Bull. Environ. Contam. Toxicol. (1991) 47:15–22

Environmental Contamination and Toxicology

Effect of an Addition of Sodium Sulfite on the Mutagenicity of Chlorinated Solutions of Aquatic Humic Substances

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Dechlorination with sulfur dioxide (SO_2) or, more generally, with S_{IV} species ($NaHSO_3$, Na_2SO_3) is now employed in a number of drinking water treatment plants in Paris to eliminate the bad taste due to excessive free chlorine concentrations present in water at the reservoir outlets (Maquennehan and Clause 1987). Recent studies have reported a decrease in the mutagenic activity (Wilcox and Denny 1985 ; Cheh et al. 1980) and the total organic halogen content (Morlay et al. 1990 ; Sadiki et al. 1990 ; Croué et al. 1989) of chlorinated samples of drinking water and of solutions of humic substances which were dechlorinated with sulfur dioxide derivatives. The aim of this study was to understand better the effects of dechlorination treatments with S_{IV} species and especially sodium sulfite (Na_2SO_3) on the mutagenic activity of chlorinated drinking water. For this purpose, laboratory experiments were carried out with aqueous solutions of isolated aquatic humic substances. These compounds account for the bulk of organic matter present in most surface waters and are known to be precursors of many of the mutagens and halogenated organics which are produced during the chlorination stage in drinking water treatment (Horth 1989 ; Meier et al. 1983).

MATERIALS AND METHODS

The experiments were carried out with aquatic humic substances (AHS) which were isolated from a dam water by XAD-8 resin extraction (Thurman and Malcolm 1981). The elemental analysis of the unfractioned extract (mixture of fulvic and humic acids) gave a carbon content of 49.5 % by weight. Concentrations of chlorine in the stock solution of sodium hypochlorite and free chlorine in chlorinated samples of AHS were measured by iodometry and colorimetry (o-tolidine and DPD methods) respectively. To determine the effect of S_{IV} species on the mutagenic activity, an appropriate volume of a standard solution of sodium sulfite (0.1-0.2 *M*) was added to the chlorinated samples of AHS. All the solutions (solutions of AHS or 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 prior to use.

In the first part of this study, the mutagenicity tests were performed directly on concentrated aqueous solutions of AHS to avoid any artefact due to the extraction-concentration method used. In the second part, experiments were carried out with dilute solutions of AHS in order to show the effects of a partial (residual of chlorine) or total (excess of reducer) dechlorination on the mutagenic activity. To this end, mutagenicity tests were performed on XAD-8/XAD-2 resin extracts.

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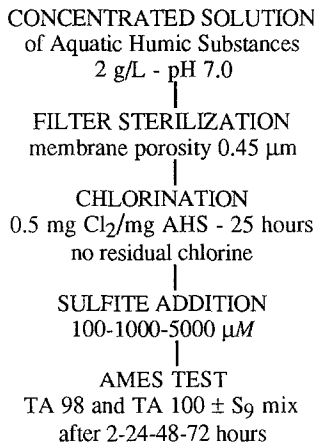

Figure 1-a

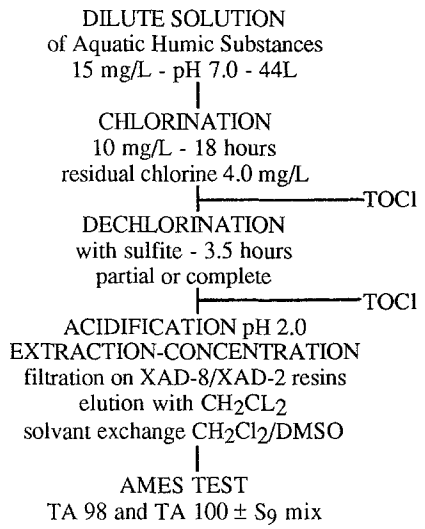

Figure 1-b

Figure 1 : Procedures used for the preparation of the aqueous samples from a concentrated solution of AHS (Figure 1-a) or of the organic extracts obtained from a dilute solution of AHS (Figure 1-b) to be submitted to the Ames test.

