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Effect of Sodium Sulfite on the Mutagenicity of Chlorinated Drinking Water

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It is a well known fact that numerous organohalogenated compounds are formed during the chlorination (preoxidation or final disinfection) of drinking water. Some of these compounds have been proved to be mutagenic (Horth 1989). Recent studies have suggested that a treatment with S_{IV} species (SO_2, $NaHSO_3$, Na_2SO_3) could reduce the mutagenic activity and the total organic halogen content (AOX) of chlorinated samples of drinking water (Cheh et al. 1980 ; Bauman and Stenstrom 1989). A partial dechlorination with sulfur dioxide (SO_2) has been employed in a number of drinking water treatment plants in Paris to eliminate the bad taste due to excessive free chlorine concentrations in the water before distribution.

The aim of this work was to determine the effect of dechlorination treatments with sodium sulfite (Na_2SO_3) on the mutagenic activity and of the AOX content of chlorinated drinking water. Preliminary studies carried out with aqueous solutions of isolated aquatic humic substances (which are the main precursors of the mutagens and the halogenated organics produced during chlorination in drinking water treatment) showed that the addition of sodium sulfite could reduce the mutagenic activity (AMES test) and the AOX content of the chlorinated solutions of humic substances (Morlay et al. 1991). Our purpose was to determine the effect of different treatments with sodium sulfite on the mutagenicity and on the AOX content of chlorinated natural waters.

MATERIALS AND METHODS

The experiments were carried out with four natural waters : two raw and two treated surface waters. The first raw water studied was from a pond situated in a protected area near Poitiers called "Le Pinail". This water has a high humus content (Dissolved Organic Carbon : DOC = 15.4 ± 0.3 mg C/L). The water of the river Clain which flows through Poitiers was the second raw water considered. The water was sampled at its arrival at the water treatment plant and had not yet received any treatment. This water presented a DOC content of 7.8 ± 0.1 mg C/L. Before use, the two raw waters were filtrated in the laboratory on membranes of 10 and 1 µm porosity (the DOC contents were measured on the filtrated waters). The treated surface waters were sampled from two drinking water treatment plants located near Paris, at Ivry-sur-Seine and Orly. Figure 1 illustrates the flow diagrams for the treatment of the river Seine water at Ivry-sur-Seine and Orly. The water was sampled after the ozonation step and before the final disinfection with chlorine.

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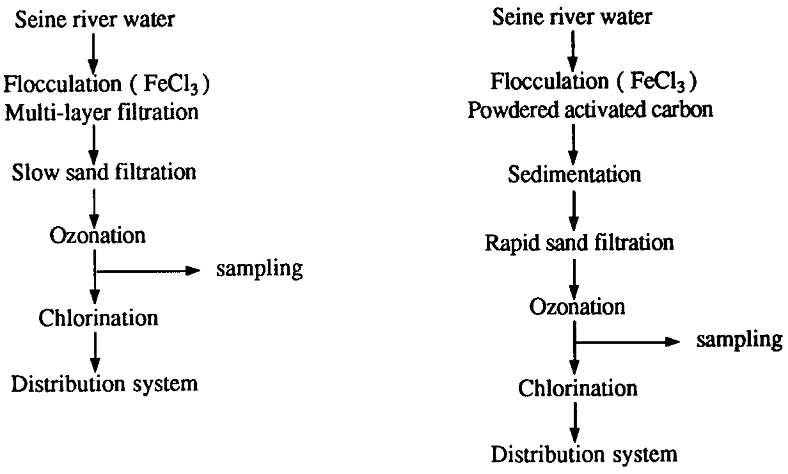

Figure 1-a

Figure 1-b

Figure 1. Flow diagram for the water treatment at Ivry-sur-Seine (Figure 1-a) and at Orly (Figure 1-b)

Table 1. Physico-chemical characteristics of the raw and treated waters before chlorination

Origin	Date of sampling	pH	DOC (mg C/L)	UV_{254} nm (/cm)	NH_4^+ (mg/L)
Le Pinail[a]	01.06.88	7.1 ± 0.1	15.4 ± 0.3	0.605 ± 0.025	-
Le Clain river[a]	10.10.90	7.8 ± 0.1	7.2 ± 0.2	0.055 ± 0.001	-
Seine river[b]	06.11.90	7.7 ± 0.1	1.4 ± 0.2	0.042 ± 0.005	0
(Ivry-sur-Seine)	19.11.90	7.7 ± 0.1	2.0 ± 0.2	0.050 ± 0.005	0
Seine river[b]	03.12.90	7.6 ± 0.1	2.0 ± 0.2	0.031 ± 0.005	0
(Orly)	10.12.90	7.7 ± 0.1	1.9 ± 0.2	0.023 ± 0.005	0.17

a : raw water b : treated water

These treated waters have a low DOC content (DOC = 1.4 to 2.0 mg C/L). The main characteristics of these four waters are given in table 1.

