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Studies on mutagenicity and disinfection by-products in river drinking water disinfected with peracetic acid or sodium hypochlorite

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Abstract The aim of this research was to study at a pilot plant the influence of peracetic acid (PAA) on the formation of mutagenic compounds in river waters used for human consumption. The results obtained using PAA were compared to those for the most commonly used disinfectant, sodium hypochlorite (NaClO). Ames test and three genotoxicity plant tests, *Allium* tests and *Tradescantia*/micronuclei (TRAD/MCN) test, were used to evaluate the mutagenic activity of disinfected water samples. Chemical analysis, using gas chromatography/mass spectrometry (GC/MS), was also performed to identify disinfection by-products (DBPs). A slight bacterial mutagenicity was found in raw river water and similar activity was detected in disinfected water samples. Plant tests gave genotoxicity only for raw river water. DBPs identified in PAA-treated water included carboxylic acids, a few non-halogenated alcohols and carbonyl-containing compounds, whereas some potentially mutagenic halogenated by-products were found in NaClO-treated samples. Although PAA appears to be promising for water potabilization, these results must be confirmed with different source waters and with higher concentrations of PAA.

Keywords Disinfection; disinfection by-products; drinking water; mutagenicity; peracetic acid

Introduction

Several previous studies have detected many organic mutagenic/carcinogenic compounds in drinking waters (Meier, 1988; Monarca and Pasquini, 1989; Richardson *et al*., 1994; Monarca *et al*., 1998; Richardson, 1998; Richardson *et al*., 1999a). The origin of these toxic compounds is not only industrial, agricultural and urban pollution but also the disinfection of drinking water, particularly when surface water is chlorinated (World Health Organization, 1996). In 1974 it was first found that water chlorination forms many mutagenic/carcinogenic by-products derived from the reaction of chlorine with organic compounds (humic and fulvic acids) naturally present in surface water (Rook, 1974; Meier, 1988; International Agency for Research on Cancer, 1991; Richardson, 1998; WHO, 1996). Several epidemiological studies have found a correlation between chlorinated drinking water consumption and gastrointestinal cancer (Morris, 1995). More recently some researchers have conducted epidemiological studies in Finland, USA and Taiwan and found an association between the mutagenicity of chlorinated drinking water and cancer of the urinary and gastrointestinal tract (Ijsselmuiden *et al*., 1992; Koivusalo *et al*., 1994, 1995; Schenck *et al*., 1998; Tao *et al*., 1999). For this reason some alternative disinfectants are being explored in an attempt to reduce these potential health risks (Monarca *et al*., 1998; Richardson, 1998).

The aim of this research was to study the influence of a new disinfectant, peracetic acid (PAA, CH_3-CO-OOH), on the formation of mutagens in river water used for human consumption. PAA is a strong disinfectant and has shown a recent increase in use for

disinfecting wastewater (Baldry and French, 1989; Baldry *et al*., 1991; Lefevre *et al*., 1992; Baldry *et al*., 1995) and its applicability to drinking water is currently under study. River water samples were disinfected with PAA or NaClO at a pilot plant and studied with a combined approach using the Ames test and three genotoxicity plant tests (root anaphase aberration assay and root/micronuclei assay in *Allium cepa* and TRAD/MCN test). Chemical characterization of DBPs by GC/MS was also performed.

Materials and methods

Description of the pilot treatment plant

Water samples were taken before and after disinfection at a pilot drinking water treatment plant supplied by river water. The pilot plant, which worked over a mean flow of 400 L/h, utilized a pre-disinfection system followed by a flocculation treatment (6 mg/L of ferric chloride) and filtration using granular activated carbon (GAC). The water samplings were carried out at the raw river water inlet and before GAC filtration. The contact time between the water and the oxidant was approximately 90 minutes. PAA-disinfected water (3 mg/L) and NaClO-disinfected water (1.5 mg/L) were concentrated on C_{18} cartridges and then tested for mutagenicity (Ames test) and for the presence of chemical DBPs. Non-concentrated water samples were also studied using three *in situ* genotoxicity plant tests.