Filter-sterilized concentrated solutions (cellulose nitrate membranes of 0.45 µm porosity) of AHS (2 g/L) were chlorinated under buffered conditions (pH = 7.0 ; µ = 0.4 *M* ; 20°C) at a chlorine to carbon weight ratio of 0.5/1.0 (i.e. 1.0 g Cl_2/L) (Figure 1-a). The ionic strength of the AHS solution was high (0.4 *M*) to avoid any variation of pH by chlorination. After a 251/2 hours reaction (no more free chlorine was present) in 100-mL stoppered flasks, sulfite (filter-sterilized solution of sodium sulfite) was added at doses corresponding to 0, 100, 1 000 and 5 000 µ*M* concentrations without altering the pH value by more than 0.1 unit. Mutagenicity testing was performed directly on the aqueous samples (0 to 2 mL/plate) after a 2, 24, 48 and 72 hours reaction. The samples were stored in the dark, at 20°C. The pH of each sample was checked at the end of the experiment.

In the second part of this study, the mutagenic activity of chlorinated and dechlorinated dilute solutions of AHS was determined on the corresponding XAD-8/XAD-2 resin extracts. Six flasks containing 44 litres of a buffered dilute solution (15 mg/L ; pH = 7.0 ; µ = 0,01 *M*) were chlorinated at a chlorine to carbon weight ratio of 0.67/1.00 (i.e. 10.0 mg Cl_2/L) (Figure 1-b). After a 18 hours reaction, the average free chlorine concentration in each flask was 4.1 ± 0.1 mg/L. Different amounts of sulfite were then added to obtain either a partial or total dechlorination. 31/2 hours later, the free chlorine concentration was measured in each flask and neutralized by the addition of a sodium thiosulfate solution when necessary. The content of a flask, to be taken as reference of the partial dechlorination for the further determination of the mutagenic activity, was dechlorinated with a stoichiometric amount of sodium thiosulfate just after the 18 hours chlorination. The acidification to 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 2 L/h (10 ± 1 BVH). Prior to use, the organic macroporous resins (Rohm and Haas) were purified by several washes with ultra-pure water to eliminate fine particles, soxhlet extractions with methanol (60 hours) and ether (72 hours) and rinsed with dichloromethane and

Table 1-a : Effect of the addition of sodium sulfite at different doses and of the contact time on the mutagenic activity of concentrated solutions of AHS. The slope values (expressed as revertants per mL of aqueous sample) were obtained with TA 98 and TA 100 tester strains without S9 mix.

Contact time (hours)	Revertants / mL							
	TA 98 [Sulfite]$_0$ (μM)				TA 100 [Sulfite]$_0$ (μM)			
	0	100	1000	5000	0	100	1000	5000
2	65	48	12	0	426	221	107	(±)24
24	-	20	(±)5	0	-	242	62	(±)14
48	-	(±)9	0	0	-	125	(±)24	(±)18
72	-	(±)8	0	0	-	105	(±)22	0

Table 1-b : Effect of the addition of sodium sulfite and of the contact time on the mutagenic activity of concentrated solutions of AHS. The slope values (expressed as revertants per mL of aqueous sample) were obtained with TA 98 and TA 100 tester strains with S9 mix.

Contact time (hours)	Revertants / mL			
	TA 98 [Sulfite]$_0$ (μM)		TA 100 [Sulfite]$_0$ (μM)	
	0	1000	0	1000
2	(±)5	0	184	49
24	0	0	167	(±)47
72	(±)5	0	126	(±)27

ultra-pure water. The adsorbed organics were then eluted 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. The total organic chlorine concentrations (TOCl) were measured on the aqueous samples before and after dechlorination (Figure 1-b ; Table 2). The mutagenicity testing was carried out after a solvant exchange (dimethylsulfoxide).

The mutagenicity tests were conducted at the "Laboratoire d'Hygiène de la Ville de Paris" with *Salmonella typhimurium* tester strains TA 98 and TA 100 following the methods described by Maron and Ames (1983). The tests were performed in duplicate at 5 dose levels, both in the absence and in the presence of a rat-liver microsomal fraction containing cofactors (S9 mix). Positive and negative controls were included in each test. Mutagenic activity was quantitated by the regression analysis of the linear portion of the dose-response curve. In this manner, the slope (expressed as revertants per mL of sample in the case of concentrated solutions of AHS or expressed as revertants per L of the initial aqueous solution in the case of dilute soultions of AHS) was calculated and used for establishing the data for comparisons. The observation of a dose-related increase of 2-fold or more above background was used as the criterion of a positive mutagenic response. Samples quoted (-) were not mutagenic and those quoted (±) could have given a positive response if higher doses had been tested.

