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Genotoxicity of the disinfection by-products resulting from peracetic acid- or hypochlorite-disinfected sewage wastewater

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Abstract

Wastewater disinfection is routinely carried out to prevent the spread of human pathogens present in wastewater effluents. To this aim, chemical and physical treatments are applied to the effluents before their emission in water bodies. In this study, the influence of two widely used disinfectants, peracetic acid (PAA) and sodium hypochlorite (NaClO), on the formation of mutagenic by-products was investigated. Wastewater samples were collected before and after disinfection, in winter and in summer, at a pilot plant installed in a municipal wastewater-treatment plant. Samples were adsorbed using silica C_{18} cartridges and the concentrates were tested for mutagenicity in the *Salmonella typhimurium* reversion test with strains TA98 and TA100. Non-concentrated water samples were tested with two plant genotoxicity assays (the *Allium cepa* root anaphase aberration test and the *Tradescantia*/micronucleus test). Mutagenicity assays in bacteria and in *Tradescantia* showed borderline mutagenicity in some of the wastewater samples, independent of the disinfection procedure applied. Negative results were obtained in the *A. cepa* anaphase aberration test. These results indicate that, in the conditions applied, wastewater disinfection with PAA and NaClO does not lead to the formation of significant amounts of genotoxic by-products.
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Keywords: Wastewater; Disinfection; Peracetic acid; Mutagenicity; Bacterial reversion assays; *Allium*; *Tradescantia* assays

1. Introduction

Chlorine disinfection is widely used in the tertiary treatment of urban wastewaters, with the aim of reducing microbial contamination and preventing the spread of pathogens into the environment. However, chlorine can react with natural organic substances (humic and fulvic acids) present in surface waters, giving rise to numerous volatile and non-volatile disinfection by-products (DBP) with mutagenic and/or carcinogenic activity (Rook, 1974; Daniel et al., 1993; Glaze et al., 1993; DeMarini et al., 1995; Meier et al., 1996). It is therefore necessary to search disinfectants alternative to chlorine which are effective against microbial contamination of wastewater, and concurrently reduce DBP.

Peracetic acid (PAA) is a proposed alternative to chlorine for the disinfection of urban effluents. PAA was

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found to be effective against bacteria and viruses present in urban wastewater, and slightly affected by the presence of organic matter in the medium (Baldry and French, 1989; Baldry et al., 1991, 1995; Lefevre et al., 1992). Disinfection of surface water used for human consumption with PAA has recently been shown to produce only carboxylic acids, devoid of mutagenic properties (Monarca et al., 2002). However, no investigation on the influence of PAA on the formation of DBP from wastewater disinfection has been carried out.

Short-term mutagenicity assays have been widely used to assess the formation of genotoxic DBP from wastewater disinfection. To this aim, both bacterial mutagenicity tests (Saxena and Schwartz, 1979; Meier and Bishop, 1985; Meier et al., 1987; Monarca et al., 2000) and plant genotoxicity assays (Rank and Nielsen, 1994; Monarca et al., 2000) have successfully been used to evaluate the genotoxicity of DBP. In this work, the formation of DBP in municipal wastewater samples disinfected with PAA or chlorine (NaClO) has been investigated with in vitro bacterial reversion assays and with plant genotoxicity tests.

2. Materials and methods

2.1. Description of the plant

The experimentation was carried out in a pilot plant installed at a municipal wastewater-treatment plant located in Rome (Italy). The municipal plant uses a conventional sewage-treatment system based on screening, primary clarification, aeration and biological oxidation through activated sludge, secondary clarification, and chlorination. Detailed information on the structure of the apparatus has been reported elsewhere (Veschetti et al., 2003). Residence times (t_R) of each tank were determined by injecting rapidly a tracer (6 l of a saturated solution of sodium chloride) into the sewage stream at the inlet of the pilot plant and by measuring the electrical conductivity at the exit of the tanks. Instantaneous flow rates of the tracer added to the sewage were also recorded during the determination of the conductivity profiles.

2.2. Wastewater treatments

NaClO and PAA solutions containing 5% or 15% of technical-grade disinfectant were supplied by Zarrelli (Rome, Italy) and Solvay Interox (Brussels, Belgium), respectively. Their actual concentration was determined daily by iodometric analyses before starting tests. Treatment conditions (final concentration of PAA or NaClO in wastewater and time of contact) were as follows:

- First treatment: 4 mg/l for 37 min;
- Second treatment: 4.1 mg/l for 26 min;
- Third treatment: 2 mg/l for 37 min;
- Fourth treatment: 4 mg/l for 26 min.