Concentrations of chlorine in the stock solution of sodium hypochlorite and free chlorine in chlorinated samples of raw or treated water were measured by iodometry and colorimetry (DPD method). To determine the effect of sodium sulfite on the mutagenic activity, an appropriate volume of a standard solution was added to the chlorinated samples. The solutions of Na_2SO_3 were prepared in ultra-pure water supplied by a Millipore (Milli-Q + Milli-RO 4) water purification system. The solutions of sodium sulfite were prepared just before use. The mutagenic activity of the samples was extracted on organic macroporous resins (Rohm and Haas). Prior to use, the resins were purified by several washes with ultra-pure water to eliminate fine particles, soxhlet extractions with methanol (60 hours), ether (72 hours) and dichloromethane (48 hours) and rinsed with methanol and ultra-pure water. Four glass flasks containing 10 L of the filtrated water of Le Pinail were chlorinated (pH = 7.1) at a rate of 15.0 mg Cl_2/L and at ambient temperature so that no more free chlorine was present in the samples after a 24-hr reaction time. Sodium sulfite was then added in two of the four flasks at a concentration of 10^{-4} M before the filtration of all the samples on a XAD-4 resin at the water pH or at pH 2.0. The acidification with concentrated HCl was done half-an-hour after the addition of sodium sulfite. Two non-chlorinated water samples were also filtrated on the XAD-4 resin at pH 7.1 or pH 2.0. Samples were prepared every day so that each final extract corresponded to a water volume of 45 L. The extraction of the mutagenic activity of the samples was carried out by filtration through resin cartridges (50 mL) at an average flow rate of 0.4 L/hr. The adsorbed organics were then eluted with 4 volumes of dichloromethane. Extracts were dried on anhydrous sodium sulfate and concentrated from 200 to 10 mL approximately in a rotary evaporator (water bath temperature = 40 °C).

For the river Clain water and for the two treated waters, the chlorination, dechlorination and extraction steps were carried out with three reactors of a capacity of 200 L. These glass reactors equipped with a stirring device were used on site for the treated waters. The volume of each sample was 200 L. The river Clain water was chlorinated at a rate of 3.8 mg Cl_2/L and at ambient temperature (between 15 and 20 °C). After a 15-hr reaction time, the average free chlorine concentration in each reactor was 1.27 ± 0.02 mg/L. The treated waters of Ivry-sur-Seine and Orly plants were chlorinated at a rate of 2.1 to 2.8 mg Cl_2/L and at ambient temperature (between 10 and 15 °C) to obtain a free chlorine concentration of 1.1 to 1.3 mg/L after a 6-hr contact time (the highest chlorination rate of 2.8 mg Cl_2/L was necessary only in one case where the treated water contained ammonia ; Table 1). Different amounts of sodium sulfite were then added in some of these water samples to obtain either a partial (presence of free chlorine) or a total dechlorination. 2.3 hours (river Clain water) or 3 hours later (treated waters), the residual free chlorine concentration was measured in each reactor and the oxidant was neutralized by the addition of a stoichiometric amount of a sodium thiosulfate solution. The content of one flask, to be taken as reference for the further determination of both the mutagenic activity level and the AOX content, was dechlorinated with a stoichiometric amount of sodium thiosulfate at the end of the chlorination reaction time. The acidification at pH 2.0 of all the samples was carried out with concentrated HCl before filtration through XAD-8 + XAD-2 (100 mL of each one) resin cartridges at an average flow rate of 10 L/h. The elution was carried out with 1 volume of acetone and 2 volumes of dichloromethane. Extracts were dried on anhydrous sodium sulfate and concentrated from 500 to 30 mL approximately in a Kuderna-Danish apparatus (water bath temperature = 65 °C) equipped with a Snyder column.