Sample concentration

The water samples (50–60 L) collected before and after disinfection were paper-filtered to remove suspended solids to avoid silica cartridge block. Ames tests on the extracts of particulates adsorbed on paper filters always gave negative results. After filtration the water samples were acidified with HCl (pH 2.5), because mutagenicity found in chlorinated drinking water is primarily due to acidic compounds (Monarca *et al*., 1985). The concentration of water was carried out at a 10–15 mL/min. flow rate on C_{18} cartridges (Sep-Pak Plus tC_{18} Environmental Cartridges, Waters Chromatography) according to the Environmental Protection Agency 525.2 method (1994) (US EPA, 1994). The cartridges had previously been washed with 5 mL of ethyl acetate, 5 mL of dichloromethane, 10 mL of methanol, and 10 mL of distilled water. Three litres of water were adsorbed on each cartridge, and then the cartridges were dried in a flow of nitrogen and eluted with 5 mL ethyl acetate and 5 mL dichloromethane. The two eluates were reduced to 1 mL by rotary evaporation and dried by a gentle stream of nitrogen. Finally the concentrates, equivalent to 40–50 L, were studied using the Ames test and the extracts, equivalent to 10 L, were analyzed by means of GC/MS.

Ames test

The adsorbates were dissolved in dimethylsulphoxide (DMSO) and tested in duplicate at increasing doses (corresponding to 1, 2 and 3 L of equivalent volume per plate) with the Ames test, using *Salmonella typhimurium* TA98 and TA100 strains, with and without *in vitro* microsomal activation (S9 mix) to detect indirect and direct mutagenic compounds (Maron and Ames, 1983). The results were evaluated according to the *Standard Methods for the Examination of Water and Wastewater* (1998).

Plant genotoxicity tests

Allium cepa tests. Equal-sized (2–2.5 cm diameter) young bulbs of common *Allium cepa* were used for the *Allium* root anaphase aberration assay (Fiskesjo, 1988) and the *Allium* root/micronuclei assay (Grant, 1982). Onion bulbs germinated directly in water samples for 72 hours were analyzed. Afterwards, the onion bulbs were maintained in Hoagland's solution for 24 hours (recovery time) and the roots were fixed in 1:3 acetic acid–ethanol

solution for 24 hours, and stored in 70% ethanol. Slides were scored for the mitotic index (1,000 cells per slide), for anaphasic aberrations, namely bridges, laggard chromosomes and fragments (800 anaphasic cells per sample), and for micronuclei. Micronucleus frequency was determined by examination of 1,000 cells per slide (5,000 per sample). Analysis of variance was performed for data analysis. Furthermore, each sample was compared with the negative control using Dunnett's test for multiple comparisons as commonly suggested for these experimental studies (Fiskesjo, 1988).

TRAD/MCN test. In the TRAD/MCN test young inflorescences were immersed in the water samples for 24 hours. After 24 hours of recovery time and 24 hours of fixing in 1:3 acetic acid–ethanol solution (Carnoy's), the buds were stored in 70% ethanol. Natural mineral water (total dissolved solids: 74 mg/L; COD: 0.3 mg/L) was used as a negative control. Slides were observed to score micronuclei frequencies in early tetrads, expressed as MCN/100 tetrads. Data were statistically analysed by means of the *F*-test for analysis of variance, and the significant difference (at the $p<0.05$ level) between the negative control and a series of treated groups was determined with Dunnett's test for multiple comparisons (Ma *et al*., 1994).

Chemical analyses

Chemical characterization of DBPs using GC/MS was performed on the same concentrates tested for mutagenicity.

Results and discussion

PAA- and NaClO-disinfected river water samples showed a low bacterial mutagenicity with TA98-S9, similar to that found in raw water (Table 1).

The unconcentrated water samples tested using the *Allium cepa* assays showed a weak genotoxicity only in raw water (Table 2). Statistical analysis on chromosomal aberrations carried out by means of Dunnett's test gave a very significant p value ($p<0.01$) for raw water compared with distilled water. The disinfected samples exhibited a lower frequency of aberrations than the raw water samples. This may be due to the destruction of unknown genotoxins in the raw water by the disinfectants. Visible toxic effects (root malformations and inhibition of growth) were found on roots exposed to treated waters, being similar for NaClO and PAA disinfection treatments (data not shown).

Negative results were also found with the TRAD/MCN test (Table 3). Variance analysis and Dunnett's test showed that the frequency of micronuclei was similar in the negative control and in the tested samples.