At the end of treatment period, both untreated and the disinfected wastewater samples were added with ferrous sulfate ($FeSO_4$) to eliminate free disinfectants. Unconcentrated water samples, with and without $FeSO_4$, were tested in toto in the plant genotoxicity tests. Samples tested in bacterial assays were concentrated as described below.

2.3. Concentration of wastewater samples

Wastewater samples collected before and after disinfection treatments were added with $FeSO_4$, then passed on filter paper (Whatman 5) to eliminate the suspended solids, acidified with hydrochloric acid at pH 2–2.5, and passed on trifunctional silica C_{18} cartridges (Sep-Pak Plus tC_{18} Environmental Cartridges, Waters Chromatography) according to US EPA 525.2 method (US Environmental Protection Agency, 1995). 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. Two litres of wastewater samples were adsorbed on each cartridge, which were then dried under a flow of nitrogen and eluted with 5 ml ethyl acetate and 5 ml dichloromethane. The eluates were reduced to a small volume by means of a rotating vacuum evaporator, mixed and dried under nitrogen flow. The dry residue was dissolved in dimethylsulfoxide (DMSO) and stored in the dark at −20 °C.

2.4. Chemical analyses

Duplicate wastewater samples were collected in 500-ml glass bottles at the inlet and outlet of the pilot plant. Sampling operations were repeated three times at each programmed experimental condition to determine the reproducibility of the results. Sodium sulfite (0.50 g) was introduced in all bottles containing the disinfected wastewater in order to reduce the residues of the oxidizing agents. One sample was acidified with 2 ml of concentrated sulfuric acid (96%). Collected samples were stored at approximately 4 °C and subsequently analyzed.

Total organic carbon (TOC), adsorbable organic halogens (AOX) and ammonia were determined in the acidified samples, while nitrite, nitrate and total suspended solids (TSS) were dosed in the remaining samples. All these analytic procedures were carried out

Table 1
Physico-chemical and chemical characteristics of the sewage before the disinfection

Parameter		Treatment no.			
		1	2	3	4
Temperature	°C	15.0	13.9	15.0	19.1
pH		7.60	6.58	7.60	6.89
Electrical conductivity at 25 °C	mS/cm	0.84	0.88	0.84	0.84
Dissolved oxygen	mg/l	4.5	5.2	4.5	5.0
E_h	mV	282	315	283	302
Turbidity	NTU	9.6	5.4	14.3	6.1
TSS	mg/l	18	9	17	ND
TOC	mg/l C	53	59	60	ND
AOX	μg/l Cl	43	53	21	ND
NH_4^+	mg/l	0.20	0.46	4.14	ND
NO_2^-	mg/l N	0.29	0.36	0.21	0.33
NO_3^-	mg/l N	6.5	5.8	8.8	3.9

ND: not determined.

as described in the literature (Standard Methods 4500-Cl G, APHA, 1998a). Water temperature, pH, electrical conductivity, dissolved oxygen and redox potential (E_h) with respect to the standard-hydrogen-electrode potential were measured in the field with a multi-parametric probe during sampling operations. Table 1 lists physico-chemical and chemical composition of the sewage employed during the tests.

Free and total chlorine in the effluent treated with NaClO and residual PAA in wastewater disinfected with PAA were determined, every 10 min, by automated flow-injection analyzers located at the exit of the pilot plant. Disinfectant residues were dosed by applying the DPD colorimetric method (Standard Methods 4500-Cl G, APHA, 1998a) after decomposing H_2O_2 by means of catalase. Hydrogen-peroxide residue in wastewater treated with PAA was determined spectrophotometrically.

2.5. Bacterial mutagenicity test

Wastewater concentrates were tested in duplicate at increasing doses (corresponding to 60, 120, 240 and 480 ml of water per plate) with *Salmonella typhimurium* strains TA98 and TA100, with and without exogenous metabolic activation by Aroclor-induced rat liver S9 (Ames et al., 1975). Two experimental procedures were used for treatment of tester strains, the microsuspension method (Kado et al., 1983) and the standard preincubation method (Maron and Ames, 1983).

With the *microsuspension method* (Kado test), overnight cultures of tester strains in nutrient broth were concentrated 10-fold by centrifugation and resuspended in 0.1 M phosphate buffer (pH 7.4); 100 μl of concentrated bacteria ($3–6 \times 10^8$ viable cells) were incubated for 90 min at 37 °C in 13 × 100 mm test tubes together with 100 μl of S9 Mix (10% v:v) or phosphate buffer, and 6–48 μl of DMSO solution of water concentrate.

