	0.614
16	chlorobutenedioic acid	SC	0.656
17	chlorobutanedioic acid	SC	0.682
18	dichloropropanedioic acid	SC*	0.688
19	3,3,3-trichloro-2-hydroxypropanoic acid	T	0.693
20	2,3-dichloro-4-oxopentenoic acid	T	0.744
21	2,2-dichlorobutanedioic acid	SC	0.767
22	2,3-dichlorobutenedioic acid	SC	0.798
23	isomer of compound 22	SC	0.803
24	dichlorinated compound	-	0.874
25	2,2-dichloropentanoic acid	C	0.898
26	methylfurandicarboxylic acid	C	0.944
27	benzenedicarboxylic acid	SC	0.965
28	2,3-dimethoxybutenedioic acid	T	0.971
29	2-chloro-3-dichloromethyl-butenedioic acid	SC	0.981
30	l-chlorododecane	SC	1.000
31	isomer of compound 27	SC	1.027
32	2,3,4,4-tetrachloro-2-pentenedioic acid	T	1.041
33	4-oxoheptanoic acid	T	1.068
34	trichlorinated compound	-	1.089
35	2-carboxy-3,5,5,5-tetrachloro-4-oxo-pentanoic acid	T	1.100
36	2,3,3,5,5,5-hexachloro-4-hydroxy-pentanoic acid	T	1.137

Peak	Compound	ID[1]	RRT[2]
37	isomer of compound 35	T	1.169
38	trichlorinated compound	-	1.207
39	2,3-dicarboxybutenedioic acid	T	1.229
40	benzenetricarboxylic acid	SC	1.235
41	phthalate	T	1.257
42	isomer of compound 40	-	1.275
43	trichlorinated compound	-	1.284
44	methylbenzenetricarboxylic acid	C	1.291
45	trichlorinated compound	-	1.321
46-53	benzenetracarboxylic acid and glyoxylic acid isomers of 46	C	1.364
54-58	benzenepentacarboxylic acid and glyoxylic acid isomers of 54	C	1.523
59-68	fatty acids and alkanes	C	
69	dibromomethane	SC*	0.174
70	bromodichloromethane	SC*	0.179
71	chlorodibromomethane	SC*	0.274
72	1,2-dibromoethane	SC*	0.287
73	bromoethanoic acid	SC*	0.314
74	1,3-bromochloropropane	T*	0.363
75	tribromomethane	SC*	0.393
76	2-chlorobutenoic acid	T*	0.416
77	isomer of compound 76	T*	0.437
78	bromochloroethanoic acid	SC*	0.443
80	1,2-dibromopropane	T*	0.471
81	dibromoethanoic acid	SC*	0.540
82	1,2-bromochloropropanoic acid	T	0.550
83	butanedioic acid	SC	0.588
84	tribromoethanoic acid	SC*	0.759
85	dibromopropanedioic acid	T	0.831
86	bromochlorobutenedioic acid	T	0.876
87	isomer of compound 86	T	0.879
88	2,4-dichloro-2-bromo-3-oxobutanoic acid	T*	0.899
89	2-carboxy-3-bromobutanoic acid	T	0.909
90	dibromobutenedioic acid	T	0.951
91	2-chloro-3-bromobutanedioic acid	T	0.965
92	2,5-dichloro-4-bromo-3-oxopentanoic acid	T*	1.008
93	2-chloro-4-bromo-3-oxopentanedioic acid	VT	1.025
94	not-halogenated compound	-	1.028
95	4,5-dibromo-2-chloro-3-oxopentanoic acid	VT*	1.067
96	not-halogenated acid	-	1.082
97	2,5-dibromo-6-chloro-3-oxopentanoic acid	VT*	1.097

Peak	Compound	ID[1]	RRT[2]
98	halogenated acid	-	1.107
99	X-CO-CHBr-CH_2-COOH	T	1.171
100	X-CO-CHCl-COOH	T	1.174
101	X-CO-CHBr-CH_2-COOH	T	1.226
102	X-CO-CHBr-CH_2-CH_2-COOH	T	1.249
103	dibrominated compound	-	1.262
104	phthalate	T	1.313
105	halogenated acid	-	1.321
106	X-CO-CHCl-COOH	T	1.449
107	X-CO-CHCl-COOH	T	1.513

[1]; SC=Standard Confirmed; C=Confident; T=Tentative; VT=Very Tentative.
[2]; Retention time relative to the internal standard 1-chlorododecane.
*; Elemental composition confirmed by GC/AED.

tween chlorinated, brominated and mixed chloro/bromo compounds. In GC/AED the GC-effluent enters a high-temperature microwave induced helium plasma. The organic compounds are first decomposed into their elemental constituents, and then the atoms are exited to a higher energy level. Once a sufficient number of atoms is exited, atomic emission signals can be recorded at element specific wavelengths. In our case the emissions of bromine, chlorine, carbon and hydrogen were recorded simultaneously. A multi-element chromatogram of the high chlorine dose experiment with bromide, is given in figure 3. The peak heights of trichloro-, and dibromoacetic acid, and the internal standard n-chlorododecane, were used to calculate the relative elemental response factors and thus the empirical formula's of the compounds. The results of the GC/AED analysis generally confirmed the MS-interpretations.

