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Monitoring of Genotoxicity in Drinking Water Using *in vitro* Comet Assay and Ames Test

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Summary

A screening strategy for evaluation of genotoxic potential of drinking water has been proposed in the present work. Genotoxicity assays with tap water collected at three different sampling points in Ljubljana drinking water region are presented here. *In vitro* alkaline version of the comet assay was performed with human HepG2 and Caco2 cell lines and protozoa *(Tetrahymena thermophila)* cells. Parallel genotoxicity evaluation on the same samples was carried out by the Ames test (with/without exogenous metabolic activation) using *Salmonella typhimurium* TA97a, TA100 and TA1535 strains. Nonconcentrated and concentrated water samples were tested in both bioassays, and chemical analyses were performed to check the contents of pesticides and nitrates. There was no indication of genotoxic activity in any of the drinking water samples according to the Ames test. The results of the comet assays showed differences and possible genotoxic potential among the water samples tested on different cell types, which were, however, statistically not significant, except in two cases. Statistical analyses showed the comet assay was more sensitive than the Ames test for genotoxicity detection in drinking water samples.

Key words: genotoxicity, drinking water, *in vitro* comet assay, Ames test

Introduction

The pollutants (*i.e.* industrial chemicals, biocides, agrochemicals, pharmaceuticals, *etc.*), which exhibit genotoxic activity, continue to be deposited into the environment and as such are creating a need for sensitive assays to monitor their accumulation and impact on the ecosystems and human health. Epidemiological studies have shown that there is a correlation between genotoxicity of drinking water, focused mostly on chlorination by-products, and increased cancer risks (*1–3*).

Among the numerous biomarkers of genotoxicity used to evaluate DNA damage as a consequence of exposure to environmental pollutants, the DNA damage detected by the alkaline comet assay is particularly interesting. The comet assay, as first described by Östling and Johanson (*4*) and further developed by Singh *et al.* (*5*) and Olive *et al.* (*6*), is a sensitive method used for visualisation and quantitative analysis of DNA damage in individual cells, regardless of chromosome size, number, or mitotic status. Any nucleated cell can be used for comet assay. Numerous studies have demonstrated its capacity to detect low levels of DNA damage, its requirement for few cells (*i.e.* a few hundred), and its relatively low cost (*7–9*).

The Ames test (*Salmonella* assay) is an *in vitro* test, very well validated and widely used as a screening test for evaluation of chemicals for mutagenicity (*10*) and most widely used test system for water monitoring. It reveals the gene mutation inducing ability (mutagenicity). Its disadvantages are that the concentration of the samples is important, metabolic activation homogenates

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have to be added and the system is insensitive towards certain groups of compounds such as halogenated hydrocarbons and heavy metals (*1*). Overall, the Ames test exerts relatively high sensitivity and specificity.

Single test assays for detecting genotoxic effects cannot fulfill the criteria of high sensitivity and high specifity to predict human health hazard. This is also true for the analysis of complex environmental samples such as water, air and soil samples. The combination of appropriate mutagenicity and genotoxicity tests for the control of drinking water is still a matter of debate and different test strategies have been suggested (*11,12*). In the present study a combination of two biological assays based on bacteria, protozoa cells and two mammalian cell lines (Caco2 and HepG2) was used. The combination of bioassay results together with chemical analysis would give us more certain results concerning human health risk related to the consumption of drinking water. Because of the potential genetic risks that humans are exposed to by the daily intake of drinking water and because of the potential accumulation of the harmful effects, it is very important to regularly monitor the drinking water. We assume that these battery tests complement each other in regard to their sensitivity to environmental genotoxins and are useful in detecting low genotoxic levels which are expected in drinking water samples.