With the *standard preincubation method*, 100 μl of overnight bacterial culture in nutrient broth were incubated with the water concentrate dissolved in DMSO together with 500 μl of S9 Mix or buffer for 60 min at 37 °C.

With both methods, at the end of incubation 2 ml of molten (42 °C) top agar with 0.5 mM histidine and biotine was added to each tube, the content obtained was mixed and poured onto minimal medium plates. Revertant colonies were hand counted after 48 h of incubation at 37 °C. All determinations were made on duplicate plates in two independent experiments. Positive controls were included in each assay, as well as controls of samples and S9 sterility.

The data obtained were expressed as *mutagenicity ratio,* dividing the average revertants/plate by the spontaneous mutation rate. Results were considered positive if two consecutive dose levels or the highest non-toxic dose level produced a response at least twice that of the solvent control doses, and at least two of these consecutive doses showed a dose-response relationship (APHA, 1998b).

2.6. Plant genotoxicity tests

Tradescantia-micronucleus test. Unconcentrated wastewater samples were tested in the *Tradescantia*-micronucleus test using the *Tradescantia* clone #4430 (Ma et al., 1994) groups of 20 cuttings with young inflorescences were partially immersed with their stem in the wastewater samples for 24 h. After exposure, inflorescences were maintained on mineral water for 18 h

(recovery time) and then for 24 h of fixing in 1:3 acetic acid–ethanol solution (Carnoy's). After that, the buds were stored in 70% ethanol. Natural mineral water stored in glass bottles was used as a negative control. As an index of genetic damage, micronuclei were scored in meiotic pollen mother cells in slides prepared from the buds. Generally about 300 tetrads were scored from each of the 7 slides in each experimental group. The micronucleus frequencies were calculated by dividing the total number of micronuclei (MCN) by the total number of tetrads scored and expressed as MCN/100 tetrads. Data were statistically analyzed by ANOVA *F*-test and the significance (at 0.05 level) between the negative control and the series of treated groups determined with Dunnett's *t*-test. To evaluate the effect of $FeSO_4$, the results obtained with each disinfectant with and without $FeSo_4$ were compared by Students's *t*-test.

Allium cepa anaphase aberration test. For the *Allium* root anaphase aberration assay (Grant, 1982; Fiskesjo, 1985) equal-sized young bulbs of common *A. cepa* (2–2.5 cm diameter) were used. Bulbs were exposed to wastewater samples for 72 h. The roots were fixed in 1:3 acetic acid–ethanol solution for 24 h, and stored in 70% ethanol. As a marker of genotoxicity, anaphase aberrations (bridges, laggard chromosomes and fragments) were scored in 800 anaphasic cells per sample. The mitotic index was determined in 1000 cells to evaluate the effect of treatments on cellular division rate. Moreover, a few macroscopic parameters (modifications in root consistency and form, formation of tumors, hook roots, twisted roots) were recorded as an index of toxicity. Data on anaphase aberrations and mitotic index were analyzed for statistical significance by the χ^2 test. Root growth in different experimental groups was compared using Student's *t*-test.

3. Results

3.1. Bacterial mutagenicity assays

The results obtained in the microsuspension bacterial reversion assay with three series of disinfected wastewater samples are presented in Table 2. Wastewater concentrates showed variable toxicity to bacterial strains, more evident toward strain TA100, not consistently associated to the disinfection procedure applied. Similarly, tested samples elicited variable, although weak, mutagenic response which was apparently unrelated to wastewater disinfection. No mutagenic activity was observed in experiments with concentrates from *first treatment*, prepared from wastewater disinfected under relatively severe conditions (4 mg/l of disinfectants for 37 min). On the other hand, borderline mutagenicity toward strain TA98 was observed in assays with concentrates from the *second* and the *third treatments* (relative to water samples treated with 4.1 mg/l for 26 min and with 2 mg/l of disinfectants for 37 min, respectively), independently from the disinfectant used (NaClO, PAA, or none).