The mutagenic activity of the samples was also determined to see if the addition of bromide resulted in an increased TA-100 acitivity. In general, the results in the Ames test without metabolic activation showed that the samples of the high chlorine dose experiments were 2 times more mutagenic than those of the low chlorine dose experiments. In both cases however, the

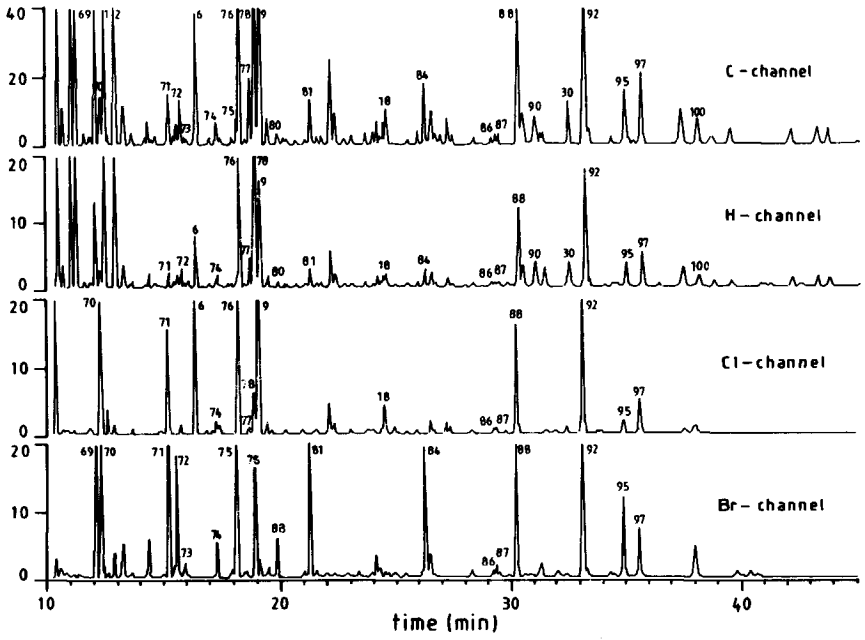

Fig. 3. The multi-element chromatogram of the GC/AED analysis of a sample of chlorinated/brominated humic acid. The emissions of carbon, hydrogen, chlorine and bromine were recorded simultaneously and are shown in that order. Compounds that were identified with GC/AED are indicated in table I.

addition of bromide during chlorination led to a 2-3 times higher mutagenic activity of the samples. When the Ames test was performed with metabolic activation (S9-mix) the mutagenic activity was substantially lower. The mutagenicity of the samples is shown in figure 4.

The most important mutagen identified in chlorinated drinking water so far is MX (17,18). However, analysis of chlorinated drinking water showed that many brominated compounds were present and that the majority of the volatile mutagens was in fact brominated (5,6,9). Therefore it may be considered likely that brominated MX and EMX analogues, BMX and

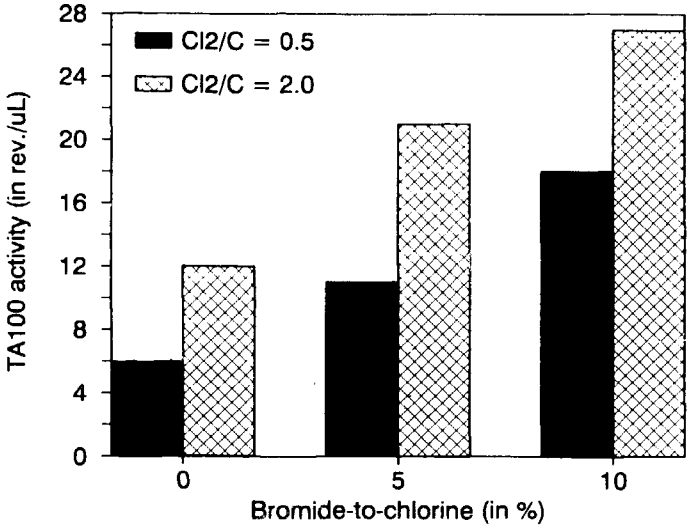

Fig. 4. The mutagenic activity (TA100) in the Ames test without metabolic activation of the chlorinated/brominated humic acid extracts. The chlorine-to-carbon and bromide-to-chlorine ratios are indicated.

