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In vitro Genotoxicity of Chlorinated Drinking Water Processed from Humus–Rich Surface Water

A. Liimatainen[1] and T. Grummt[2]

[1]Department of Environmental Hygiene and Toxicology, National Public Health Institute, P.O.B. 95, 70701 Kuopio, Finland and [2]Research Institute for Hygiene and Microbiology, Heinrich-Heine-Strasse 12, DDR-9933 Bad Elster, GDR

Chlorination by-products of drinking waters are capable to induce sister chromatid exchanges (SCE) and chromosome aberrations (CA) in vitro, in addition to their mutagenic activity in the Ames' test (Athanasiou and Kyrtopoulos 1983; van Kreijl et al. 1983; Meier and Bull 1984; Al-Sabti and Kurelec 1985; Wilcox and Williamson 1986).

Finnish drinking waters, processed from humus-rich surface water using chlorine disinfection, have been found to be highly mutagenic in the Ames' test (Vartiainen and Liimatainen 1986; Vartiainen et al. 1987; Vartiainen et al. 1988). The highest activities have been found in the acidic, non-volatile fraction of the water concentrates using tester strain TA100 without metabolic activation by S9mix. The mutagenicities have varied between 500 and 14000 induced revertants per liter. These figures are one to two magnitudes higher than those reported elsewhere.

We studied five Finnish drinking water samples for their potency to exert genotoxic effects, SCEs and CAs, in mammalian cells in vitro (human peripheral lymphocytes and Chinese hamster lung fibroblasts).

MATERIALS AND METHODS

Drinking water concentrates were prepared as described in detail by Vartiainen et al. (1988). Five drinking water samples of 192 l were collected from the same source (a tap at the University of Kuopio, Finland) during spring and summer in 1986. The samples were allowed to stand until they were free of residual chlorine. The samples were then acidified to pH 2

Send reprint requests to Ari Liimatainen at Department of Pharmacology and Toxicology, University of Kuopio, P.O.B. 6, SF-70211 Kuopio, Finland

(conc. HCl) and adsorbed on XAD 8 resin (50 ml, 20 - 50 mesh, 06446, Fluka AG), using 24 l portions of water (flow rate 0.67 bed volumes per minute). After each portion the column was eluted with ethyl acetate. The combined eluates were evaporated in a Rotavapor, and the volume was adjusted to 4 ml with ethyl acetate. Small aliquots of the concentrates (corresponding to 10 - 12 l of water) were used in the genotoxicity assays after exchanging the solvent to dimethyl sulfoxide (DMSO).

Mutagenicities of the samples were tested by the method of Ames et al. (1975) and Maron and Ames (1983) using tester strains TA97, TA98 and TA100 without metabolic activation. Metabolic activation was not used, because it was shown earlier that it reduces the activity (Vartiainen and Liimatainen 1986). Methyl methane sulfonate (MMS) for TA100 and 4-nitrophenylene-o-diamine (4-NPD) for TA97 and TA98 were used as positive control mutagens. The solvent (DMSO) served as negative control for all strains. For each sample 5 doses (12.5 to 200 ml of water equivalents per plate) were tested using two plates per dose. The mutagenicities are presented as induced revertants per liter, calculated from the slope of the linear part of the dose-response curve.

The cytogenetic assays were performed as described by Galloway et al. (1985). Metabolic activation was not used.

Chinese hamster lung fibroblasts (FAF cell line) were cultivated in darkness for two cell cycles at 37°C in Eagle's minimal essential medium (Eagle-MEM, 10 ml) containing 15% calf serum and antibiotics (penicillin 100 U/ml and streptomycin 100 µg/ml).

Drinking water concentrates (10 µl), corresponding to doses of 100 and 200 ml of water, and bromodeoxyuridine (BrdU) (final concentration 10 µM) were added 24 hours after culture initiation. Incubation was continued for additional 24 hours. Colcemide (final concentration 0.1 µg/ml) was added 3 hours prior to the end of the test. Mitotic cells were harvested with trypsin and subsequently treated with hypotonic solution of KCl (75 mM) and fixed with methanol/acetic acid (3:1). Air dried, coded slides were stained according to the method of Perry and Wolff (1974). For the determination of frequencies of sister chromatid exchanges and chromosome aberrations, 50 M2 cells and 100 M1 cells, respectively, per dose level were scored. Gaps and endoreduplications were recorded but not included in totals (breaks, terminal deletions, exchanges, rearrangements, pulverised cells, uncoiled, dicentric

and ring chromosomes, and heavily damaged cells containing ten or more aberrations). DMSO was used as negative control (10 µl). Triethylenemelamine (TEM) as positive control was used at 0.25 µg/ml for aberrations and 15 ng/ml for sister chromatid exchanges.