Materials and Methods

Water sampling

Three different parts of Ljubljana drinking water region were selected as sampling sites where tap water samples were taken (samples marked 1, 2 and 3). The chosen sites for taking water samples were the same as the sites for regular monitoring for pesticides and chemical analyses done by the Institute of Public Health of the Republic of Slovenia (Table 1). Sampling was performed according to the recommended standard method (*13*). The sampling was done at one occasion in November 2003 and charcoal filtration was carried out as a disinfection process. Water samples were transported to the laboratory in 500-mL glass flasks and stored at –20 °C for further testing. Prior to the genotoxicity bioassays 0.9 % NaCl was added to the samples in order to avoid hypoosmotic shock, which causes cytotoxic effect resulting in false positive genotoxic effects later on. The negative control in all biotests performed was 0.9 % sterile physiologic solution prepared from MilliQ water.

The concentrating of drinking water samples was done according to the producer´s guidance on XAD4 columns containing copolymer of styrene divinyl benzene (*14*). Concentration of the water samples was performed with XAD4 resins with a concentration factor of 1:1000 (*14*). Concentrated water samples and non-concentrated water samples were then tested in triplicate. Water samples concentrated 1000× were diluted with sterile MilliQ water to final concentration of 50×. Chemical analyses of nitrates and pesticides were performed as a part of regular drinking water monitoring by the National Institute of Health of the Republic of Slovenia, Department of Sanitary Chemistry, the accredited institution for chemical analyses of drinking water in Slovenia.

Methods of the analyses are a part of the Annex to the Accreditation Document No. L-025. Nitrates were analysed by Standard Method SM 4500 B, while pesticides were analysed by gas chromatography with mass spectrometry detection.

Cells and cell cultures

Epithelial colon cancer cells (Caco2, obtained from Istituto Zooprofilatico Sperimentale, Brescia, Italy) and human hepatoma cell line (HepG2 cells, obtained as a gift from Prof. Dr. Knasmueller, Institute of Cancer Research of the University of Vienna) were grown in multilayer culture at 37 °C in humified atmosphere of 5 % CO_2 in Dulbecco's Modified Eagle's Medium (DMEM)

Table 1. Chemical analyses of nitrates, pesticides and their degradation products in drinking water samples

	Sample 1	**Sample 2**	**Sample 3**	**MAC**
γ(nitrate)/(mgNO_3/L)	16.8	9.9	9.0	**50**
γ(atrazine)/(μg/L)	0.08	0.05	0.05	**0.1**
γ(desethyl atrazine)/(μg/L)	0.09	0.05	0.05	**0.1**
γ(desisopropylatrazine)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(propazine)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(prometryne)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(simazine)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(terbutyl azine)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(terbutryne)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(bromacil)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(metolachlor)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(ametrin)/(μg/L)	0.05	0.05	0.05	**0.1**
γ(2,6-dichlorobenzamide)/(μg/L)	0.09	0.05	0.05	**0.1**
γ(desethylterbutylazine)/(μg/L)	0.05	0.05	0.05	**0.1**

MAC – maximum allowed concentration

supplemented with 10 % foetal calf serum (FCS) and antibiotic (0.1 % gentamycin) in culture flasks for 8 days (cell density: 10^6–10^8 cells/mL). Medium was changed every 2 days. Single cell suspensions were prepared with 0.25 % trypsin-EDTA solution and finally resuspended in DMEM medium, supplemented with 10 % FCS medium.

Tetrahymena thermophila, a micronucleate strain, was originally obtained from Microbiotest (Belgium) as a part of the Protox F^{TM} kit. *T. thermophila* cells were cultivated in a semidefined medium for protozoa (*15*) at 30 °C for 72 h. The cells grew in liquid culture as motile unicellular ciliates below the surface of the medium. Before incorporation of the cells into the agarose layers of the comet slides, the cells were concentrated by centrifugation in Falcon tubes for 3 min at 300 × *g* and 4 °C.

The dye-exclusion test with Trypan blue (*16*) was used to examine the viability of cells before the comet assay was performed. The number of live and dead cells in a sample of approximately 300 cells was counted in a Neubauer counting chamber using a light microscope at 200× magnification. Cell cultures with >90 % relative viability were used for genotoxicity evaluations.