BEMX, are also formed. In a recent study we found that 3,5-dihydroxybenzaldehyde is an efficient precursor of MX and EMX. We chlorinated this compound in the presence of bromide and analysed the extract with GC/MS, to see if any BMX and BEMX compounds would be formed. The results of the GC/MS analysis showed that in addition to MX and EMX brominated analogues were produced also. In principle five brominated MX and EMX analogues may be formed. However, in the extracts only three BMX compounds, BMX-1, BMX-2 and BMX-3, could be identified with certainty. The structure of these compounds was already shown in figure 1. Their identification was confirmed by comparison with mass spectra of Horth. In addition to the BMX compounds, the isomeric BEMX compounds were tentatively identified. A part of the chromatogram of the extract of 3,5-DHBA is shown in figure 5. The peaks of the BMX and BEMX compounds are indicated, while the mass spectra of the methylated BMX and BEMX

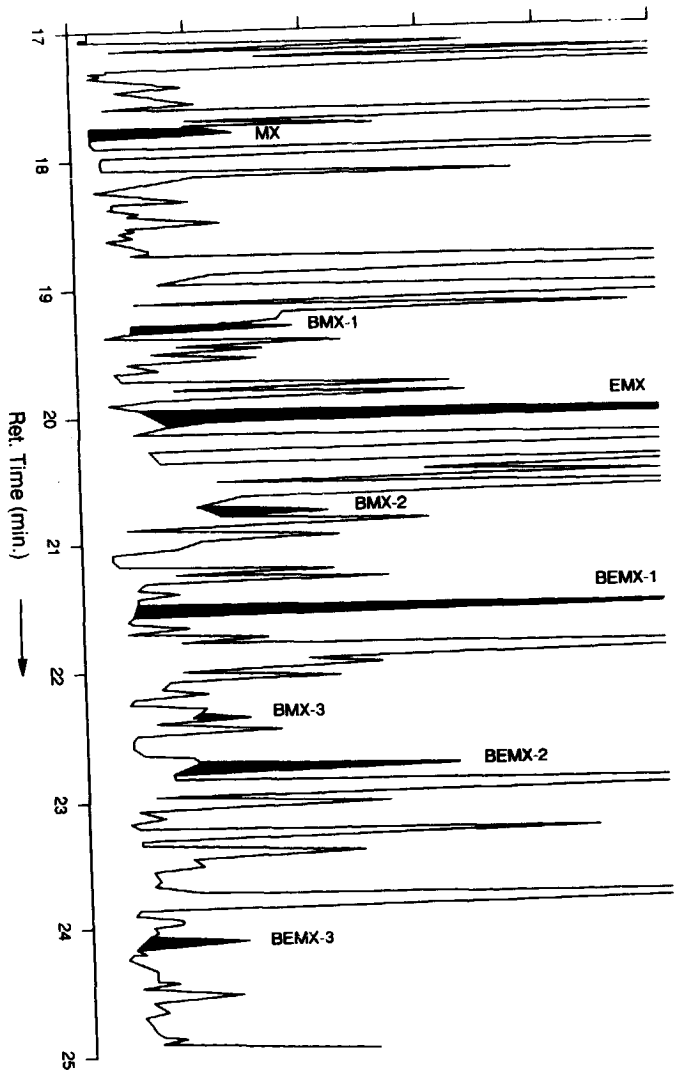

Fig. 5. Part of the chromatogram of the GC/MS analysis (scan mode) of chlorinated/brominated 3,5-dihydroxybenzaldehyde showing the peaks of MX, BMX-1, BMX-2 and BMX-3, and their isomeric EMX forms.