For lymphocyte assay, a venipuncture blood sample was donored by a healthy, non-smoking female person. Plasma (1 ml) of a heparinized blood sample was added to 10 ml of Eagle-MEM containing 15 % calf serum, antibiotics, and phytohaemagglutinin (2 %, Fluka). The samples (10 µl) were added. Cultures with BrdU in a final concentration of 10 µg/ml were incubated for 72 - 75 hours, and cultures without BrdU for 45 - 50 hours, at 37 °C. Colcemide (0.2 µg/ml) was added 3 hours prior to harvesting of the cells. Preparation and staining, as well as scoring of metaphases for SCEs and CAs, were carried out as described above for hamster cell assay.

RESULTS AND DISCUSSION

Mutagenicities of the drinking water samples are shown in Table 1. TA100 was the most sensitive strain. The activities are high, but not exceptional in Finland (Vartiainen et al. 1988). None of the samples showed any toxicity at the dose levels tested.

Table 1. Mutagenic activities (-S9) of the drinking water samples. The values are expressed as induced revertants per liter.

Sample number	Sampling date	Mutagenicity TA97	TA98	TA100
1	20.03.1986	1600	310	4300
2	09.04.1986	1550	240	4990
3	07.05.1986	1140	160	2000
4	01.07.1986	1630	360	5970
5	21.07.1986	1810	510	7360
DMSO[1]		138	22	123
MMS	0.125 µl/plate[2]	-	-	348
4-NPD	2 µg/plate[2]	273	305	-

[1]means of 4 plates;
[2]means of 2 plates, spontaneous mutations have not been subtracted

The samples induced both sister chromatid exchanges and chromosome aberrations in hamster cell assay (Table 2). The commonest chromosome damages were

interchanges. In human lymphocyte assay (Table 3), sister chromatid exchanges were induced, but no chromosome aberrations were found. The induction of SCEs, however, confirms the results in hamster cell assay.

Table 2. Induction of sister chromatid exchanges (SCEs) and chromosome aberrations (CAs) by the water concentrates in Chinese hamster lung fibroblasts.

Sample number	Dose (ml)	Number of SCEs/cell	% cells with CAs	
			excluding gaps	including gaps
1	100	14.00	7	9
	200	27.32	10	12
2	100	13.04	8	11
	200	25.72	11	14
3	100			
	200	c y t o t o x i c		
4	100	12.21	4	6
	200	23.04	6	8
5	100	13.21	7	8
	200	27.05	10	14
DMSO	10 µl	7.02	2	4
TEM	0.15 µg	32.00		
	2.5 µg		17.2	19.6

Doses higher than 200 ml equivalents of water could not be tested due to cytotoxicity, observed as loss of clone forming ability of the cells. Loss of cell-to-cell contact, appearance of "gaps" between the cells and changes in cell morphology were observed. At 200 ml dose level (corresponding to 0.02 l water per ml test medium) it was difficult to find good metaphases for scoring. High doses influenced on chromosome condensation, causing short chromosomes and separated chromatids. The same effect was reported also by Wilcox and Williamson (1986).

Sample number 3 was the most cytotoxic one. No results were obtained even at the lowest dose (100 ml) in hamster cell assay.

Finnish drinking water samples, which were mutagenic in the Ames' test, were shown to induce also sister chromatid exchanges and chromosome aberrations in

Table 3. Induction of sister chromatid exchanges (SCEs) and chromosome aberrations (CAs) by the water concentrates in human peripheral lymphocytes.

Sample number	Dose (ml)	Number of SCEs/cell	% cells with CAs	
			excluding gaps	including gaps
1	100	10.72	0	1
	200	20.03	0	1
2	100	11.03	1	3
	200	18.21	0	2
3	100	10.32	0	2
	200	19.44	0	3
4	100	10.16	0	4
	200	21.00	0	1
5	100	14.84	0	2
	200	19.92	0	3
DMSO	10 µl	6.04	0	2
TEM	0.15 µg	31.21		
	2.5 µg		15.8	20.4

vitro at considerably lower dose levels than in the studies carried out in Greece (Athanasiou and Kyrtopoulos 1983), USA (Meier and Bull 1984), the Netherlands (van Kreijl et al. 1983) and England (Wilcox and Williamson 1986).