In the hypothesis that the toxic effects observed with the microsuspension procedure might lead to an underestimation of water mutagenicity, a new series of assays was carried out using the standard preincubation protocol. The results obtained with wastewater samples treated for 26 min with 4 mg/l of disinfectants (*fourth treatment*) (Table 3) show borderline increases of revertant colonies in strain TA98, which exceeded twice negative control values with samples treated with NaClO (with and without S9) and PAA (only without S9). Weak direct mutagenicity in strain TA98 was also observed in extracts of particulate matter harvested on filters used for the prefiltration of untreated wastewater (Table 3).

3.2. Tradescantia-micronuclei test

In the *first treatment*, both untreated and NaClO-disinfected wastewater, without $FeSO_4$, elicited significant mutagenicity in comparison with the negative control mineral water (Table 4). However, the mutagenicity of the NaClO-disinfected sample did not differ significantly from that of raw water. The addition of $FeSO_4$ reduced the genotoxicity of NaClO-disinfected water.

In the *third treatment*, only the sample of PAA-disinfected water supplemented with $FeSO_4$ induced a significant mutagenic effect in comparison with the negative control. However, none of disinfected samples differ significantly from untreated wastewater (Table 4).

In the *fourth treatment,* no mutagenicity was found in any sample in comparison with the negative control. Besides, a statistically significant reduction of micronuclei was found in PAA-disinfected water in comparison with raw water (Table 4). Addition of $FeSO_4$ did not influence the genotoxic activity of wastewater samples.

3.3. A. cepa anaphase aberration test

Tests with the *A. cepa* anaphase aberration test always gave negative results: the disinfectant treatments did not induce increases of chromosomal anaphase aberrations in root cells (Table 5). Only in the *third treatment* the mitotic index for PAA-disinfected water presented lower values in comparison with negative control, showing a reduced cell division. In NaClO-disinfected water, supplemented with $FeSO_4$, a lower mitotic index was also found.

The observation of macroscopic parameters of roots exposed to the samples suggests that all the examined samples caused formation of apical root tumors, and in

Table 2
Results of bacterial mutagenicity assays on wastewater adsorbates—microsuspension procedure

Treatment	Disinfectant	Dose (l/plate)	Mutagenicity ratio			
			TA98		TA100	
			−S9	+S9	−S9	+S9
1	None	0.12	1.2	1.2	1.4	1.0
		0.24	0.8	1.3	1.1	0.9
		0.48	1.2	1.3	1.1	0.6
	NaClO	0.12	0.9	1.3	1.2	0.6
		0.24	0.9	1.2	1.2	0.8
		0.48	0.9	1.2	1.2	0.8
	PAA	0.12	1.0	1.3	1.2	1.0
		0.24	1.3	1.6	1.2	1.0
		0.48	0.6	0.8	0.7 t	0.5 t
2	None	0.06	4.8	1.7	0.8	0.8
		0.12	5.6	1.7	0.7	0.5
		0.24[a]	3.6 t	2.2	tox	tox
	NaClO	0.06	3.2	2.2	1.1	1.1
		0.12	4.5	2.6	0.6	0.7
		0.24[a]	2.5 t	3.3	tox	tox
	PAA	0.06	3.5	2.0	0.9	1.0
		0.12	4.0	2.1	0.7	0.7
		0.24[a]	5.0	2.9	tox	tox
3	None	0.06	2.1	1.2	0.9	1.1
		0.12	2.5	1.5	1.0	0.7
		0.24[a]	0.6 t	1.2	tox	0.5 t
	NaClO	0.06	3.0	1.3	1.0	1.2
		0.12	3.0	1.6	0.8	1.2
		0.24[a]	1.0	1.8	tox	0.7
	PAA	0.06	2.3	1.0	1.3	1.4
		0.12	2.7	1.4	1.0	1.6
		0.24[a]	2.3	1.5	tox	0.8

t, partially toxic (tiny background lawn of growth).
tox: completely toxic (no bacterial growth).
Spontaneous controls (range): TA98 (−S9), 20–43; TA98 (+S9), 26–35; TA100 (−S9), 126–200; TA100 (+S9), 190–249, Positive controls: TA98 −S9 (4-nitro-*o*-phenylendiamine 5 μg), > 1000 revertants/plate; TA98 + S9 (2-amino anthracene 1 μg), > 500 revertants/plate; TA100 −S9 (NaN_3 5 μg), > 1000 revertants/plate; TA100 +S9 (benzo(a)pyrene 5 μg), > 1000 revertants/plate.
[a]Higher dose (0.48 l/plate) completely toxic.

particular non-disinfected wastewater was toxic on root growth, producing twisted and hook roots and poor consistency roots. PAA disinfection in the *third* and the *fourth treatment* had only a slight effect on the formation of these morphological abnormalities (data not shown).

