Acta Veterinaria (Beograd), Vol. 45. No. 4, 227–234, 1995.

UDK 619:612.014.4

INFLUENCE OF ORGANIC CONDENSATE FROM DRINKING-WATER ON MITOTIC ACTIVITY AND SCE FREQUENCY IN CULTURED HUMAN LYMPHOCYTES

N. ĐELIĆ*, D. ZIMONJIĆ*, B. SOLDATOVIĆ*, and V. ADAMOVIĆ**

*Department of Biology, Faculty of Veterinary Medicine Bul. JA 18, Belgrade, Yugoslavia
*Department of Sanitary Chemistry, Institute of Public Health of Serbia, Belgrade, Yugoslavia

(Received, 4. April 1995.)

The organic condensate prepared from the Belgrade drinking water supply network was examined for genotoxic activity in cultured, PHA-activated, human peripheral blood lymphocytes.

The whole organic drinking water condensate caused a dose-dependent increase in SCE frequency. The lowest concentration (20μl/ml) of the whole condensate did not show cytotoxic and/or cytostatic effects, whereas, at higher concentrations (50μl/ml and 100 μl/ml) a significant decrease in mitotic activity was observed. Three fractions of drinking-water organic condensate (neutral, acidic and basic) had different effects. The neutral fraction increased the SCE frequency significantly, but did not change the mitotic index in comparison to the control level. The acid fraction increased the SCE frequency and decreased the mitcotic index, whereas the basic fraction suppressed cell proliferation but did not change the SCE frequency.

In our opinion, the different chemical composition of these three fractions is responsible for the differences in their genotoxic and cytotoxic activities.

Key words: drinking water, pollutants, genotoxicity, sister chromatid exchange (SCE), mitotic index (MI).

INTRODUCTION

It is estimated, that the human body consumes about 40000 liters of drinking water during its 50-year lifetime. Since drinking water can contain more than 2 200 different organic chemicals (Bull, 1981), there is serious concern about their possible effects on human health. The list of organic substances detected in water is continuously increasing due to the rapid development of new, sensitive and accurate analytical techniques. Most organic pollutants remaian present in water even after boiling and repetitive evaporation (Adamović, 1984).

There are different sources of contamination in water, but these can be generally classified into three groups: contaminants in raw water, chemicals

introduced into water through treatment (chlorination, fluoration ect.) and chemicals formed, or unintentionally introduced through water distribution (Meier, 1988).

The treatment of drinking water is an inevitable measure for preventing infective diseases, improving its taste and removing the natural colouring caused by humic substances. Chrorination is most commonly used to fulfill these requirements. However, in spite of these positive effects, it seems that this introduction of chlorine is the major factor responsible for the creation of highly reactive chemicals, and some of these have strong genotoxic potential. Namely, during water chlorination, trihalomethanes (THM's) are produced in relatively high amounts. The most commonly formed THM's are:$CHCl_3$ (chloroform), $CHCl_2Br$, $CHClBr_2$ and $CHBr_3$. In addition, there is a large number of other halogenated hydrocarbons and other chemicals with a more complex structure (chlorophenols, haloketones, haloacetonitriles, haloacetic acids, dichloroacetic acid, trichloroacetic acid etc., Meier, 1988). The THM's and other chlorinated products are considered to be the most active mutagenic and carcinogenic chemicals. During chlorination, precursors of these chemicals are either naturally occuring in water (i.e. humic substances ect.) or introduced via wastewaters. The chlorination of humic substances results in the formation of the acidic, chlorinated furanone (3-chloro-4- (dichloromethyl)-5-hydroxy-2- (5H)- furanone, usually refered to as MX). Some experiments have shown that MX represents a major mutagen in both chlorinated drinking water and in chlorinated humic acid solutions (Backlund et al. 1989).

The aim of our experiments was to evaluate the genotoxic and cytotoxic effects of the water organic condensate by employing the SCE in vitro assay. The water organic condensate was prepared from the Belgrade drinking water-supply network.

MATERIALS AND METHODS

The samples for evaluation of the genotoxic properties of drinking water were prepared in the following way.