In vitro comet assay

Comet assays with Caco2 and HepG2 cells were performed according to the method described by Duthie *et al.* (*17*) and Uhl *et al.* (*18*), respectively. The alkaline version of the original Singh *et al.* (*5*) comet assay protocol, modified for protozoa cells, was performed according to the method described by Lah *et al.* (*19*). Briefly, to achieve a uniform background rough microscope slides were first coated with up to 400 μL of 1 % normal melting point agarose (NMP). They were left to air dry overnight. The supportive (second) agarose layer (0.6 % NMP agarose) was solidified on ice and the collected Caco2 or HepG2 or protozoa cells were immobilized in the third layer. Approximately $2 \cdot 10^4$ cells were mixed with 0.7 % low melting point agarose (LMP) and spread over the slides as the third layer. After removing the cover glass, the slides were covered with 500 μL of 0.5 % LMP agarose (the fourth layer) to prevent nuclear DNA from escaping during cell lysis and electrophoresis.

Four-layered slides with incorporated cells were first submerged into water samples for 20 min and then 1-hour incubation in alkaline lysis buffer followed. After that the slides were submerged in electrophoretic buffer (pH>13) to unwind the nuclear DNA for 1 h and then subjected to electrophoresis in the same buffer. The electrophoresis was carried out at 2 V/cm and 300 mA; the duration of electrophoresis depended on cell type (30 min for Caco2 and HepG2 cells, and 5 min for *T. thermophila* cells). After electrophoresis the gels were neutralized in 400 mM Tris-HCl (pH=7.5) for 15 min. The damaged DNA travelled toward the anode during electrophoresis and formed an image of a »comet« tail. After staining the slides with ethidium bromide (20 μg/mL) the comets were detected and quantified as described below.

Ames test/Salmonella assay

The Ames test was carried out as standard plate incorporation test (*10*) with *Salmonella typhimurium* strains TA97a, TA100 and TA1535 with and without *in vitro* microsomal activation (by S9 rat liver homogenate). Mutagenic activities were expressed as induction factors, *i.e.* as multiples of the background levels. The results were considered positive if the tested sample produced a response which was at least twice as high as the one found with the negative control. For positive controls 4-nitroquinoline-N-oxide (4NQNO) and 2-amino fluorene (2AF) were used in dimethyl sulfoxide (DMSO) as solvent; for methylmethane sulphonate (MMS) and sodium azide, water was used as solvent. After 48-hour incubation of agar plates at 37 °C, counting of bacterial colonies was performed.

Data collection and statistical analysis of the comet assay results

For quantitative analysis of nuclear DNA damage with Caco2, HepG2 cells and *T. thermophila*, the slides were viewed at 200× magnification with an epifluorescence microscope (Olympus BX 50) using a BP 515–560 nm filter and a BA 590 nm barrier filter. Microscopic images of comets were captured by a digital camera (Hammamatsu Orca 2) connected to a computer, and the comets were scored using Komet 5.0 Computer Software (*20*).

Among the parameters available for the analysis of the comets, olive tail moment (OTM) was chosen as the most relevant measure of genotoxicity. Tail length and the percentage of DNA in comet tails and heads were collected. These values were used to calculate OTM, using the relationship: OTM = (tail mean – head mean) × tail % DNA/100 in arbitrary units (*6*). Analysis was restricted to OTM as this parameter takes into consideration the intensity profile of the DNA signal and percentage of DNA in the comet heads and tails.

Images of 50 comets were collected from each of the two replicate slides per sample, OTMs were calculated, and the significance of treatment-related differences tested using SAS/STAT statistical software version 8E (*21*). Descriptive statistics was determined by the MEANS procedure. OTM records were tested for normal distribution with the UNIVARIATE procedure. Bauer *et al.* (*22*) suggested that the distribution of OTMs obeys a chi-square (χ^2) distribution. The chi-square distribution, which is a special case of gamma distribution, fitted well to our data. As a consequence, data were then analysed by the GENMOD procedure (Generalised Linear Models), which allows distributions other than a Gaussian one. Statistically significant differences between groups were evaluated by the linear contrast method.

Statistical analysis for the Ames test

Genotoxic activities were expressed as induction factors *i.e.* as multiples of the background levels. Statistical significance was evaluated with Kruskal-Walis test (non-parametric ANOVA) for differences between treatment groups and Dunnett's C multiple comparison for differences from the negative control.