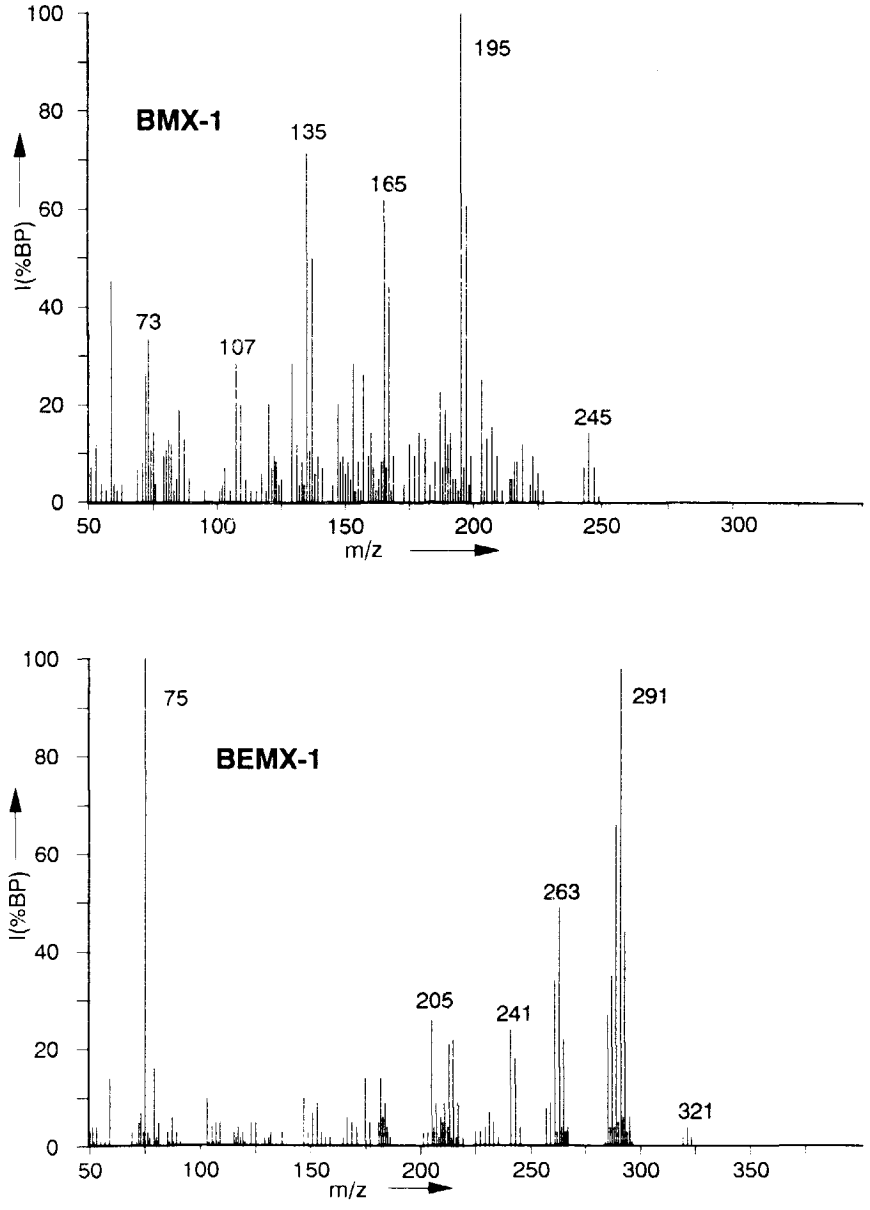

Fig. 6a. The mass spectra of methylated BMX-1 and BEMX-1.

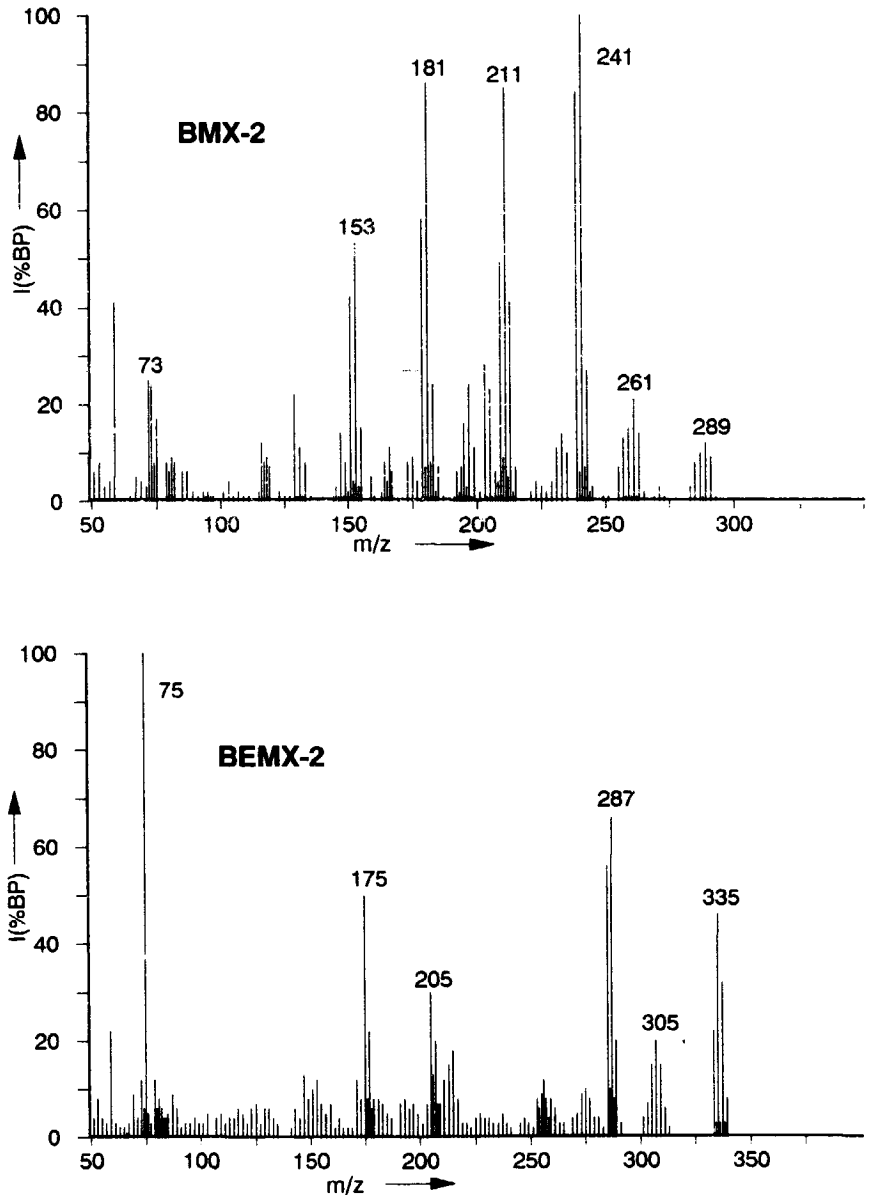

Fig. 6b. The mass spectra of methylated BMX-2 and BEMX-2.