Drinking water was preconcentrared by adsorption on the macroreticular resin XAD-2 (Rohm and Hass, PA, USA), packed in two glass columns placed one above the other. The height of the column adsorbant was 250 mm, and the diameter 10 mm. The entire quantity of water was 400 liters per probe. The water flow rate from one to the other column was about 80 ml/min. Elution of the adsorbed organic phase was achieved with purified diethylether (200 ml.) The concentration was performed by subsequent evaporation at room temperature down to the volume of 25 ml (25 ml of eluate = 400 lit. of water). From that, 2,5 ml of eluate (2,5 ml = 40 lit. of water) was fully evaporated, and the residue dissolved in 300 ml of DMSO (dimethylsulfoxide). This was then used as a "total organic condensate". The 20 ml of eluate was separated into three fractions: acidic, neutral and basic. DMSO was added to each fraction to the volume of 25

ml. From these, 5 ml were taken (5 ml = 64 lit. of water), fully evaporated and dissolved in 300μl of DMSO. In the following way.

Tests were performed in vitro, using short-time cultures of lymphocytes from human peripheral blood. Human lymphocytes were set up using heparinised whole blood from healthy female donors, in Parker 199 medium containing 30% inactivated calf serum,) 0,04 mg/ml of PHA (Difco laboratories), 100 IU penicillin, 100 μg/ml streptomycin and 25 μM 5-bromo-2'-deoxyuridine (Sigma Chemical Co.): At the beginning of incubation, water organic condensate was added to one group of vials at concentrations of 20, 50 and 100 μl/ml. Another group of vials was treated by the different fractions (acidic, neutral and basec) of previously separated water condensate, at concentrations of 100 μl/ml.

Cultures were kept in the dark at 37^{0}C, for a further 72 hours. Four hours before harvesting, colcemide (Ciba) was added at the final concentration of 0,5 μg/ml. After centrifugation (1750 rpm for 8 min) cells were treated with a hypotonic (0,075 M) solution of KCl, and then fixed in 3:1 methanol-acetic acid for three repetitive cycles of centrifugation and resuspension. Finally, the cell suspension was dropped on to microscopic slides, air dried and aged for a further 96 hours before staining.

Differential staining for the inspection of SCE rate was performed according to the FPG procedure, described by Perry and Wolff (1974).

For each experimental concentration, as well as for controls, 30 well-spread mitoses (Wulff et al., 1984) were inspected for SCE scoring, while the mitotic activity was analysed according to the standard procedure. Statistical processing of the experimental values (Student's t-test), as well as the curve fitting and function analyses were performed on a computer.

RESULTS AND DISCUSSION

The activity of the three fractions (acidic, neutral and basic) in constant concentrations of 100 μl/ml on the SCE frequency and mitotic index is shown in Figure 1. The acidic and neutral fraction increased the SCE/cell significantlly ($p<0,001$). The basic fraction caused a slight, insignificant increase in the SCE. Therefore we conclude that the acidic and neutral fraction influence the SCE frequency, whereas the basic one does not have such an effect. These results agree with some previously reported experimental data (Zimonjić et. al. 1986).

The mitotic index (MI) decreased when basic fraction was used ($p<0,001$) and although the acidic one showed the same effect the level of statistical significance was lower ($p<0,01$). The neutral fraction did not change MI significantly (Figure 1). Obviously, the basic and acidic fractions had cytotoxic and/or cytostatic effects in our experiments.

The other part of our experiments was designed to evaluate the effects of the total water organic condensate (WOC) on the SCE and MI values using various concentrations of WOC (20, 50 and 100 μl/ml). Figure 2 shows the influence of WOC on the SCE frequency. All three experimental concentrations effectively increased the SCE ($p<0,001$). This increase was concentration-de-