Table 1 Bacterial mutagenicity (Ames test) of river water treated with PAA or NaClO

Water samples	Doses (L/plate)	Mutagenicity TA98 –S9	TA98 +S9	TA100 –S9	TA100 +S9
	1	–	–	–	–
Raw river water	2	+	–	–	–
	3	–	–	–	–
	1	+	–	–	–
PAA-disinfected	2	+	–	–	–
	3	+	+	–	–
	1	+	–	–	–
NaClO-disinfected	2	+	–	+	–
	3	–	–	–	–
Negative control[a]		–	–	–	–

+ = mutagenic (at least twice the revertants/plate for negative control);
[a]200 µL DMSO

Table 2 Anaphase aberrations and frequency of micronuclei in *Allium cepa* roots exposed directly to river water samples

Water samples	Anaphase aberrations	Micronuclei
Raw river water	+	–
PAA-disinfected water (3 mg/L)	–	–
NaClO-disinfected water (1.5 mg/L)	–	–
Negative control[a]	–	–

+ = statistically significant vs. distilled water according to Dunnett's *t*-test ($p<0.01$);
[a]distilled water

Table 3 Frequency of micronuclei (MCN) in early tetrads of *Tradescantia* inflorescences exposed to raw and disinfected river water

Water samples	Micronuclei
Raw river water	–
PAA-disinfected water (3 mg/L)	–
NaClO-disinfected water (1.5 mg/L)	–
Negative control[a]	–

[a]distilled water

Gas chromatography/mass spectrometry was used to identify DBPs (Table 4) in the river water samples. Many compounds that are common pollutants or are used in industry have been found to be DBPs. Non-halogenated carboxylic acids have also been shown to be DBPs, but they are also frequently found in untreated raw source waters (Richardson, 1998). The DBPs identified in the PAA-treated water, in which no halogenated compounds were found, appear to be consistent with the low level of mutagenic activity observed. The presence of tributyl phosphate probably derives from phosphonates used as PAA stabilizers. On the contrary, the water samples treated with NaClO showed the presence of

Table 4 Disinfection by-products identified by CG/MS in treated river water

PAA	NaClO
benzoic acid	4-bromo-2,6-di-tert-butylphenol
decanoic acid	bromochloroacetic acid*
2-ethyl-1-hexanol	bromodichloromethane*
lauric acid	bromoform*
myristic acid	bromotetramethylbenzene
nonanal	bromotrimethylbenzene
nonanoic acid	decanoic acid
octanoic acid	dibromoacetic acid*
	dibromoaminobenzoic acid
	dibromochloromethane*
	dichloroacetic acid*
	hexanedioic acid
	lauric acid
	myristic acid
	nonanal
	nonanoic acid
	octanoic acid
	1,1,1,3,3-pentachloro-2-propanone*
	1,1,3,3-tetrachloro-2-propanone*
	tribromoaniline
	tribromophenol
	trichloroacetic acid*

* Genotoxic agent

halogenated by-products. Some of these identified compounds are suspected of being mutagenic and/or carcinogenic (Fujie *et al*., 1990; Bull and Kopfler, 1991; Patterson *et al*., 1995; Giller *et al*., 1997; Nelson *et al*., 2001).

Conclusions

This study on river waters disinfected in a pilot plant showed that raw river waters were slightly mutagenic with the Ames test, similar to the PAA- or NaClO-disinfected waters. Therefore, it was impossible to show any increase in bacterial mutagenicity due to disinfection. Exposure of *Allium cepa* roots to unconcentrated river water samples showed an increase of chromosomal aberrations in raw river water; this activity was reduced after disinfection with the two compounds. The results show that this *in situ* test would appear to be promising for monitoring drinking water before and after disinfection. The concentrations of disinfectants used for treating these water samples seem to be unable to produce mutagenicity detectable with the short-term mutagenicity tests. On the contrary, gas chromatography/mass spectrometry analyses allowed the identification of specific disinfection by-products formed in disinfected water samples. The dominant by-products produced by PAA disinfection were found to be carboxylic acids, which are not recognized as mutagenic, whereas the water samples treated with NaClO showed the presence of halogenated by-products. Some of these identified compounds are known as mutagenic/carcinogenic agents (Fujie *et al*., 1990; Bull and Kopfler, 1991; Patterson *et al*., 1995; Giller *et al*., 1997; Nelson *et al*., 2001).

In conclusion, in these preliminary studies PAA appears to be promising for potential application in the pre-disinfection phase. These results need to be confirmed, however, in particular, pilot and full-scale plant studies with different source waters and with higher concentrations of PAA should be performed to allow a better evaluation of this disinfectant.

The biological/chemical approach used allowed a rapid and thorough study of the different disinfection treatments and their effect on water mutagenicity and DBP formation. Short-term genotoxicity tests reveal different genetic end-points and different types of genotoxins and, together with chemical analyses, provide additional, useful information on the formation of compounds that are potentially hazardous to human health.

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