Results and Discussion

The results of the Ames test (with/without exogenous metabolic activation) on nonconcentrated (1, 2, 3) and 50 times (50×) concentrated (1C, 2C, 3C) water samples are presented in Table 2. According to the EPA (*23*) and GenPharmTox (*24*) guidelines, a mutagenic potential of a test item, tested with the Ames test, is assumed if the mutant frequency is 2.0 or higher. A dose-effect relationship could underline this conclusion. A possible mutagenic potential is assumed if the quotient ranges between 1.7 and 1.9 in combination with dose-effect relationship. No mutagenic potential is assumed if all quotients range between 1.0 (or lower) and 1.6. A nonexistent dose-effect relationship could underline this conclusion. In our study none of the results of the Ames test (+S9 and –S9) exceeded the critical value of 2.0 and all the quotients ranged below 1.6, therefore the Ames test did not prove genotoxic potential in any of the drinking water samples. However, an increase in the induction factor can be seen in the presence of S9 homogenate (+S9), but the statistical significances of genotoxic potential in any of the water samples according to the negative control were not proven ($p>0.05$). TA97a, TA100 and TA1535 tester strains detect possible mutagenic changes at G-C (guanine-cytosine) sites within target histidine genes. It is clear from the literature that some mutagenic carcinogens also modify A-T (adenine-thymine) base pairs (*10*) and TA102 strain could detect point mutations at A-T sites, within multiple copies of *hisG* genes. Different results might be obtained if other tester strains were selected.

The alkaline version of the comet assay was used for the analysis of genotoxicity on Caco2, HepG2 and *T. thermophila* cells. General procedure involves subjecting cells to high pH>13 so that the nuclear DNA unwinds, and as a consequence alkaline electrophoresis is performed. Fragmented DNA migrates from the nuclear region (comet head) towards the anode, forming a comet tail. After staining with a fluorescent DNA-binding dye (*i.e.* ethidium bromide), fluorescence microscopy is used to evaluate the comets. The length of the comet's tail and its fluorescence intensity are directly correlated with the extent of nuclear DNA damage. The results of the *in vitro* comet assay on *Tetrahymena thermophila*, Caco2 and HepG2 cells are presented in Fig. 1 and Table 3. The results are presented as box-and-whisker plots, where OTM presents the degree of DNA damage caused by the water sample. The statistically significant increase of genotoxicity was proven for concentrated sample 2 ($p<0.05$) on both human cell lines, nonconcentrated sample 2 caused slight, but statistically not significant increase in genotoxicity on protozoa cells, although the chemical analyses did not show any exceeding of maximum allowed concentration (MAC) for the tested compounds in these

Table 2. Results of the Ames test with the strains TA97a, TA100 and TA1535 of three nonconcentrated (1, 2, 3) and 50× concentrated (1C, 2C, 3C) drinking water samples expressed as revertants/plate and induction factors (*i.e.* multiple of negative control)

Sample	TA97a		TA100		TA1535	
	Revertants/plate	Induction factor	Revertants/plate	Induction factor	Revertants/plate	Induction factor
			-S9			
Negative control	33±11	1	313±13	1	14±4	1
	Mean±SD	IF±SD	Mean±SD	IF±SD	Mean±SD	IF±SD
1	33±8	1.00±0.26	305±16	0.97±0.05	13±3	0.94±0.23
1C	33±11	1.00±0.33	305±13	0.97±0.04	12±4	0.78±0.39
2	33±6	1.01±0.17	320±9	1.02±0.02	13±3	0.92±0.24
2C	33±10	1.01±0.31	317±26	1.01±0.08	9±1	0.69±0.11
3	33±10	1.01±0.30	321±28	1.02±0.08	13±2	0.91±0.31
3C	32±7	0.96±0.22	316±17	1.00±0.05	11±4	0.77±0.26
m(4NQO) = 50 μg/plate	142±37	4.63±2.10	–	–	–	–
m(MMS) = 2.5 μg/plate	–	–	1060±106	3.3±0.3	–	–
m(sodium azide) = 1.5 μg/plate	–	–	–	–	151±13	10.64±0.94
			+S9			
Negative control	37±15	1	289±19	1	8±3	1
	Mean±SD	IF±SD	Mean±SD	IF±SD	Mean±SD	IF±SD
1	44±8	1.17±0.22	296±19	1.02±0.06	9±2	1.16±0.30
1C	45±8	1.22±0.21	292±12	1.00±0.04	9±4	1.17±0.52
2	45±6	1.20±0.15	264±9	0.91±0.03	10±2	1.26±0.28
2C	42±10	1.13±0.27	262±19	0.91±0.06	8±2	0.99±0.31
3	26±23	0.70±0.62	257±25	0.88±0.08	9±3	1.14±0.43
3C	37±8	1.00±0.23	278±17	0.96±0.05	10±3	1.25±0.39
m(2AF) = 10 μg/plate	142±55	4.27±0.53	524±93	1.80±0.32	158±21	19.17±2.47