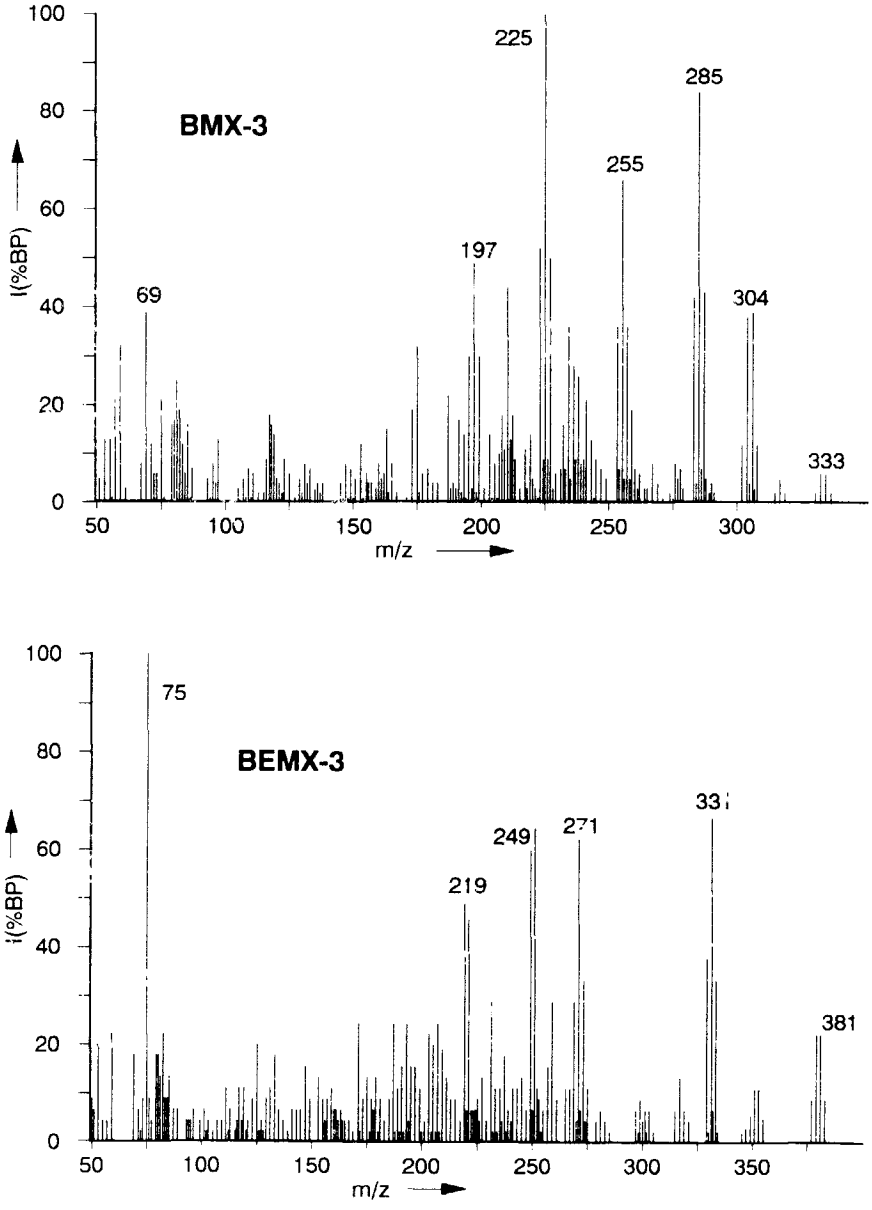

Fig. 6c. The mass spectra of methylated BMX-3 and BEMX-3.

compounds are given in figure 6.

The halogenated compounds identified after chlorination of humic acid with and without bromide, which are given in table I, were not considered to be responsible for the total mutagenic activity of the samples. Since MX is formed mainly as a result of the reaction of chlorine with humic materials, MX was expected to be present in the samples. In samples where bromide was present during chlorination BMX compounds may be expected also. This was also suggested by the results of the Ames test with metabolic activation, which showed a diminished activity, a result that is typical for MX. Therefore, a part of the extracts of chlorinated/brominated humic acid was methylated using methanol/sulfuric acid and was analysed with GC/MS in the SIM-mode for MX, BMX-1, BMX-2 and BMX-3. Since the pure BMX compounds were not available, only MX could be quantified correctly in the samples. The relative respons factor of the BMX compounds was expected to be comparable and therefore set to 1.00. In this way at least a rough etimate of their concentrations in the samples is found. In the samples where no bromide was present during chlorination only MX was observed.