4. Discussion

Short-term mutagenicity tests are widely applied in the analysis of complex environmental mixtures as sensitive tools for the detection of trace amounts of contaminants or unknowns endowed with genotoxic properties. Among environmental matrices, water has extensively been investigated. Most studies focussed on drinking water, where the occurrence of genotoxic contaminants and chlorine by-products has intensively been studied by means of tests in bacteria (Alink, 1982; Monarca et al., 1985; Vartiainen and Liimatainen, 1986; Wilcox and Williamson, 1986; Meier, 1988; Galassi et al., 1989; Monarca and Pasquini, 1989; Monarca et al., 1998) and plant systems (Helma et al., 1994; Monarca et al., 1998).

Even though drinking water has certainly a greater potential to impact human health, also wastewater contamination may also pose health risks, e.g. in case of its re-use in agricultural practice. Moreover, it is

Table 3
Results of bacterial mutagenicity assays on wastewater adsorbates and particulate extracts—preincubation procedure

Treatment	Disinfectant	Dose (l/plate)	Mutagenicity ratio			
			TA98		TA100	
			−S9	+S9	−S9	+S9
4 (Wastewater adsorbate)	None	0.12	1.5	1.2	1.4	1.0
		0.24	1.5	1.4	1.5	1.1
		0.48	1.7	1.4	0.9 t	0.9
	NaClO	0.12	2.3	1.9	1.2	0.9
		0.24	2.0	2.0	1.3	1.0
		0.48	1.4	2.0	1.2	1.0
	PAA	0.12	1.6	1.6	1.3	0.9
		0.24	2.3	1.9	1.3	1.1
		0.48	1.6	1.5	1.2	0.9
4 (Particulate extract)	None	0.12	2.0	2.2	1.2	1.1
		0.24	4.1	3.3	1.2	0.9
		0.48	4.8	4.2	1.5	0.5 t
	NaClO	0.12	1.2	0.9	1.0	0.9
		0.24	1.6	0.9	1.2	0.9
		0.248	2.0	1.4	1.0	0.9
	PAA	0.12	1.3	1.1	1.1	0.9
		0.24	1.8	1.7	1.2	1.0
		0.48	2.7	1.7	1.1	0.9 t

t, partially toxic (tiny background lawn of growth).
Spontaneous controls (range): TA98 (−S9), 16–21; TA98 (+S9), 16–18; TA100 (−S9), 74–110; TA100 (+S9), 106–160, positive controls: TA98 −S9 (4-nitro-*o*-phenylendiamine 5 μg), > 500 revertants/plate; TA98 + S9 (2-amino anthracene 1 μg), > 500 revertants/plate; TA100 −S9 (NaN_3 5 μg), > 1000 revertants/plate; TA100 +S9 (benzo(a)pyrene 5 μg), > 500 revertants/plate.

Table 4
Frequency of micronuclei in early tetrads of *Tradescantia* inflorescences exposed to urban wastewater

Sample	MCN/100 tetrads (mean ± SD)		
	Treatment		
	1	3	4
Negative control (Mineral water)	1.6 ± 0.8	3.4 ± 1.9	2.9 ± 1.8
Untreated wastewater	7.2 ± 3.4**	10.4 ± 6.7	6.0 ± 3.5
NaClO-disinfected	9.1 ± 4.2**	4.1 ± 3.0	3.4 ± 1.5
NaClO-disinfected + $FeSO_4$	2.8 ± 1.1	1.3 ± 1.5	4.5 ± 3.2
PAA-disinfected	5.6 ± 4.2	4.5 ± 5.9	2.4 ± 1.1***
PAA-disinfected + $FeSO_4$	2.8 ± 1.2	11.8 ± 6.3*	2.2 ± 1.1***

*Significantly different from negative control ($p < 0.05$, Dunnett's *t*- test); **Significantly different from negative control ($p < 0.01$).
***Significantly lower than untreated water ($p < 0.05$, Dunnett's *t*- test).

conceivable that wastewater represents a better substrate for by-product formation during disinfection, due to the high content of organic precursors. Previous investigation on urban and industrial wastewaters (Meier and Bishop, 1985; Meier et al., 1987; Monarca and Pasquini, 1989; Nielsen and Rank, 1994; Saxena and Schwartz, 1979) did not address specifically the formation of genotoxic DBP in treated effluents. In this work, the possible generation of mutagenic by-products was investigated in urban wastewater disinfected with NaClO, currently the most used disinfecting agent, and PAA, a proposed alternative to chlorine for disinfection and sanitation purposes. To this aim, two in vitro assay systems were applied, namely the analysis of wastewater