TOCl analyses were carried out with a DOHRMANN DX-20A Total Organic Halogen Analyser equipped with four dual carbon adsorption modules and a

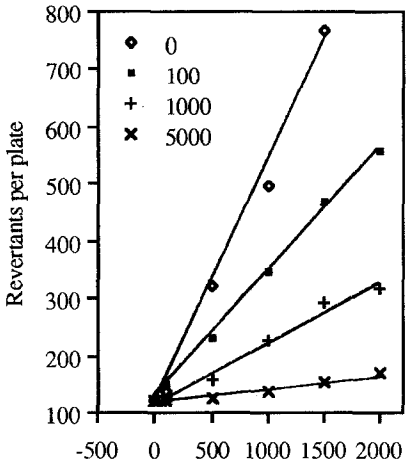

Figure 2-a

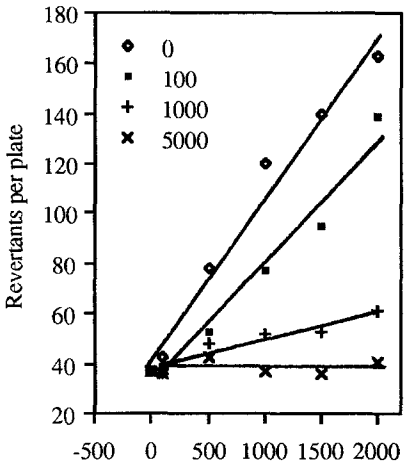

Figure 2-b

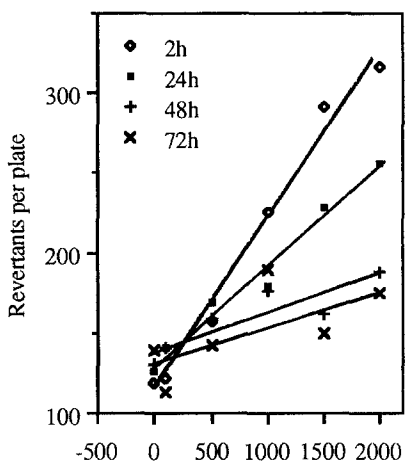

Figure 2-c

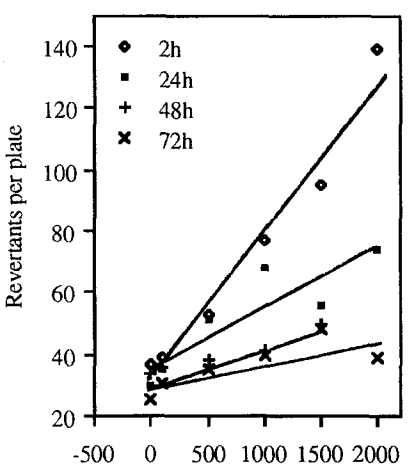

Figure 2-d

Figure 2 : Mutagenic activity without S9 mix of dechlorinated AHS concentrated solutions : influence of the sulfite dose (Figure 2-a : TA 100 ; Figure 2-b : TA 98 - reaction time = 2 hr) and of the reaction time (Figure 2-c : TA 100 - sulfite dose = 1 000 µ*M* ; Figure 2-d : TA 98 - sulfite dose = 100 µ*M*).

microcoulometric titration cell. After a given reaction time, the residual chlorine, when present, was neutralized with sodium thiosulfate and the samples were acidified (pH = 1.4) with concentrated HNO_3. The TOCl measurements were made within a period of 8 hours. The reproducibility of TOCl analyses (in the range 10 - 300 µg/L as Cl) was 2 % for a 100-mL tested sample.

RESULTS AND DISCUSSION

In the first part of this study, the mutagenic activity of the samples was determined directly on the concentrated aqueous solutions of AHS ([AHS] = 2 g/L)

Table 2 : Effect of a partial (samples no. 1, 2 and 3) or complete (samples no. 4, 5 and 6) dechlorination on the TOCl concentration and on the mutagenic activity (TA 98 and TA 100 ± S9 mix) of dilute solutions of AHS. The percentage of variation in the mutagenic activity are mentioned in parentheses.