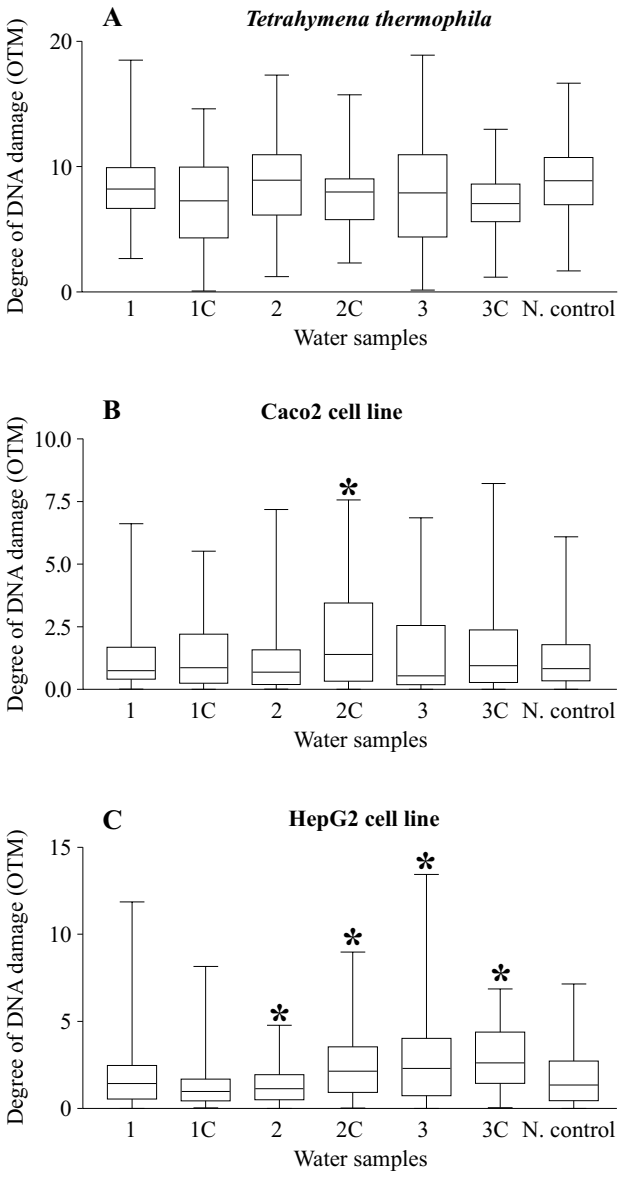

Fig. 1. Nuclear DNA damage in *T. thermophila* (**A**), Caco2 (**B**) and HepG2 (**C**) cells (presented as OTM) treated with nonconcentrated (1, 2, 3) and 50× concentrated (1C, 2C, 3C) drinking water samples. Results from 100 comets for each water sample are shown as box-and-whisker plots. The OTM values measured at the test concentrations are shown as boxes that include 50 % of the data. The top and bottom of the boxes mark 25th and 75th percentiles, the inner line marks the median value. One fourth of the data above the 75th percentile and 25 % of the data below the 25th percentile are marked as »whiskers«, limited by the maximum or minimum values

* Statistically significant differences according to negative control (N. control), $p<0.05$

water samples (Table 1). The statistically significant increase of genotoxicity was proven also for nonconcentrated and concentrated sample 3 on HepG2 cells ($p<0.005$).