Table II. MX and BMX analogues found in the extracts of humic acid chlorinated in the presence of bromide.

Sample	Cl_2/C molar ratio	% Br rel. to Cl_2	MX mg/L	BMX-1 mg/L	BMX-2 mg/L	BMX-3 mg/L
1	0.5	0	0.2	-	-	-
2	2.0	0	0.5	-	-	-
3	0.5	5	0.2	0.1	0.1	-
4	2.0	5	0.2	0.3	0.2	0.1
5	0.5	10	0.2	0.2	0.2	0.1
6	2.0	10	0.4	0.3	0.3	0.2

- = not detected

In the other samples MX was found together with the brominated analogues BMX-1, BMX-2 and BMX-3, depending on the amount of bromide added. With a bromide-to chlorine molar ratio of 0.1 the amounts of MX, BMX-1 and BMX-2 are comparable, while the amount of BMX-3 is somewhat lower. The results are given in table II.

Horth studied the mutagenic activity of BMX and found that they were strong Ames mutagens, comparable to MX (11). The TA100 activity of MX is reported in several studies and was found to be 5600 rev/nmole, or 26 rev/ng (8). Horth reported that BMX-2 and BMX-3 were 1.3 times more mutagenic than MX, while BMX-1 was found to be only 0.1 times as mutagenic as MX. We used these numbers and the concentrations in table II, to estimate the contributions of MX and the BMX compounds to the total mutagenic activity of the samples. The results of these calculations are given in table III. In samples 1 and 2, where no bromide was present during chlorination, MX accounted for 80 and 110% of the observed mutagenicity of the samples. However, in the other samples, where bromide was present during chlorination, MX could explain no more than 50% of the total mutagenic activity. The calculation of the contribution of the individual BMX compounds showed that these compounds may be responsible for the remaining part of the mutagenic activity. In all cases, the sum of the activities of MX, BMX-1, BMX-2 and BMX-3, could explain 70 to 110% of the total mutagenicity of the samples.

Table III. The contribution of MX and BMX analogues to the total mutagenic activity (TA100) of the extracts of chlorinated/brominated humic acid.

Sample	measured TA100 activity rev/μL	calculated contributions of MX rev/μL	BMX-1 rev/μL	BMX-2 rev/μL	BMX-3 rev/μL	total/ measured activity in %
1	6	5	-	-	-	83
2	12	13	-	-	-	108
3	11	5	0	3	-	73
4	21	5	1	7	3	76
5	18	5	0	7	3	83
6	27	10	1	10	6	100

- = not detected

MX is formed during disinfection of drinking water mainly as a result of the reaction of chlorine with humic materials. The identification of many brominated compounds, among which BMX, after aqueous chlorination of humic acid in the presence of bromide, suggests that BMX may also be

present in finished drinking waters. However, these compounds were never analysed and their presence, not to mention their concentrations, in finished drinking waters is unknown. Therefore, the individual BMX compounds have to be studied, and a method for their analysis in real drinking water samples has to be developed. This has to be a part of future work in this area.

7.4 Conclusions

The chlorination of humic acid in the presence of bromide resulted in the production of many brominated and mixed bromo/chloro compounds. The type of compounds formed is similar to previously identified chlorinated products. Trihalomethanes, halogenated aliphatic acids and diacids, and trihalomethane precursors were major chlorination products. The presence and elemental composition of several of the brominated compounds was also confirmed by the use of an atomic emission detector. Three brominated analogues of MX, BMX-1, BMX-2 and BMX-3, and the isomeric brominated analogues of EMX could be identified in the extract of 3,5-dihydroxybenzaldehyde, when chlorinated in the presence of bromide. The samples of chlorinated/brominated humic acid were analysed for MX and its brominated analogues. When no bromide was present during chlorination, MX accounted for the total mutagenic activity of the samples. If bromide was present during chlorination, the mutagenic activity increased significantly and could be attributed for less than 50% to MX itself. However, brominated analogues were also identified and may account for the remaining mutagenicity of the samples. The results of this study indicate that brominated organohalogen compounds are important disinfection by-products and that brominated MX analogues may explain that part of the mutagenic activity of chlorinated drinking water that is not accounted for to date.

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Summary

Chlorine is used for the disinfection of drinking water since the beginning of this century and has made a substantial contribution to the maintenance of public health. However, in 1974 it was found that trihalomethanes (THMs) were produced during chlorine disinfection of drinking water, as a result of the reaction of chlorine with humic materials. This discovery, and the development of advanced analytical techniques, led to the identification of many other chlorination by-products. Chlorination of drinking water also results in an increased mutagenic activity of finished drinking waters, which indicates the presence of compounds that may be hazardous to the health of the consumer. The nature and extent of the reaction of chlorine with organic substances in water, and the consequences of this reaction, are described in chapter 1.