Sample	1	2	3	4	5	6
Dechlorination rate $[SIV]_0/[Cl_2]_0$ (mol/mol)	1 (thiosulfate)	0,91	0,98	1	2	10
Residual Cl_2 (mg/L) and TOCl (µg/L) after 18-hr chlorination	4,22 1205	3,99 1280	4,12 1218	4,08 949	4,07 949	3,98 937
Residual Cl_2 (mg/L) and TOCl (µg/L) after 31/2-hr dechlorination	0 -	0,35 1567	0,10 1468	0 928	0 879	0 885
Slopes TA 100-S9 mix (revertants/L)	3691	3350 (-9)	3612 (-2)	3052	2269 (-26)	1629 (-47)
Slopes TA 100+S9 mix (revertants/L)	1882	2338 (+24)	2267 (+20)	2883	2002 (-31)	980 (-66)
Slopes TA 98-S9 mix (revertants/L)	537	518 (-4)	426 (-21)	463	429 (-7)	238 (-49)
Slopes TA 98+S9 mix (revertants/L)	355	388 (+9)	353 (-1)	355	279 (-21)	157 (-56)

(Figure 1-a). Two series of experiments were conducted in rigourously similar conditions in order to determine the response of the two tester strains in the absence (Table 1-a) or in the presence (Table 1-b) of the rat-liver microsomal fraction S9 mix. In the first case (absence of S9 mix), blanks were prepared, including the non-chlorinated AHS solution, the phosphate buffer solution and a solution of sodium sulfite corresponding to the highest dose used (5 000 µ*M*).

The results (Tables 1-a and 1-b) showed that :
- blanks did not present any mutagenic activity with TA 98 and TA 100 tester strains in the absence of S9 mix (data not shown) ;
- the chlorination of the AHS solutions induced a significant genotoxicity with the two strains in the absence of S9 mix and only with TA 100 tester strain in the presence of S9 mix. The largest responses were always obtained with TA 100 without S9 mix (slope values of 426 and 65 revertants per mL of sample with TA 100 and TA 98 tester strains respectively in the absence of S9 mix, slope values of 184 and 5 revertants per mL of sample with TA 100 and TA 98 tester strains respectively in the presence of S9 mix) ;
- the introduction of the lowest dose of sodium sulfite ($[SO_3^{2-}]_0$ = 100 µ*M*) could reduce, in 2 hours, the genotoxicity observed on the two tester strains in the absence of S9 mix. In the presence of S9 mix and for the sulfite dose tested ($[SO_3^{2-}]_0$ = 1 000 µ*M*), the genotoxicity observed could be reduced on TA 100 tester strain only (the initial genotoxicity level obtained with TA 98 was too low to draw up the same conclusion) ;
- an increasing sulfite dose (Figures 2-a and 2-b) and/or an increasing reaction time (Figures 2-c and 2-d) contributed to decrease more the mutagenic activity observed on the two tester strains in the absence of S9 mix.

In the second part of this work, experiments were carried out with dilute solutions of AHS ([AHS]) = 15 mg/L) in order to confirm the results previously obtained with concentrated solutions by the addition of an excess of reducer and to study the effects of a partial dechlorination (presence of residual chlorine) on the mutagenic activity. The mutagenicity tests were performed with TA 98 and TA 100 tester