In each case, the total organic halogen concentrations (AOX) were measured on the aqueous samples before and after dechlorination. The mutagenicity testing was carried out after a solvent exchange (dimethylsulfoxide). The Ames mutagenicity tests were conducted at the "Laboratoire d'Hygiène de la Ville de Paris" with Salmonella thyphimurium tester strains TA98 and TA100 (with or without metabolic activation S_9 mix) following the method described by Maron and Ames (1983). The procedure of this test and of the AOX analysis have already been described (Morlay et al., 1991).

RESULTS AND DISCUSSION

In all the experiments, extracts were prepared by washing freshly purified resins with the corresponding elution solvents. These extracts did not exibit any toxic or mutagenic activity (data not shown).

Table 2. Effect of the chlorination and of the addition of sodium sulfite on the mutagenic activity (net revertants/L) of Le Pinail water extracted at pH 7.1 and 2.

	Extraction at pH 7.1			Extraction at pH 2.0	
Tester strain	Raw water	Chlorinated water	Chlorinated water + sulfite	Chlorinated water	Chlorinated water + sulfite
TA100 - S9 mix	0	460	256	2 400	2 728
TA 98 - S9 mix	(±) 2	160	41	616	378
TA100 + S9 mix	0	232	56	813	959
TA98 + S9 mix	0	(±) 9	12	109	96

In the first part of this work, we studied the effect of an addition of sodium sulfite on the mutagenic activity level and on the AOX content of chlorinated raw water samples (Le Pinail ; no residual free chlorine). We also examined the effect of pH on the recovery of the mutagenic activity by filtration on the XAD-4 resin. The results obtained (Table 2) showed that :

- the raw water filtrated at pH 7.1 did not exibit any mutagenicity for the two strains, with or without metabolic activation ;
- the extracts (pH 7.1 and pH 2.0) of chlorinated and sulfite treated water samples were mutagenic for the two tester strains and the metabolic activation always decreased their mutagenicity ;
- the extracts prepared by filtration of the water samples at neutral pH (pH 7.1) showed genotoxicity levels lower than those of the extracts obtained by filtration at pH 2.0 (Figure 2). This could be observed for either tester strain, with or without metabolic activation ;
- the mutagenicity levels obtained by filtration of the chlorinated and sulfite treated water samples at pH 2.0 were of the same order of magnitude. For the extracts prepared by filtration at neutral pH, a mutagenic activity appeared after chlorination of the raw water and the addition of sodium sulfite decreased this genotoxicity by a factor 4 on TA98 tester strain and by a factor of 2 approximately on TA100 tester strain without metabolic activation. The decrease of the mutagenic activity observed at neutral pH can be explained by the fact that sulfite ion is more reactive towards mutagenic compounds than bisulfite ion or sulfurous acid (pK HSO_3^-/SO_3^{2-} = 7.2 at 25 °C; pK H_2SO_3/HSO_3^- = 1.9 at 25 °C).

Moreover, the determination of the AOX content of the samples revealed an important production of organohalogenated compounds by chlorination (1 900 ± 100 µg Cl/L) but did not show any significant decrease in the concentration of these compounds by the addition of sodium sulfite at a 10^{-4} M concentration and for a 2-hr contact time (the precision of the analysis was estimated to be about 10 %).

In the second part of this work, we investigated the effect of dechlorination treatments (partial or complete elimination of the residual chlorine) with sodium sulfite on the mutagenic activity and on the AOX concentration of chlorinated samples of raw or treated waters. The effects of a total dechlorination were studied on the chlorinated raw water of the river Clain . The effects of a partial or a complete dechlorination were examined on the two treated waters. Two rates of partial dechlorination and of total dechlorination (stoichiometric or with the addition of an excess of reducer) were studied. A reference sample was prepared in each series of dechlorinated water samples. The results obtained showed that :