Table 5
Root length, anaphase aberrations and mitotic index in *Allium cepa* exposed to urban wastewater

Sample	Treatment								
	1			3			4		
	Root length (cm)	Anaphase aberrations (%)	Mitotic index (%)	Root length (cm)	Anaphase aberrations (%)	Mitotic index (%)	Root length (cm)	Anaphase aberrations (%)	Mitotic index (%)
Negative control (mineral water)	2.6±1.1	1.6	6.2	2.8±0.8	1.7	9.3	2.7±1.3	0.9	5.4
Untreated wastewater	3.2±0.4	1.5	7.2	1.3±1.6	2.3	9.2	2.1±0.8	1.3	6.4
NaClO-disinfected	2.8±1.3	1.0	9.2	1.9±1.3	2.2	11.2	2.5±0.3	0.9	6.0
NaClO-disinfected + $FeSO_4$	3.5±0.7	1.0	6.8	1.9±1.2	2.9	8.3**	2.0±0.7	0.8	6.6
PAA-disinfected	2.6±0.9	2.1	7.6	2.3±1.0	2.9	6.2*	2.2±0.8	1.4	6.7
PAA-disinfected + $FeSO_4$	2.6±0.6	1.9	7.4	3.1±1.3	3.1	8.5	2.2±0.7	0.7	5.5

*Significantly different from negative control (χ^2 test, $p<0.05$).
**Significantly different ($NaClO+FeSO_4$ vs. NaClO) according to χ^2 test ($p<0.05$).

concentrates in bacterial reversion tests, and the assay of unconcentrated water samples in plant systems. The former approach, which is the most widely used in the mutagenetic analysis of acqueous matrix, allows the efficient detection of trace amounts of organic genotoxic components through their concentration on proper absorbents, allowing the assay of equivalent volumes of water samples otherwise untestable. On the other hand, cytogenetic tests on plants allow the detection of clastogens, including inorganic compounds, in the whole acqueous matrix. The two test methods address different genetic end-points, i.e. point mutations due to base pair substitution or base insertion/deletion, and cytogenetic damages due to the alteration of chromosome integrity or segregation. Current knowledge suggests that both end-points have similar relevance and involvement in pathological processes, and therefore both need to be investigated in genotoxic hazard identification (Muller et al., 2003). Due to the specificity of the genotoxic profile of chemical mutagens, which rarely affect different end-points with the same efficiency, the two methods applied herein are expected to work in a complementary way, providing only partially overlapping results. Therefore, discrepancies in the experimental results, as observed in this study, have not to be considered as incongruence, but a consequence of the specific mode of action of the agents tested. In this study, mutagenicity assays in bacteria showed for some of the wastewater extracts, borderline mutagenicity, which was apparently unrelated to the applied disinfection procedure. Such results, together with the variable toxicity levels observed in different samples, are likely to highlight significant day-to-day variation in the composition of sewage. In any case, the genotoxic response elicited by wastewater extracts was quite low, and partially suppressed in the presence of a mammalian metabolizing system (S9).

Some genotoxic activity was found by examining in toto water samples with the *Tradescantia-micronucleus* test. Raw and NaClO-disinfected wastewater from the *first treatment* and PAA-disinfected wastewater added with $FeSO_4$ from the *third treatment* showed an increased frequency of micronuclei in *Tradescantia* tetrads. In some samples, a reduction of genotoxicity was observed in samples added with $FeSO_4$, both after disinfection with NaClO (*third treatment*) or with PAA (*fourth treatment*). On the other hand, complete negative results were obtained in the *A. cepa* anaphase aberration test, which appeared to be less sensitive than the *Tradescantia*-micronucleus assay to water mutagens.

5. Conclusion

The results obtained in this work and in previous investigations (Monarca et al., 2000, 2002) suggest that sewage disinfection with moderate doses of PAA does

not lead to the formation of significant amount of genotoxic DBP. However, further experiments with different urban effluents and using higher doses of PAA are required for a final evaluation of the applicability of PAA for wastewater treatment. In any case, taking into account the variable chemical composition of urban wastewaters, the routinary monitoring of genotoxicity and toxicity of treated wastewaters is recommended, in order to minimize human and environmental exposure to noxious pollutants.

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