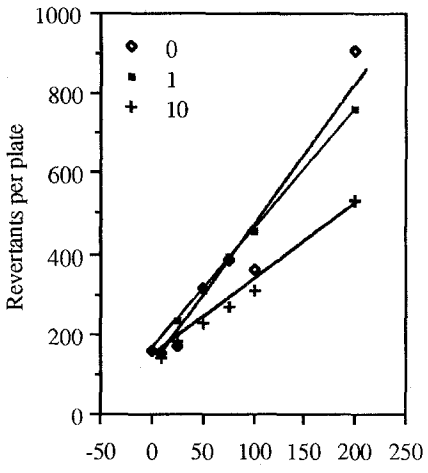

Figure 3-a

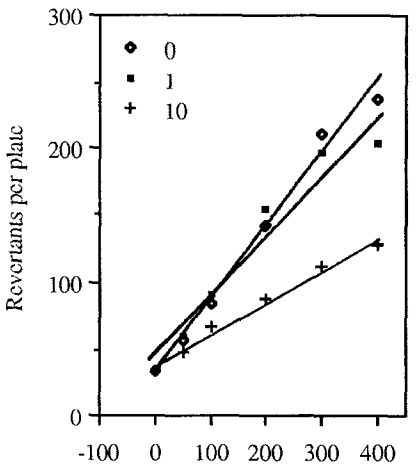

Figure 3-b

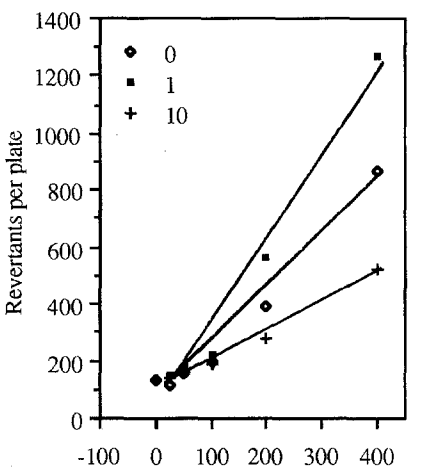

Figure 3-c

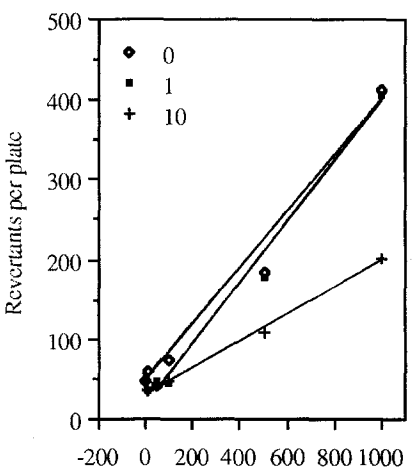

Figure 3-d

Figure 3 : Mutagenic activity without (Figure 3-a : TA 100 ; Figure 3-b : TA 98) or with (Figure 3-c : TA 100 ; Figure 3-d : TA 98) S9 mix of extracts obtained from dechlorinated AHS dilute solutions : effect of dechlorination rates of 0, 1 or 10-fold the stoichiometric dose of sodium sulfite.

strains in the absence of S9 mix on the corresponding XAD-8/XAD-2 resin extracts (Figure 1-b). Moreover, the total organic chlorine concentrations (TOCl) were measured on aqueous aliquots sampled before and after the addition of sodium sulfite (samples no. 2-6 ; Table 2) or thiosulfate (sample no. 1 ; Table 2).

Because of the important volume of each aqueous sample (44 L), two series of experiments were carried out : the partial dechlorination was studied on samples number 1, 2 and 3 (Table 2) while the addition of a stoichiometric dose or of an excess of reducer was studied on samples number 4, 5 and 6 (Table 2). The corresponding results are presented and interpreted separately.

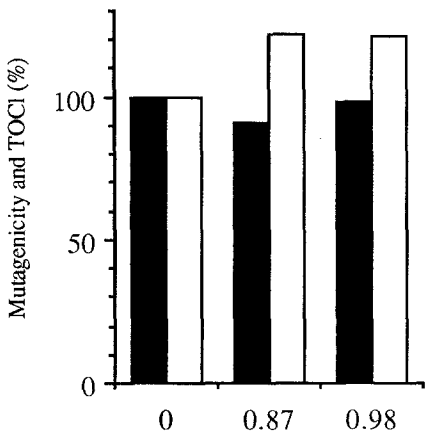

Figure 4-a

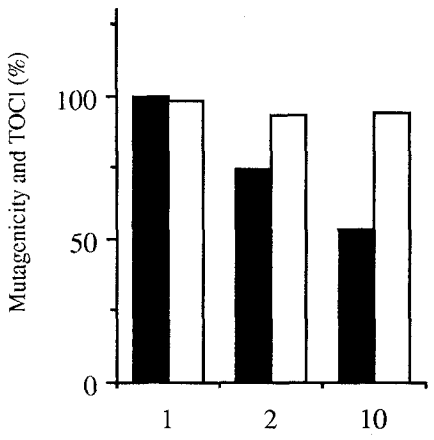

Figure 4-b

Figure 4 : Effect of a partial (Figure 4-a) or complete (Figure 4-b) dechlorination on the TOCl concentration and on the mutagenic activity (TA 100 without S_9 mix) of dilute

The results obtained from the partial dechlorination (first series of experiments ; Table 2) showed that :

- the TOCl levels measured after a 3 1/2 hours dechlorination were higher than those determined before the addition of sulfite (21 and 22 % for samples no. 2 and 3 respectively). These results can be explained by the fact that the chlorine demand of AHS samples is not satisfied after a 18 hours chlorination. Consequently, a fraction of the free chlorine present in the partially dechlorinated samples can react with the organic matter and lead to an increase in the TOCl concentration during the 3 1/2 hours dechlorination ;
- the mutagenic activity observed on TA 98 and TA 100 tester strains with and without S_9 mix did not decrease significantly (less than 10 % for samples no. 2 and 3 compared to sample no. 1). The slope values obtained for sample number 1 on TA 100 with S_9 mix (1882 revertants per L) and for sample number 3 on TA 98 without S_9 mix (426 revertants per L) seem to be abnormally low. This could be explained by a poor recovery of the mutagenic compounds during the extraction-concentration procedure or by an abnormal variability in the response of the strains.