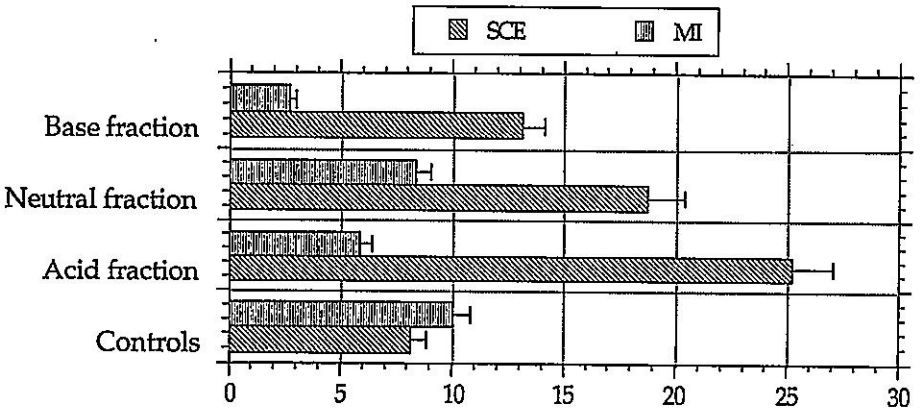

Figure 1. The changes of mitotic activity (MI) and SCE frequency in cultures of human lymphocytes caused by three different fractions of water organic condensate.

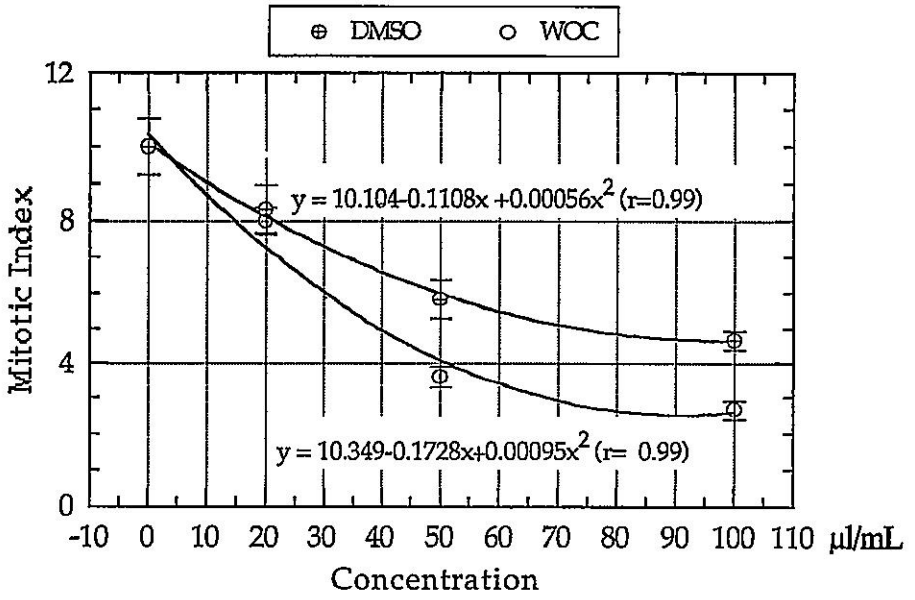

Figure 2. The influence of DMSO and whole drinking water organic condensate on SCE frequency in cultures of human lymphocytes.

pendent. The fitted curve equation for WOC is $y=8,159 + 0,486x - 0,009x^2 + 0,000064x^3$ and the correlation coefficient $r=0,99$. DMSO (negative control) at the same concentration was also used, but it did not change the SCE significantly

(the corresponding fiffted curve equation is: $y = 8,159 - 0,0406x + 0,0013x^3 - 0,0000095x^3$, $r = 0,99$).

The change of MI after the application of WOC at concentrations of 20, 50 and 100 μl/ml are shownin Figure 3. The concentrations of 50 ml/ml and 100 μl/ml were strong enough to decrease the MI ($p<0,001$). On the other hand the concentration of 20 μl/ml caused only a small insignificant suppression of MI. The fiffted curve equation for WOC is: $y = 10,349 - 0,1728x + 0,0009x^2$ ($r = 0,99$), and for DMSO: $y = 10,104 - 0,1108x + 0,00056x^2$ ($r=0,99$). It is interesting to note that DMSO suppressed the MI at concentrations of 50μl/ml ($p<0,01$) and 100μl ($p<0,001$)..