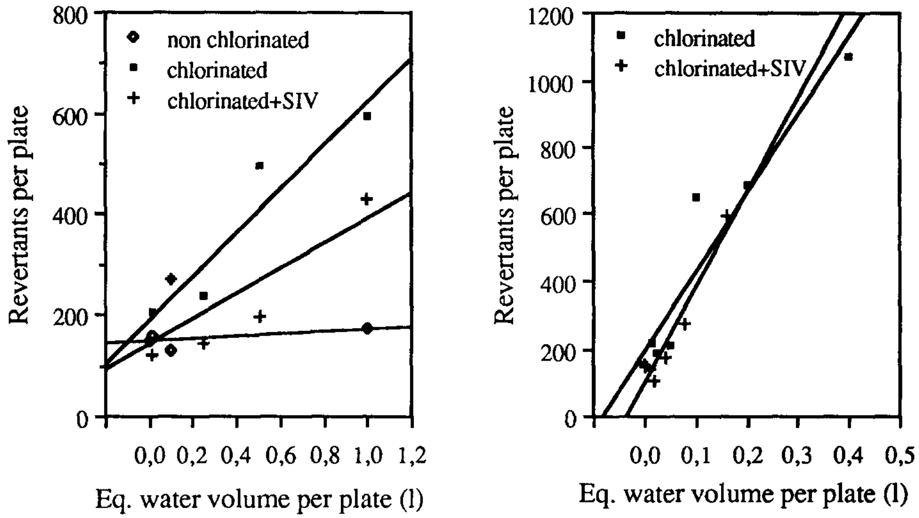

Figure 2-a

Figure 2-b

Figure 2. Mutagenic activity with strain TA 100 without metabolic activation of extracts obtained from Le Pinail chlorinated water before and after sodium sulfite addition (Figure 2-a : filtration at pH 7.1 ; Figure 2-b : filtration at pH 2.0)

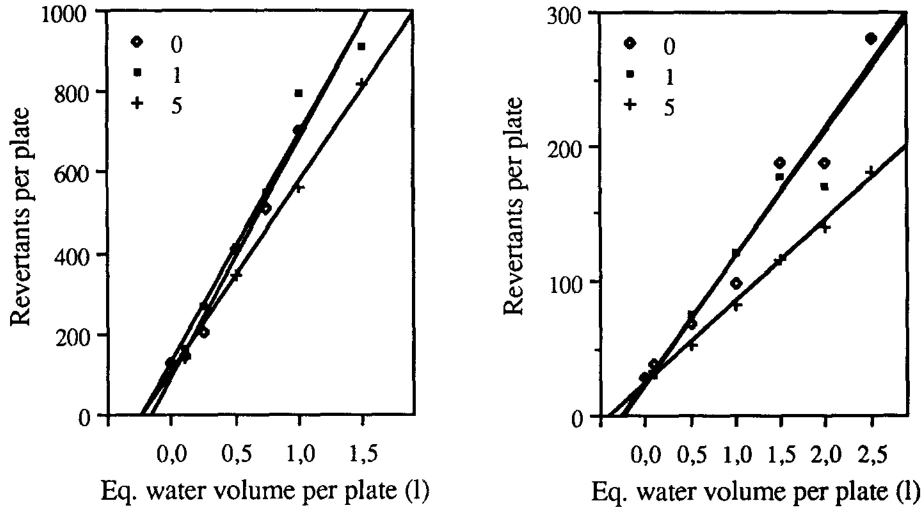

Figure 3-a

Figure 3-b

Figure 3. Mutagenic activity with strains TA 100 without metabolic activation (Figure 3-a) and TA 98 without metabolic activation (Figure 3-b) of extracts obtained from Le Clain water dechlorinated with sodium sulfite (1 or 5-fold the stoichiometric dose) and with sodium thiosulfate (0 : stoichiometric dose)

- a partial or complete dechlorination (even with an excess of sodium sulfite) did not decrease significantly the AOX content of the chlorinated raw or treated water samples (Table 3 ; dechlorination time = 2.3 or 3 hours). For these measurements, the precision of the analysis was evaluated to be about 10 % ;