The results obtained from the addition of a stoichiometric dose or of an excess of reducer (second series of experiments ; Table 2) showed that :

- the TOCl levels measured after the 3 1/2 hours dechlorination were slightly lowered (less than 8 % in all cases). These changes of the TOCl concentrations by a partial or complete dechlorination (excess of sulfite) at neutral pH were previously observed on other dilute AHS solutions ([AHS] = 5 mg/L) (Morlay et al. 1990) ;
- the mutagenic activity observed on the two tester strains with and without S_9 mix significantly decreased after the 3 1/2 hours contact time with an applied dose of sulfite 10-fold the stoichiometric one (sample no. 6 compared to sample no. 4 which received a stoichiometric sulfite dose) (Figure 3). The order of magnitude of this drop in the mutagenic response was approximately 50 %.

Elsewhere, the comparison of the results obtained from the mutagenic activity determinations and those obtained from TOCl measurements indicate that the

decrease in the genotoxicity observed in fully dechlorinated samples can be attributed to the destruction by sulfite of non halogenated mutagenic compounds or of highly mutagenic halocarbons which are present in trace amounts in the samples and which therefore account for a small part of the TOCl content (Figure 4).

The results obtained with chlorinated solutions of AHS show that a drecrease in the mutagenic activity can be obtained under neutral conditions after sulfite addition. These results also indicate that a partial dechlorination with sulfite has no significant effect neither on the total organic chlorine level nor on the mutagenicity of chlorinated samples of AHS. However, further experiments with drinking water samples are necessary in order to confirm the data obtained for the study of the effects of a dechlorination treatment with S_{IV} species on the mutagenicity of treated waters.

Acknowledgments : The authors thank Ms. M.L. Pesle, Ms. C. Lachenal, Ms. F. Bardant (Laboratoire d'Hygiène de la Ville de Paris) and Mr. D. Rigomier (University of Poitiers) who contributed to the performance of the mutagenicity tests. We also thank Mr. E. Dreyfus for correcting the manuscript.

REFERENCES

Cheh AM, Skochdopole J, Heilig C, Koski P, Cole L (1980) Destruction of direct-acting mutagens in drinking water by nucleophiles : implications for mutagen identification and mutagen elimination from drinking water. In : Jolley RL et al. (eds) Water Chlorination : Environmental Impact and Health Effects, vol 3. Ann Arbor Science Publ, MI (USA), pp 803-815

Croué JP, Reckhow DA (1989) Destruction of chlorination byproducts with sulfite. Environ Sci Technol 23 : 1412-1419

Horth H (1989) Identification of mutagens in drinking water. Aqua 38 : 80-100

Maquennehan F, Clause J (1987) Chloration et déchloration des eaux de Paris. L'Eau, l'Industrie, les Nuisances 113 : 63-66

Maron DM, Ames BN (1983) Revised methods for the Salmonella mutagenicity test. Mutation Research 113 : 173-215

Meier JR, Lingg RD, Bull RJ (1983) Formation of mutagens following chlorination of humic acid ; a model for mutagen formation during drinking water treatment. Mutation Research 118 : 25-41

Morlay C, De Laat J, Dore M, Courtois Y, Houel N, Montiel A (1990) Dechlorination with sodium sulfite : effect on the TOX concentration and the mutagenicity of chlorinated solutions of aquatic humic substances. In proceedings of the Sixth European Symposium "Organic micropollutants in the aquatic environment". Lisbon (Portugal), May 22-24, 1990

Sadiki A, De Laat J, Dore M, Montiel A, Houel N (1990) La déchloration des eaux potables : étude de la réactivité des dérivés du soufre IV sur les composés organiques en milieu aqueux dilué. J Fr Hydr, 21, n°1 : 77-92

Thurman EM, Malcolm RL (1981) Preparative isolation of aquatic humic substances. Environ Sci Technol 15 : 463-466

Wilcox P, Denny S (1985) Effect of dechlorinating agents on the mutagenic activity of chlorinated water samples. In : Jolley RL et al. (eds) Water Chlorination : Chemistry, Environmental Impact and Health Effects, vol 5. Lewis Publ., Inc., Chelsea, MI (USA), pp 1341-1353

Received August 3, 1990 ; Accepted January 11, 1991.