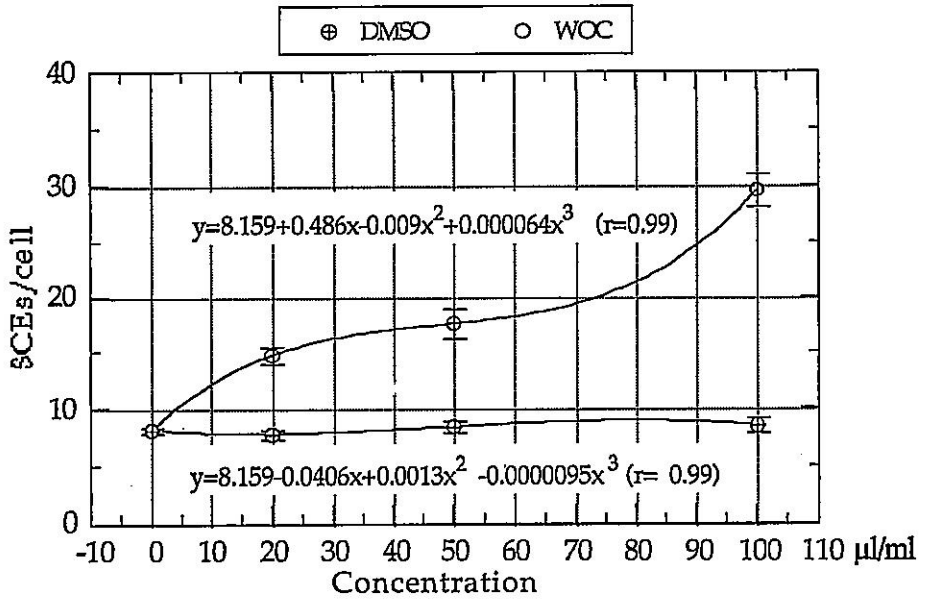

Figure 3. The mitotic index values in cultures of human lymmphocytes treated with DMSO and whole drinking water organic condensate.

An increase in the SCE frequency is a sensitive method in the detection of DNA damage caused by various chemical mutagens and/or carcinogens (Popescu et al. 1982). On the basis of our experimental results, we assume that different fractions (acidic. neutral and basic) probably have different chemical composition of genotoxins, i. e. the acidic and neutral fractions contain the majority of mutagenic and/or carcinogenic substances. The whole WOC increased the SCE frequency with all three concentrations applied. Therefore, it is logical to assume that the mixture of genotoxins in WOC is strong enough to cause such an effect, and that the possible synergistic effects might be of importance in the area of low concentration (20 μl/ml).

The changes in MI imply that the substances responsible for cytotoxic and/or cytostatic effect are present mainly in the basic fraction of WOC (Figure 1). The whole WOC suppresses the MI at concentrations of 50 and 100 μl/ml, whereas the concentration of 20 μl/ml does not have such an effect. DMSO (negative control) decreased the MI in concentrations of 50 and 100 $\mu\lambda$/ml. Both the WOC and DMSO have cytotoxic and/or cytostatic effects, but those of the former are more pronounced.

Although there is a wide variety of organic and inorganic chemicals in drinking water, their concentration is very low. That is the reason why even the very potent mutagens do not exhibit genotoxicity in different assays (McMichael, 1990). In addition, some authors entertain the opinion that in most or all drinking waters the concentrations of carcinogenic substances are very low and there is no realistic evidence that they have caused harm to humans and animals (Dayan, 1993). The problem of mutagenicity testing of drinking waters can, at least partially, be solved by preconcentration before testing. The most widely employed method of sample preconcentration involves adsorption of organic molecules from water on Amberlite XAD resins (Onodera et al., 1993). After adsorption, organic solvents are used for elution. It is a convenient and relatively inexpensive technique, useful for processing large volumes of water in a short period of time.

It is interesting to note, that there is experimental evidence that some drinking water organic concentrates can induce carcinoma in mice and rats (Truhaut et al., 1979). In addition, epidemiological studies of the human populations exposed to relatively high amounts of genotoxins in water have demonstrated that some types of cancer (bladder, colon, rectum) might occur more frequently (Williamson, 1981).

