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Ames and Sister Chromatid Exchange Tests of Organic Extracts from Drinking Water

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The finding of mutagenic and carcinogenic organic compounds in source water (Pelon et al. 1977) and drinking water (Loper 1980) has caused concern due to their potential effects on human health. Traditionally, the impact on water quality from the discharge of toxic pollutants into water resources has been evaluated by measurement of specific chemical pollutants. Furthermore, the estimation of the magnitude of risk from the presence of specific pollutants requires knowledge of the toxicological properties of each pollutant. An alternative approach to water quality assessment involves the use of biological test systems for determining the toxicological impact of drinking water pollutants.

The Ames test (Maron and Ames 1983) has been widely used for the evaluation of mutagenicity from organic pollutants in drinking water (Noot et al. 1989; Meier 1988). Moreover, it has proved to be suitable to correlate mutagenicity and carcinogenicity (Prival 1983), useful for the evaluation of complex mixtures such as organic extracts from water, fast, sensitive, inexpensive and requiring small sample volumes. The use of bacterial cells is the main disadvantage associated with this test for the evaluation of human risks. Theoretically, the response of mammalian cells may more closely approximate that of human cells than does the response of bacterial cells, because of substantial differences in cellular membranes and in the organization and packaging of DNA (Lockard et al. 1982).

Mammalian cell system short-term tests fill the gap between the frequently used bacterial assays and the whole animal short-term bioassays (Putman et al. 1983). The Sister Chromatid Exchange (SCE) test with human lymphocytes is less subjective, more rapid, and less costly than classical cytogenetic scoring methods (Latt et al. 1981). At present, many reports deal with two or more tests to evaluate the human and environmental risks of compounds from complex mixtures (Dieter et al. 1990).

This paper, shows the comparison of the results obtained by Ames and SCE tests from organic extracts of Barcelona's drinking water (N.E. Spain).

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MATERIAL AND METHODS

Barcelona's drinking water is coming from the chlorinated disinfection treatment of surface water from Llobregat river. This river is extremely polluted, bearing effluents from various industries including textile, salt works, surfactants and domestic wastewaters. Organic compounds present in raw and drinking water have been already described elsewhere (Rivera et al. 1986; Guardiola et al. 1991).

Amberlite XAD-2 resin (Daignault et al. 1988) was mixed and washed with methanol until it became clear (Junk et al. 1974). A methanol slurry containing 1000 ml of cleaned resin was packed in a glass column (40 mm x 600 mm); bed volumes were designed to accommodate 1200 ml of drinking water. XAD-2 column was washed by passing 5 l of Milli-Q water (pH=2 with 0.1 N HCl) after sampling.

500 L of drinking water samples were continuously acidified to pH 2.0 with HCl analytical grade and intermittently passed through the packed XAD-2 column at a flow rate of 100 ml/min. After sampling, the XAD-2 column was washed with 2 L of Milli-Q distilled water (pH=2 with 0.1 N HCl) and the residual water was blown off from the column by dry nitrogen. The column was eluted with 4 L of diethyl ether (DEE) and 4 L of methanol (ME). The extracts were dehydrated and evaporated to a small volume.

The diethyl ether extract (DEE) was fractionated into neutral (NDEE) and acidic (ADEE) fractions according to the method described by Coleman et al (1980). Aliquots for bioassays were dissolved in DMSO.

The *Salmonella typhimurium* TA98 and TA100 strains were used for the Ames test. Assays were carried out without S9 activation system (Onodera et al. 1989) as described by Maron and Ames (1983). The mutagenic properties of the strains were verified using positive (0.5 µg of sodium azide and 2-nitrofluorene) and negative (100 µl of DMSO and sodium phosphate buffer) controls as part of each experiment. They were performed in duplicate.

The Ames test results were given in genotoxicity ratio (GR): revertants on the test plates to those on the negative control (spontaneous revertants) and equivalent liter of water extract (Onodera et al. 1989). Thus, the spontaneous revertants (SR) is the GR = 1 (zero genotoxic value). The criterion for detection of mutagenicity in experimental samples was a dose dependent response curve exceeding the zero genotoxic value by at least two-fold. The toxicity detection was a negative dose dependent response curve.