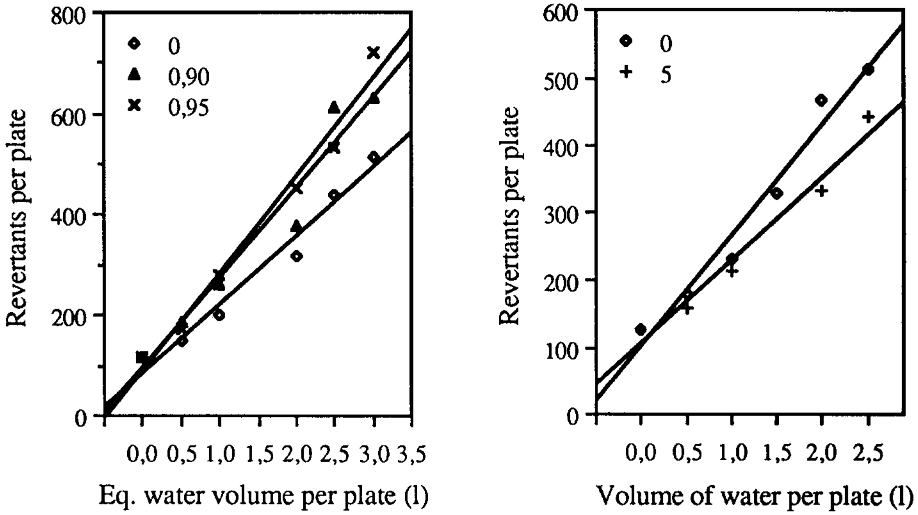

Figure 4-a

Figure 4-b

Figure 4. Mutagenic activity with strain TA 100 (without metabolic activation) of extracts obtained from Ivry water partially (Figure 4-a) or completely (Figure 4-b) dechlorinated with sodium sulfite (0.90-0.95 or 5-fold the stoichiometric dose) or with sodium thiosulfate (0 : stoichiometric dose)

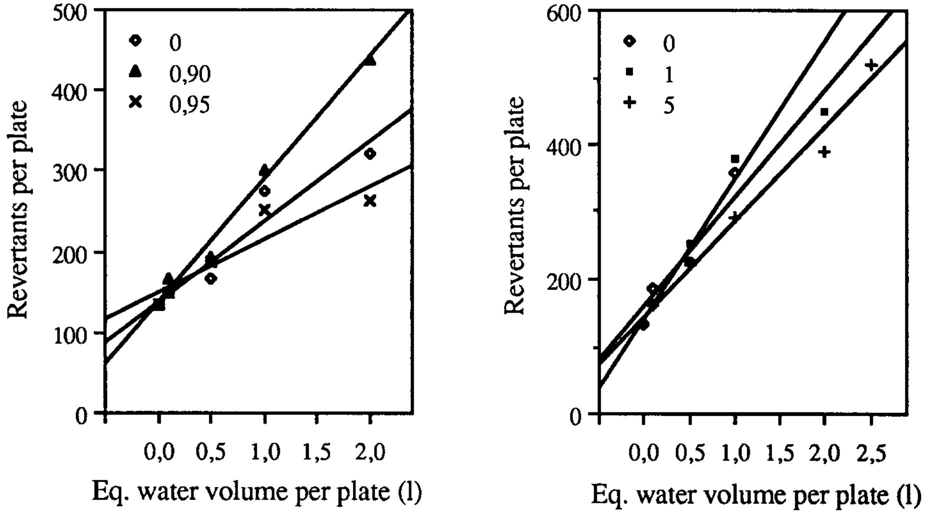

Figure 5-a

Figure 5-b

Figure 5. Mutagenic activity with strain TA 100 (without metabolic activation) of extracts obtained from Orly water partially (Figure 5-a) or completely (Figure 5-b) dechlorinated with sodium sulfite (0.90, 0.95, 1 or 5-fold the stoichiometric dose) or with sodium thiosulfate (0 : stoichiometric dose)

- a partial or stoichiometric dechlorination ($[SO_3^{2-}]_0/[Cl_2] \leq 1$ mole/mole) did not decrease significantly the mutagenicity level of the samples (Table 3 ; Figure 3, 4 and 5 ; samples n° 2, 3 and 5 compared to samples n°1 and 4). Indeed, laboratory experiments carried out with a chlorinated dilute solution of aquatic humic

Table 3. Chlorination and dechlorination conditions of the river Clain raw water and of the river Seine treated water (Ivry-sur-Seine and Orly treatment plants) ; AOX content and mutagenic activity with strains TA 100 and TA 98 (without metabolic activation) of the chlorinated and dechlorinated samples