Chapter 2 describes the aqueous chlorination of cyanoacetic acid. While chlorinated acids were the end products of the reaction, several N-chloro amides and dichloroacetonitrile were found as intermediates. The latter compound also showed to be responsible for the observed mutagenic activity of the reaction mixture. Dihaloacetonitriles (DHANs) are active mutagens, whose presence in Dutch drinking waters was unknown. Therefore, we developed a method to analyse these compounds together with the better known and more frequently measured THMs. The results are presented in chapter 3. In most cases the brominated and mixed chloro/bromo compounds were in the majority. Brominated analogues of chlorination by-products are formed when bromide is present in the raw water.

Some chlorination products are explained by the chlorination of amino acids. However, most amino acids are found covalently linked in proteins which may be in free form or bound to humic materials. Chapter 4 shows the results of a study of the chlorination of proteins and the protein fraction of humic materials. Although the reactivity of proteins towards chlorine is generally considered to be low, many chlorinated and non-chlorinated products were formed. Protein material seems to contribute significantly to the chlorination produts of humic material.

THMs represent only a minor part of the total halo-organics while the larger part exists as non-volatile polar compounds. The dominant non-volatile compounds are halo-acetic acids (HAAs), and chapter 5 describes a method for their analysis. Several Dutch drinking waters, prepared from different source waters were analyzed and HAAs were found to be present in

all drinking waters prepared from surface water. As with the DHANs and THMs, brominated and chloro/bromo HAAs were the most prominent in chlorinated drinking waters.

Chlorine disinfected drinking waters are known to contain the Ames-mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, often referred to as MX. It is an extremely strong mutagen, responsible for a significant part of the total mutagenic activity of chlorinated water. In chapter 6 it is demonstrated that 3,5-dihydroxybenzaldehyde, a structure expected to be present in humic acid, is an efficient precursor for MX and its geometric isomer EMX. A possible pathway for their formation was suggested. While many compounds identified in chlorinated drinking water are brominated, most studies focussed on chlorinated products. Chapter 7 presents the results of a study of the chlorination of humic acid in the presence of bromide. GC/MS analysis resulted in the identification of more then 100 compounds while chlorinated and brominated compounds could also be detected selectively by the use of an atomic emission detector. The mutagenic activity was found to be 2 to 3 times higher when bromide was present during chlorination. When no bromide was present during chlorination MX accounted for the total mutagenic activity of the samples. However, if bromide was present during chlorination, brominated MX analogues were detected in addition to MX. It was estimated that MX together with its brominated analogues accounted for the total mutagenicity of the samples. The results indicate that brominated compounds are important chlorination by-products, and that brominated MX analogues may contribute significantly to the mutagenic activity of chlorinated drinking water.

Samenvatting

Sinds het begin van deze eeuw wordt chloor gebruikt als desinfectiemiddel bij de drinkwaterbereiding, wat heeft geleid tot de productie van hygienisch betrouwbaar drinkwater. Dat de chlorıng van drinkwater ook ongewenste effecten heeft bleek in 1974, toen werd gevonden dat tijdens de chlorıng bij de waterbehandeling trihalomethanen (THM) worden gevormd uit humusachtige verbindingen. Deze ontdekking, en de ontwikkeling van steeds betere analysemethoden, leidde tot de identificatie van vele andere nevenproducten van de chlorering. De toepassing van chloor leidt ook tot een verhoging van de mutagene activiteit van het gechloorde drinkwater. Mutageniteit van drinkwater is een indicatie voor de aanwezigheid van stoffen die een mogelijk gevaar voor de gezondheid van de consument inhouden. Hoofdstuk 1 geeft een overzicht van de verschillende aspecten van de reacties van chloor met natuurlijk organisch materiaal in water, en de gevolgen daarvan. Hoofdstuk 2 beschrijft de chlorering van cyaanazijnzuur in waterig milieu. Terwijl verschillende gechloreerde zuren als eindproducten van deze reactie werden aangetoond, werden N-chloor amides en dichlooracetonitril als intermediairen geidentificeerd. Vooral dichlooracetonitril is interessant omdat deze verantwoordelijk bleek te zijn voor de mutageniteit van het reactiemengsel. Dihaloacetonitrillen (DHAN) zijn mutagene verbindingen die in Nederlands drinkwater nog nooit zijn aangetoond. Daarvoor is een methode ontwikkeld waarmee de concentraties van deze verbindingen, en die van de beter bekende THM, in een aantal Nederlandse drinkwaters zijn bepaald. De analysemethode en de resultaten van de analyses zijn beschreven in hoofdstuk 3. Gebromeerde en gemengde chloro/bromo DHAN en THM bleken meer voor te komen dan de overeenkomstige gechloreerde verbindingen. Gebromeerde verbindingen worden gevormd wanneer er bromide aanwezig is in het gechloorde water.
Een aantal chlorerings producten kan worden verklaard uitgaande van amino zuren. De meeste aminozuren in water komen echter voor als eiwitten, of als aan humus gebonden eiwitachtig materiaal. De reactiviteit van eiwitten met chloor, die in het algemeen als laag werd verondersteld, wordt in hoofdstuk 4 beschreven. Bij deze reactie worden vele gechloreerde en niet-gechloreerde producten gevormd. Uit de resultaten blijkt dat eiwitachtig materiaal in belangrijke mate bijdraagt aan de vorming van bepaalde producten, met name cyaanverbindingen en 2,2-dichlooralkaanzuren, die in gechloreerd humus materiaal zijn aangetroffen. Vluchtige verbindingen zoals