Peripheral blood samples were obtained from different healthy subjects and used for the cytogenetic SCE test. The lymphocytes cultures were performed as described by Moorhead et al. (1960). The samples to be tested were added 24 h after initiation of culture, with the bromodeoxyuridine. Chromosome preparations

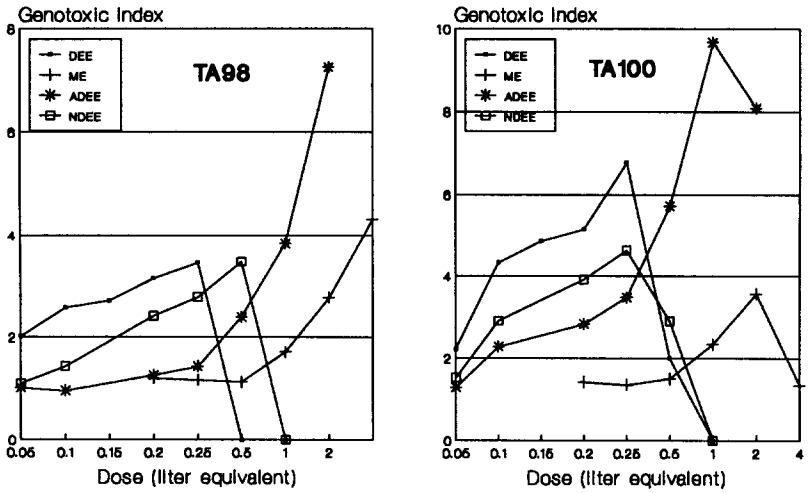

Figure 1. Ames' test results of drinking water extracts and fractions with TA98 and TA100 strains.

were made according to standard methods (hypotonic treatment with KCl 0,075 M, acetic alcohol fixation, air drying). Sister chromatid differentiation was induced by the FPG technique according to Perry and Wolff (1974).

In the SCE analysis, if possible, a total of 50 well-spread second metaphases were examined for each experimental concentration and donor. The Student's t-test was used for statistical evaluation and proliferation rate index (PRI) was calculated according to the formula of Ivett and Tice (1982).

RESULTS AND DISCUSSION

Representative dose-response curves depicting the mutagenicity of drinking water extracts and fractions in the *Salmonella* assay are shown in figure 1. In order to better compare the total levels of mutagenicity for the different samples and to see more clearly the mutagenicity activity of each extract and fraction, the mutagenicity results were also calculated in net-revertants per liter equivalent of drinking water (see table 1).

All samples showed a significant dose-related mutagenic effect on both TA98 and TA100 strains, but the TA100 strain was the most sensitive. The DEE was about 20 fold more mutagenic than ME using the TA100 strain. The former was toxic at dose levels greater than 0,25 L whereas at dose of 1 L no growth was observed at the test plates. The ME required more than 2 and 4 L respectively to obtain the same toxic effects described above for DEE. The NDEE fraction recovered 66% of the mutagenic activity and about 100% of the toxicity activity of DEE. The sum of the activity of the individual fractions ADEE and NDEE showed fairly good agreement with the mutagenicity of the DEE sample indicating that the mutagenic activities of the separate fractions were additive.

Table 1. Net His$^+$ revertants per liter equivalent of drinking water in the DEE, ME, ADEE and NDEE fractions.

	TA98 strain		TA100 strain	
SAMPLE	Net Revertants/l*	r^2	Net Revertants/l*	r^2
DEE	282	0.9256	3329	0.9961
ME	23	0.9912	191	0.9953
ADEE	90	0.9962	1325	0.9951
NDEE	163	0.92702	2210	0.9757
ADEE + NDEE	253	--	3535	--

*: Net Revertants/l=(GRliter x SR)-SR. SR: 30 revertants for TA98 and 150 revertants for TA100.

Table 2. Induction of SCEs by drinking water extracts in human lymphocytes (two donors).

Dose (liter equivalent)	nº of metaphases scored		SCE/cell±SE		PRI	
	D1	D2	Donor 1	Donor 2	D1	D2
DEE						
control	50	50	7.48±0.43	9.04±0.29	1.91	1.85
0.25	50	50	17.98±0.58*	18.34±0.48*	1.69	1.57
0.5	50	40	21.22±0.65*	21.85±0.60*	1.40	1.27
0.75	50	--	22.38±0.83*	cytotoxic	1.24	--
ADEE						
control	50	50	8.48±0.48	8.74±0.43	2.13	2.02
0.2	50	50	9.50±0.48	15.36±0.57*	1.97	1.84
0.4	50	50	13.00±0.50*	18.82±0.71*	1.88	1.98
0.6	50	50	14.08±0.78*	20.24±0.68*	1.78	1.98
NDEE						
control	50	50	9.08±0.44	6.90±0.33	1.93	2.03
0.25	50	50	14.42±0.62*	13.52±0.44*	1.43	1.75
0.5	30	50	18.86±1.08*	15.76±0.04*	1.11	1.64
0.75	--	--	M1 only(a)	cytotoxic	1.00	--
ME						
control	50	50	7.94±0.44	9.04±0.29	1.90	1.85
0.25	50	50	13.34±0.59*	13.88±0.40*	1.78	1.65
0.5	50	50	15.82±0.57*	15.68±0.66*	1.76	1.42
0.75	50	50	19.40±0.67*	15.46±0.44*	1.60	1.42
ADEE+NDEE(b)						
0.25			24.80*	29.75*		
0.5			32.40*	35.29*		