Having in mind that the genotoxins occur in drinking water mainly as a consequence of chlorination, different approaches to their elimination have been considered (Meier, 1988). There is certain evidence that mutagenicity of drinking water can be decreased by granular activated carbon (GAC) treatment (Kool et al., 1982). Other approaches include the use of alternative methods of disinfection, ozonation being the most commonly employed alternative. Ozone inactivates microorganisms more efficiently than chlorine and many water-treatment plants use ozone instead of chlorine. Ozone improves smell and taste. There are some indications that ozone produces less toxic and mutagenic compounds than chlorine (Van Hoof, 1982). However, its disadvantages are a higher cost and an absence of residual activity.

Finaly, there is still a quite different approach to the elimination of genotoxins. The direct-acting mutagens produced after water chlorination can be inactivated by neucleophilic reagents (Cheh et al., 1980, Watanabe et al., 1994). Sulfur dioxide (SO_2) is one of such substances. Anyway, the problem of water pollution is very complex and requires deeper investigation to be solved fully.

In addition to drinking, humans and animals are exposed to genotoxins in water through swimming in polluted waters and through consuming aquatic

organisms (fish, shells and other molluscs, seaweed ect.). The presence of genotoxic pollutants in source waters has been determined directly by cytogenetic analysis of aquatic species (Perin et al., 1978, Alink et al., 1980) and other organisms (Ma et al., 1985). It is important to note that genotoxins from water are accumulated through food chains. Consequently, the amounts of mutagens in aquatic food sources, especially those of animal origin, are relatively high. The problem has to be viewed in the context of its global effects on water ecosystems. Chlorination disturbs the normal communications between aquatic organisms (malfunctions in the system of ecomones, sexomones and pheromones). In addition, chemical changes in water ecosystems inferfere with normal chemotaxis in microbial communities and, consequently, affect the processes of biodegradation, i. e. regenertion of unpolluted ecosystems (Alink, 1982).

Drinking water from various sources has different chemical properties, depending mainly on the pollutants present in certain areas. The genotoxicological evaluation of drinking water should, therefore, be carried out continuously and routinely.

REFERENCES

1. *A. Adamović V. M.* 1984. Aktuelna situacija u oblasti zagađenja voda i snabdevanja zdravstveno ispravnom vodom za piće. *Hrana i Ishrana, 25,* st. 7-10.
2. *Alink G. M.,* 1982. Genotoxins in waters. *Mutagens in our environment,* pp.261-276, *Alan R. Liss Inc. 150 Fifth Ave. New York, NY 10011*
3. *Alink G. M., Frederix-Wolters E. M. H., Van der Gaag M. A., Van der Kerkhoff J. F., Poels C. L. M.* 1980. Indiction of sister-chromatid exchanges in fish exposed to Rhine water. *Mutation Research 78, pp.* 369-374.
4. *Backlund P., Wondergem E., Voogd K., de Jong A.* 1989. Mutagenic activity and presence of the strong mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2-(5H)-furanone (MX) in chlorinated raw and drinking waters in the Netherlands. *Sci-Total-Environ, 84, pp.* 273-82.
5. *Bull R., In: Saindhys.* 1981. Application of short-term bioassays in the analysis of complex environmental mixtures. *Vol. 2 Plenum Press New York pp.* 367-384.
6. *Cheh A. M., Skochdopole J., Heilig C., Koski P. M., Cole L.* 1980. Destruction of direct-acting mutagens in drinking water by nucleophiles: implications for mutagen identification and mutagen elimination from drinking water, In: R. L. Jolley et al. (Eds.) Water Chlorination, Environmental Impact and Health Effects, Vol 3, Ann Arbor Science, *Ann Arbor, Mi. pp.* 803-815.
7. *Dayan A. D.* 1993. Carcinogenicity and drinking water. *Pharmacol-Toxicol. 72 Suppl 1, pp.* 108-15
8. *Kool H. J., Van Kreijl C. F., De Greef E., Van Kranen H. J.,* 1982. Presence, introduction and removal of mutagenic activity during the preparation of drinking water in The Netherlands, *Envir. Hlth. Perspect 46, pp.* 207-214.
9. *Ma T. H., Anderson V. A., Harris M.H., Neas R. E. and Lee T. S.* 1985. Mutagenicity of drinking water detected by the Tradescantia micronucleus test. *Can J. Genet. Cytol. 27, pp.* 143-150.
10. *McMichael A. J.,* 1990. Drinking water and human cancer IIARC Sci-Publ 104, pp. 298-306.
11. *Meier J. R.,* 1988. Genotoxic activityof organic chemicals in drinking water. *Mutation Research, 196, pp.* 211-245.
12. *Onodera S., Nagatsuka A., Rokuhara T., Asakura T., Hirayama N., Suzuki S.* 1993. Re-evaluation of solid-phase adsorption and desorption techniques for isolation of trace organic pollutants from chlorinated water *J. Chromatogr. 16, 642 (1-2), pp.* 185-94.
13. *Perin A. E., Thie G. M., Alink G. M., Poels C. L. M., Koeman J. H.* 1978. Cytogenetic changes in fish exposed to water of the River Rhine, *Sci Total Environ 9, pp.* 287-291.