In most publications on genotoxic effects of water samples only one test system has been used and almost all data come from the *Salmonella* (Ames) test (*1,2*). The combination of genotoxicity/mutagenicity tests and physicochemical methodologies can be useful to indicate the potential genotoxic contaminants in drinking water more relevantly and studies have shown that mammalian cell assays are generally, but not always, consistent with the data from bacterial assays (*1,2,25*).

The presence of potential genotoxic compounds in drinking water samples was expected to be very low, so it was necessary to choose very sensitive test systems. Human cells exhibiting different degrees of specialisation and representing specific targets within the human body were chosen. The Caco2 cell line, isolated from human colon adenocarcinoma, displays many specialised enterocyte-like functions. It expresses functional brush border microvilli and tight junctions, and retains the ability to transport vitamins and ions (*26*). As HepG2 hepatoma permanent cell line with endogenous bioactivation capacity has been extensively studied as an *in vitro* model for normal human liver, this cell line seemed to be a good choice as model cells. HepG2 cells are easy to handle, they retain many of the morphological characteristic of liver parenchymal cells, and contain several enzymes responsible for the activation of various xenobiotics (*27*). These cells appear to be a practical alternative for assessing genotoxicity, because most genotoxic compounds are indirect mutagens (*28*). Preliminary studies on exposure time of HepG2 cells to water samples were done. No statistically significant differences for exposures of 20, 40 and 60 min were found, so shorter exposure time was chosen in order to modify and develop short term genotoxic bioassay (data not shown). Similar short exposure time (30 min) for genotoxicity testing of indirect mutagens on HepG2, V97 and VH10 cells was chosen by the Czech research group, where HepG2 cells appeared to be the most sensitive ones since they possess a wide spectrum of xenobiotic metabolizing activities (*29–31).*

Since *T. thermophila* organism possesses a complex eukaryotic cellular structure (*32*) it was expected that genotoxic substances which require metabolic activation to produce DNA damage would also be genotoxic in the chosen protozoa cells.

In order to test complex environmental mixtures (like wastewater or drinking water samples, soil extracts, *etc.*) that might contain some indirect acting mutagens, a representative mammalian enzyme is added to the biotest system, which may lead to enhancement of mutagenic activity of indirect mutagens. The S9 rat liver homogenate (INC Biomedicals, GmbH) was added to the Ames test system as an exogenous metabolic activating system.

A reliable assessment of health risks caused by complex mixtures of chemicals or pesticides in drinking water is of crucial importance with respect to public health issue. Therefore, during the last decades papers were published using laboratory models with bacteria, mammalian cells and even animals, to investigate the DNA damaging potency of chlorinated and non-chlorinated drinking water samples. However, as reported earlier (*33*), studies with bacteria and/or metabolically incompetent mammalian cells have some shortcomings especially regarding the interpretation of results in the frame of human risk assessment. Additionally, *in vivo* animal studies are too much time consuming and expensive.

Table 3. Genotoxic effects of water samples in *T. thermophila*, Caco2 and HepG2 cells: values of the comet parameter OTM

	1	1C	2	2C	3	3C	N. control
				Tetrahymena thermophila			
Minimum	2.66	0.07	1.22	2.31	0.14	1.17	1.68
25 % Percentile	6.66	4.31	6.13	5.76	4.39	5.60	6.96
Median	8.21	7.25	8.91	7.97	7.90	7.04	8.86
75 % Percentile	9.91	9.96	10.96	9.01	10.95	8.61	10.72
Maximum	18.49	14.61	17.30	15.72	18.88	12.96	16.65
				Caco2 cells			
Minimum	0.01	0.00	0.01	0.00	0.00	0.00	0.00
25 % Percentile	0.41	0.25	0.19	0.33	0.19	0.27	0.34
Median	0.83	0.87	0.75	1.39	0.69	0.95	0.54
75 % Percentile	1.68	2.21	1.58	3.45	2.55	2.37	1.79
Maximum	6.61	5.52	7.18	7.56	6.85	8.22	6.09
				HepG2 cells			
Minimum	0.00	0.03	0.00	0.02	0.00	0.04	0.00
25 % Percentile	0.54	0.43	0.49	0.92	0.73	1.44	0.45
Median	1.43	0.97	1.13	2.14	2.29	2.62	1.34
75 % Percentile	2.47	1.68	1.93	3.54	4.02	4.37	2.72
Maximum	11.86	8.15	4.77	8.97	13.44	6.85	7.14