	RAW WATER			SEINE RIVER TREATED WATER											
	Clain river			Ivry-sur-Seine treatment plant						Orly treatment plant					
	Complete dechlorination			Partial dechlorination			Complete dechlorination			Partial dechlorination			Complete dechlorination		
Sample	1	2	3	1	2	3	4	5	6	1	2	3	4	5	6
Chlorination rate and residual chlorine (mg Cl_2/L)	3.8 1.27[a]	3.8 1.29[a]	3.8 1.26[a]	2.1 1.33[b]	2.1 1.25[b]	2.1 1.33[b]	2.1 1.11[b]	2.1 1.25[b]	2.1 1.30[b]	2.8 1.28[b]	2.8 1.25[b]	2.8 1.31[b]	2.1 1.25[b]	2.1 1.21[b]	2.1 1.22[b]
Dechlorination rate $[SO_3^{2-}]_o/[Cl_2]$ (mol/mol)	($Na_2S_2O_3$)	1	5	($Na_2S_2O_3$)	0.90	0.95	($Na_2S_2O_3$)	1	5	($Na_2S_2O_3$)	0.90	0.95	($Na_2S_2O_3$)	1	5
Theoretical excess of sulfite introduced (μ mol/L)	-	0	71	-	0	0	-	0	73	-	0	0	-	0	69
Residual free chlorine after dechlorination (mg Cl_2/L)	0[c]	0.21[d]	0[d]	0[c]	0.29[e]	0.20[e]	0[c]	0.50[e]	0[e]	0[c]	0.31[e]	0.22[e]	0[c]	0.11[e]	0[e]
AOX before and after dechlorination (μg Cl/L)	250 283	243 278	220 214	61 69	77 73	64 70	48 -	46 120	49 47	53 55	52 56	52 56	94 78	82 93	71 70
Mutagenic activity of the dechlorinated samples (revertants/L)															
TA 100	567	602	455	124	168	190	128	712	106	155	183	143	201	233	159
TA 98	96	92	56	23	38	30	22	65	17	38	37	21	56	61	36

a : 15-hr chlorination time

b : 6-hr chlorination time

c : 5-min reaction with sodium thiosulfate (stoichiometric dose) before acidification at pH 2.0

d : 2.3-hr dechlorination time with sodium sulfite before acidification at pH 2.0

e : 3-hr dechlorination time with sodium sulfite before acidification at pH 2.0

substances(15 mg/L) had previously shown that the variability of the slope values observed for five extracts obtained in conditions rigourously identical and similar to the conditions used in the present study was about ± 10 % for the TA 98 tester strain and ± 20 % for the TA 100 one (Morlay 1991). This percentage was the sum of the variability of each step of the preparation of the extracts (adsorption, elution, drying and solvent exchange) ;

a complete dechlorination with the introduction of a large excess of sodium sulfite (sulfite dose introduced = 5 times the stoichiometric dose, i.e. an excess of reducer of $7\ 10^{-5}$ M) could decrease the mutagenicity of the chlorinated raw water of the river Clain . The slope values obtained with the chlorinated treated waters were not high enough for a significant decrease of the mutagenic activity of the samples to be observed by dechlorination with the same excess of sulfite. So no clear conclusion could be drawn from the treated waters studied and only a tendency could be observed in this case.

The abnormally high AOX value and genotoxicity level observed on the sulfite stoichiometrically dechlorinated sample of the Ivry chlorinated treated water could be explained by the fact that the residual free chlorine (0.5 mg/L) which was present after a dechlorination time of 3 hours had not been neutralized. This residual oxidant led to the formation of organohalogenated compounds during the storage of the sample (10 days at pH 2.0) and to the formation of mutagenic compounds. These last molecules could have been produced, during the filtration, in the reactor itself or by reaction of the chlorine with the resins or the organic compounds adsorbed on it. This shows the importance of neutralizing the residual free chlorine at the end of the reaction time and before the filtration on XAD resins.

The results obtained in this study show that only a complete dechlorination with a large excess of sodium sulfite could decrease the mutagenicity of chlorinated samples of raw or treated waters. Furthermore, the results indicate that a partial dechlorination treatment with sodium sulfite, as practiced in drinking water treatment plants to reduce the residual free chlorine concentration, has no effect on the mutagenicity and on the total organic chlorine content of the chlorinated water. These results are consistent with those obtained previously from chlorinated solutions of aquatic humic substances.

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