de THM vormen slechts een klein deel van de in water aanwezige organohalogeen verbindingen. Het grootste deel bestaat uit niet-vluchtige, polaire verbindingen waarvan de halo-azijnzuren (HAZ) de belangrijkste vertegenwoordigers zijn. Hoofdstuk 5 beschrijft een methode voor de bepaling van deze sterk zure verbindingen en geeft de resultaten voor verschillende Nederlandse drinkwaters. HAZ blijken in alle drinkwaters bereid uit oppervlakte water voor te komen en, net als bij de bepaling van de DHAN en THM, bleek ook hier dat gebromeerde en gemengde chloro/bromo verbindingen het meest voorkomen in gechloord drinkwater. Een verbinding die belangrijk bijdraagt aan de mutageniteit van gechloord drinkwater is 3-chloor-4-(dichloormethyl)-5-hydroxy-2(5H)-furanon, dat meestal MX wordt genoemd. MX is een extreem mutagene verbinding die, vaak samen met het isomere EMX, in zeer lage concentraties in gechloord drinkwater voorkomt. In hoofdstuk 6 wordt aangetoond dat 3,5-dihydroxybenzaldehyde, een in humus materiaal voorkomende structuur, een goede precursor voor MX en EMX is. Tevens wordt een mechanisme voor de vorming van deze belangrijke chlorerings producten gegeven. Hoewel uit analyses blijkt dat vele chlorerings producten gebromeerde verbindingen zijn, is er tot nu toe voornamelijk aandacht besteed aan gechloreerde producten. In hoofdstuk 7 wordt dan ook aandacht besteed aan de chlorering van humus materiaal in de aanwezigheid van bromide. GC/MS analyse resulteerde in de identificatie van meer dan 100 verbindingen waarbij ook onderscheid kon worden gemaakt tussen gechloreerde en gebromeerde stoffen door het gebruik van een atomaire emissie detector. De mutageniteit bleek 2 tot 3 keer hoger indien bromide aanwezig was tijdens de chlorering. Was er geen bromide aanwezig dan kon de mutageniteit van het gechloreerde humus materiaal volledig worden verklaard door MX. Was er wel bromide aanwezig dan werden behalve MX ook gebromeerde MX verbindingen aangetroffen. Uit de analyseresultaten volgt dat MX en de gebromeerde MX verbindingen samen waarschijnlijk de totale mutageniteit van de reactiemengsels verklaren. De resultaten van het onderzoek geven aan dat gebromeerde verbindingen belangrijke chloreringsproducten zijn en dat gebromeerde MX verbindingen een belangrijke bijdrage kunnen leveren aan de mutagene activiteit van gechloord drinkwater.

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Curriculum Viteae

De auteur van dit proefschrift werd geboren op 9 juli 1960 te Ootmarsum. Nadat in 1976 het eindexamen MAVO werd behaald aan de Melchior Winhof MAVO te Ootmarsum, slaagde hij in 1978 voor het eindexamen HAVO aan het Thij College te Oldenzaal. Hierna ging hij naar Groningen waar hij studeerde aan de Nieuwe Leraren Opleiding Ubbo Emmius te Groningen en in 1983 een 2e graads bevoegdheid voor scheikunde en natuurkunde behaalde. De studie werd voortgezet aan de Rijks Universiteit Groningen en, nadat in 1985 het MO-B examen voor scheikunde werd behaald, werd vervolgens in januari 1986 het doctoraal examen scheikunde (organische chemie) behaald. Het afstudeerwerk onder begeleiding van Prof. Dr. J.B.F.N. Engbers en Dr. W. Weringa, richtte zich op electron-geinduceerde omleggingen tijdens de fragmentatie in de massa spectrometrie. Nadat hij zijn militaire dienstplicht had vervuld trad hij in september 1987 als projectmedewerker in dienst van het RIVM te Bilthoven. Het project, dat zich richtte op verbindingen in drinkwater als gevolg van de chloring tijdens de drinkwaterbereiding, werd uitgevoerd in samenwerking met de vakgroep analytische chemie van de TU Delft waarbij Dr. Ir. E. de Leer als directe begeleider optrad. Daardoor kon het werk gecombineerd worden met een promotie op hetzelfde onderwerp bij Prof. Dr. L. de Galan, waarvan dit proefschrift het uiteindelijke resultaat is. Sinds augustus 1990 is Ruud Peters werkzaam bij de afdeling Analytische Chemie van MT-TNO te Delft.