*: induction of SCE significantly different from control at P<0.01. (a) only 1st. division metaphases.(b) interpolation values of ADEE. PRI=(M1x1)+(M2x2)+(M3x3)/100

Total extracts and fractions were tested for possible induction of SCE in human lymphocytes in culture without S9 activation (see table 2). All the extracts and fractions induced increased frequencies of SCE in a dose-dependent manner. Doses higher than 0.75 L equivalent of drinking water could not be tested due to cytotoxicity or reduction of PRI in DEE and NDEE. The DEE extract was the most genotoxic followed by NDEE. The strong cytotoxicity in DEE, when fractionated, was only found in NDEE. The sum of activities of separate fractions ADEE and NDEE was higher than the DEE extract, thus indicating that the SCEs activities of the separate fractions were antagonistic in the DEE extract.

In this study, the presence of microbial mutagens (Ames test) and cytogenetic (SCE induction) compounds in drinking water has been demonstrated. The results obtained by Ames test are in agreement with other authors relating the genotoxic activities with chlorinated disinfection byproducts of industrial origin (Meier et al. 1987), humic compounds (Liimatainen et al. 1988; Horth 1989) and agricultural runoff (Athanasiou et al. 1983).

Different authors (Meier 1988) studying drinking water samples found that the mutagenic activity is higher in the acidic fraction than in the neutral fraction. Hemming et al. (1986) correlated this activity in the acidic fraction with the presence of the highly mutagenic compound MX. We were unable to identify it, in our samples, although we believe that the compounds responsible for genotoxic activity are different from those found by other authors.

Liimatainen et al. (1988) and Meier et al. (1987) reported that no correlation exists between mutagenicity and induction of SCEs, because the behaviour of the samples is different in both tests. On the other hand, Zeiger et al. (1990) studied the prediction of rodent carcinogenicity with different short-term tests (Ames and SCE included) for 114 chemicals in the U.S. National Toxicology Program database. His results showed the Ames test to be the most predictive for carcinogenicity with 89% of chemicals positive in the Ames test that are also carcinogenic whereas for SCE was 67%. The concordance (proportion of carcinogens and non-carcinogens that are correctly identified by the short-term test) was 66% and 59% for Ames and SCE tests respectively. Benigni (1989) found similar results and both authors concluded that the other in vitro short-term tests did not complement the Ames's assay for predicting carcinogenicity, but were an important complement for describing the potential genotoxicity of chemicals. In spite of the high correlation of results between mutagenicity, SCE induction and rodent carcinogenicity with individual compounds, there is no evidence that genotoxic drinking water extracts in short-term tests have genotoxic activity in animal assay systems (Meier 1988).

Our results show that the genotoxic behaviour of the extracts and fractions from Barcelona's drinking water is similar. Thus, the DEE extract is the most active showing strong Ames mutagenic and SCE cytogenic activities. The ADEE and especially NDEE fractions splitted the Ames mutagenic and SCE cytogenic

activity. The NDEE extract contained all the toxic and cytotoxic activity. Only the ME extract was more positive in the SCE test compared to the Ames test results.

Both tests detected the genotoxic activity of the studied drinking water extracts and fractions to different degrees. Thus, the Ames test exhibited more response (higher ratio dose/response) and level of detection (lower dose with genotoxic effect) than the SCE tests. These differences according to Meier et al. (1987) could be explained on the basis that the compounds responsible for activity in the Ames test are not the same and probably at different concentration levels than the compounds with activity in the SCE test. Moreover, different sensitivities and performances could be associated with the different cellular organization (Lockard et al. 1982) and genotoxic anomalies detected (Putman et al. 1983).

The results from this study add to the evidence that drinking water frequently contains genotoxic chemicals. The use of humus and industrial pollutants-rich surface water as raw water together with chlorine desinfection, produces direct-acting mutagens and SCEs inductor compounds. Organic extracts from Barcelona's drinking water exhibit a similar behaviour in Ames and SCE tests with different performances. The use of both tests is useful for the evaluation of genotoxicity activities in drinking water extracts.

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