14. *Perry P., Wolff S.* 1974. New giemsa method for the differential staining of sister chromatids. *Nature 251,* 156-158.
15. *Popescu N. C., Di Paolo J. A.* 1982. The relevance of sister chromatid exchange to the induction of neoplastic cell transformation *Sister Chromatid Exchange (Ed.* S. Walff), Alan Liss, N.York, 425-460.
16. *Truhaut R., Gak J. C., Graillot C.* 1979. Recherches sur les risques pouvant resulter de la pollution chimique des eaux dalimentation - *I Water Res 13, pp.* 689. *andberg) Alan R. Liss Inc. New York pp* 425-460.
17. *Van Hoof F.* 1982. The influence of ozonation on direct acting mutagens formed during drinking water chlorination 4th Conference on Water Chlorination. *Pacific Grove* 18-23/10/81.
18. *Watanabe M., Kobayashi H., Ohta T.* 1994. Rapid inactivation of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a potent mutagen in chlorinated drinking water, by sulfhydryl compounds. *Mutat Res Apr 312(2), pp* 131-8.
19. *Williamson S. J.* 1981. Epidemiological studies on cancer and organic compounds in US drinking waters. In: Van Lelyweld H., Zoeteman B. C. J.: Water supply and health Elsevier Amsterdam pp. 187.
20. *Wulff H. C., Husum B., Plesner A. M., Neibuhr E.* 1984. Distribution of SCEs in lymphocytes in persons with normal, slightly increased and heavily increased SCEs *Mutation Research 125, pp.* 263-266.
21. *Zimonjić D. B., Adamović V. M. i Soldatović B. M.* 1986. Genotoksično dejstvo frakcija kondenzovane organske faze vode za piće sa područja Beograda na limfocite čoveka u uslovima in vitro *Hrana i Ishrana, 27.2.p* 107-109

UTICAJ KONDENZOVANE ORGANSKE FAZE VODE ZA PIĆE NA MITOTSKU AKTIVNOST I FREKVENCU SCE U KULTURAMA LIMFOCITA ČOVEKA

N. ĐELIĆ, D. ZIMONJIĆ, B. SOLDATOVIĆ i V. ADAMOVIĆ

SADRŽAJ

Ispitivana je genotoksičnost kondenzata organske faze vode za piće sa područja grada Beograda u kulturama PHA-aktiviranih limfocita humane periferne krvi.

Nefrakcionisani ("ukupni") kondenzat organske faze vode za piće prouzrokovao je doza-zavisno povećanje frekvence SCE. Najniža (20μl ml) primenjena koncentracija ukupnog kondenzata nije ispoljila citotoksične i/ili citostatske efekte, dok je pri višim koncentracijama (50μl/ml i 100 μl/ml) zapažen značajan pad mitotske aktivnosti. Tri frakcije kondenzata vode (neutralna, kisela i bazna) pokazale su različite efekte. Neutralna frakcija značajno je povećala frekvenciju SCE, ali nije dovela do značajnog odstupanja vrednosti mitotskog indeksa u odnosu na kontrolnu vrednost. Kisela frakcija je povećala frekvenciju SCE i umanjila mitotski indeks, dok je bazna frakcija ispoljila citotoksične efekte, ali nije značajno promenila nivo SCE po ćeliji.

Prema našem mišljenju, različit hemijski sastav pomenutih frakcija odgovoran je za razlike u njihovim genotoksičnim i citotoksičnim efektima.