The results are not, however, directly comparable, since the concentration procedures are different. In the studies of Athanasiou and Kyrtopoulos (1983) and Wilcox and Williamson (1986) the samples were concentrated using XAD resins without acidification of the water prior to adsorption. We have used acidification routinely since main part of the mutagenic activity was found in acidic rather than neutral fraction (Vartiainen and Liimatainen 1986, Vartiainen et al. 1987, Vartiainen et al. 1988). Also, contrary to our results, drinking water samples studied by Wilcox and Williamson caused chromosome aberrations in human lymphocytes. In the study of van Kreijl et al. (1983) compounds responsible to the genotoxic activity differ from those in our samples, because they require metabolic activation and exert their effects in tester strain TA98.

In the study of Meier and Bull (1984), unchlorinated

humic material was found to induce SCEs, whereas no mutagenicity was observed. Chlorinated waters may therefore contain different compounds with different genotoxic properties. The same conclusion was also made by Langi and Priha (1987) in their study on paper and pulp mill effluents, which contain chlorinated and unchlorinated degradation products of lignin that resembles structurally humic material. Our results seem to confirm this, since no correlation exists between mutagenicity and induction of SCEs and CAs.

Recently a strong direct acting mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone ("MX") has been identified in chlorinated drinking waters (Hemming et al. 1986). The samples used in this study were not chemically analyzed, so it is not known what is the contribution of "MX" to the observed effects. According to Kronberg and Vartiainen (1988) "MX" contributes to about one third of the mutagenicity of Finnish drinking waters.

Impurities of drinking waters, including natural material and chlorination by-products, have not been toxicologically evaluated. On the basis of our results, the use of humus-rich surface water as raw water together with chlorine disinfection, produces potent genotoxic substances, which represent a carcinogenic risk to man.

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REFERENCES

Al-Sabti K, Kurelec B (1985) Chromosomal Aberrations in Onion (Allium cepa) Induced by Water Chlorination By-Products. Bull Environ Contam Toxicol 34:80-88

Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res 31:347-364

Athanasiou K, Kyrtopoulos SA (1983) Mutagenic and clastogenic effects of organic extracts from the Athenian drinking water. Sci Tot Environ 27:113-120

Galloway SM, Bloom AD, Resnick M, Margolin BH, Nakamura F, Archer P, Zeiger E (1985) Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ Mutagen 7:1-51

Hemming J, Holmbom B, Reunanen M, Kronberg L (1986) Determination of the strong mutagen 3-chloro-4-

(dichloromethyl)-5-hydroxy-2(5H)-furanone in chlorinated drinking and humic waters. Chemosphere 15:549-556

van Kreijl CF, de Vries M, van Kranen HJ, Kool H, de Greef E (1983) Properties of mutagenic activity in river Rhine water in the Netherlands. Mutation Res 113:314

Kronberg L, Vartiainen T (1988) Ames mutagenicity and concentration of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone and of its geometric isomer E-2-chloro-3-dichloromethyl)-4-oxo-butenoic acid in tap waters. Submitted for publication in Mutation Res.

Langi A, Priha M (1987) Mutagenicity in pulp and paper mill effluents and in recipient. Proceedings of the 2nd IAWPRC Symposium on Forest Industry Waste Waters, Tampere, Finland, 9-12 June 1987

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

Meier JR, Bull RJ (1984) Mutagenic properties of drinking water disinfectants and by-products. In: Jolley RL, Bull RJ, Davies WP, Katz S, Roberts MH, Jacobs VA (eds) Water Chlorination: Chemistry, Environmental Impact and Health Effects, vol. 5, Ann Arbor Science Publishers, Ann Arbor, pp.207-220

Perry PE, Wolff S (1974) New Giemsa method for differential staining of sister chromatids. Nature 251:156-158

Vartiainen T, Liimatainen A (1986) High levels of mutagenic activity in chlorinated drinking water in Finland. Mutation Res 169:29-34

Vartiainen T, Liimatainen A, Jääskeläinen S Kauranen P (1987) Comparison of solvent extractions and resin adsorption for isolation of mutagenic compounds from chlorinated drinking water with high humus content. Water Res 21:773-779

Vartiainen T, Liimatainen A, Kauranen P, Hiisvirta L (1988) Relations between drinking water mutagenicity and water quality parameters. Chemosphere 17:189-202

Wilcox P, Williamson W (1986) Mutagenic activity of concentrated drinking water samples. Environ Health Perspect 69:141-149

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