DNA damage plays an important role in the development of diseases like hereditary deformities, degenerative diseases and cancer. Many investigators have assayed DNA damage caused by environmental factors under *in vitro* conditions using various metabolically incompetent indicator organisms, *e.g.* bacteria, yeasts and mammalian cells. However, most of environmental genotoxicants have to be converted endogenously to reactive metabolites by xenobiotics metabolising enzymes. Since most of the commonly used indicator microorganisms lack these enzymes, exogenous metabolizing systems containing rat liver microsomal enzymes are usually added to insert mammalian metabolism (*33*). To avoid the problems mentioned above, we used S9 liver homogenate for bacterial tests and parallely used HepG2 cell line, which expresses many xenobiotic metabolizing enzymes, to assess water samples genotoxicity more relevantly.

According to the chemical analyses, water sample 1 expressed the highest content of pesticide atrazine (and its derivative desethylatrazine), which was not in direct correlation with the Ames test and comet assay results. Because of such differences between the results of chemical analyses and results of biotests, a battery of tests (parallel to the already established and validated bioassays for regular monitoring of drinking water) must be used in order to properly estimate the risks humans are exposed to by daily water drinking.

Chemical analyses alone cannot predict the actual risks humans are exposed to by consuming the drinking water, because they are limited by the sensitivity and determination of usually single compounds and their derivatives, and some of these compounds or mixtures of compounds can even not be measured. Chemical data by itself cannot show the integral impact of chemical mixtures (*i.e.* environmental samples) on living organisms. Toxic and genotoxic action is the consequence of addition, synergism, antagonism and bioactivation which can be shown directly only by bioassays. The use of a battery of biotests for monitoring of environmental samples like drinking water, wastewater or soil extracts is sensitive enough to minimize false negative and specific enough to reduce false positive results, and as such these tests are a promising prospect in human risk assessment.

Conclusions

The initial results presented in this study indicate that comet assay with human cell lines and *T. thermophila* is rapid, easy to conduct and is in fact more sensitive than the Ames assay in the case of genotoxicity monitoring of drinking water. Due to increased sensitivity of the used combination of tests, negative results provide a higher predictive value in excluding possible human health hazards to mutation and cancer than the results from studies with a single test (*i.e.* the Ames test) alone.

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Provjera genotoksičnosti u pitkoj vodi primjenom komet tehnike i Amesovim testom *in vitro*

Sažetak

U radu je predložen postupak provjere mogućnosti genotoksičnosti pitke vode. Uzorci pitke vode uzimani su s tri mjesta na području Ljubljane. Alkalna komet tehnika provedena je s ljudskim HepG2 i Caco2 stanicama te sa stanicama protozoa (*Tetrahymena termophila*). Usporedno su provjeravani isti uzorci Amesovim testom (s egzogenom metaboličnom aktivacijom ili bez nje) koristeći sojeve *Salmonella typhimurium* TA97a, TA100 i TA1535. Nekoncentrirani i koncentrirani uzorci vode ispitani su pomoću oba biotesta, a kemijskom analizom utvrđena je prisutnost pesticida i koncentracija nitrata. Prema Amesovom testu nije bilo indikacije genotoksičnosti u pitkoj vodi. Rezultati komet tehnike pokazuju razlike i moguću genotoksičnost pojedinih uzoraka vode ispitanih s različitim tipovima stanica koji su, osim u dva slučaja, bili statistički beznačajni. Statistička analiza pokazala je da je komet tehnika osjetljivija od Amesova testa za otkrivanje genotoksičnosti u uzorcima pitke vode.