

Mutagenic effect of fresh water (well, rivers Fica and Minas Gerais, close to the town of Ubatã, Paraná, Brazil) in the animal test system

Maria Fernanda Piffer Tomasi Baldez da Silva, Gisele Pezente Ferrari, Fabiana de Toledo, Carmem Lucia de Mello Sartori Cardoso da Rocha and Veronica Elisa Pimenta Vicentini*

*Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Avenida Colombo, 5790, 87020-900, Maringá, Paraná, Brasil. *Author for correspondence. e-mail: arbv@brturbo.com*

ABSTRACT. Intense industrial development and population growth have been altering the quality of water and innumerable studies have been undertaken to analyze their effects on humans. Due to rivers contamination with agrotoxics, herbicides, pesticides, excess of farming chemical additives and through sewers spilling not properly treated industrial waste, investigating cytotoxic and mutagenic activity of river water becomes all-important. Bone marrow cells of Wistar rats (*Rattus norvegicus*), treated *in vivo*, through gavage, in subchronic treatment (7 days), were used as experimental animal test system to investigate the effects of well water and water from the rivers Fica and Minas Gerais, close to the municipality of Ubatã, State of Paraná, southern Brazil. Cell division index and metaphase chromosomes were analyzed. The statistical analysis showed that the experiment's water failed to alter cell division cycle of Wistar rats and did not increase number of chromosome aberrations. No cytotoxic and clastogenic effects ensued from this treatment.

Key words: mutagenesis, fresh water, Wistar rats.

RESUMO. Efeito mutagênico da água natural (poço, rios Fica e Minas Gerais, próximos à cidade de Ubatã, Estado do Paraná, Brasil) em sistema teste animal. O intenso desenvolvimento industrial e crescimento populacional têm promovido alterações na qualidade da água, sendo esta fonte de muitos estudos para análise desses efeitos no ser humano. Devido a contaminação dos rios por agrotóxicos, herbicidas, pesticidas e demais aditivos químicos utilizados nas lavouras, e pelas indústrias que despejam através de esgotos, resíduos de sua produção, que não são devidamente tratados, torna-se importante a investigação da atividade citotóxica e mutagênica da água dos rios. Neste estudo foi utilizado como sistema teste animal as células da medula óssea de ratos Wistar (*Rattus norvegicus*), tratados *in vivo*, via gavagem, de forma subcrônica (7 dias), para investigação dos efeitos das águas do poço, e dos rios Fica e Minas Gerais, próximos à Ubatã, Estado do Paraná, Brasil, analisando-se o ciclo de divisão celular e os cromossomos metafásicos. A análise estatística demonstrou que as águas não alteraram o ciclo de divisão celular dos ratos Wistar e também não provocaram aumento no número de aberrações cromossômicas, mostrando não terem neste tratamento, efeito citotóxico e nem clastogênico.

Palavras-chave: mutagênese, água natural, ratos Wistar.

Introduction

Since the 1940s chemical and physical agents have been known to cause hereditary mutations. In the 1960s, the lethal activities of radiation and chemical agents were linked to damages in genetic material. Reports on the deleterious effects caused by the increasing use of chemical products, mainly pesticides, have been published almost

simultaneously and related to cancer and mutations (Waters *et al.*, 1994).

Due to conscience-raising, mainly in industrialized countries, on environmental quality as the basis of life preservation for future generations, the concern about environment has increased during recent decades. The environment is currently

considered a heritage for all mankind (Melo and Azevedo, 1997).

Water is an essential human resource, as a biochemical component in living beings; as a life medium of various vegetal and animal species; as a representative element of social and cultural values; as a production factor of intermediate and final consumer goods. Water resources are extremely important nowadays in the development of various economic activities (Cedasp, 2003).

It is common knowledge that water is an essential human commodity, indispensable for life on earth. However, the processes triggered during the last decades of the 20th century have challenged the above statement. The extensive use of fertilizers and toxic products and the employment of large quantities of water have made agriculture accountable for the contamination of water resources, even exceeding the effects produced by industrial and urban wastes (Abes, 2002).

During the 19th century, men set fire to forests and planted crops on the ashes. Since the soil was fertile, one plantation gave place to another and the same process occurred elsewhere. When chemical fertilizers and other technological practices were introduced, the former system was abandoned and an increase in productivity ensued. However, such practices deteriorated soil and water: erosion, desertification, soil and water poisoning and river silting occurred. Toxic effects of these compounds are largely unknown, either because of the product novelty or because of technical difficulties in their precise biological evaluation. Undoubtedly, environmental genotoxicity studies should take into account and comprise these variables (Silva *et al.*, 2003).

During the last decades water resources have become scanty and the myth of perpetual abundant water and its continuous resourcefulness has been imploded. Population growth and intense urbanization have decreased water resources in quality and quantity. They became more difficult and more complex to obtain and to preserve, more expensive to distribute, provide for and increase their offer. Although water demands are on the increase, there is also a proportionate rise in inadequate use of water resources (Senra, 2003).

The improper use of agricultural toxics is another factor that damages water quality. Their intensive use pollutes soil and water, especially products that have a lasting degrading effect on the environment. These products may reach the soil during direct application, on the fall of the treated

leaves or during rains that wash the toxic off the treated leaves (Valério, 1998).

Knowledge on the distribution of toxic products in the soil is paramount to evaluate the negative effects, such as air and water pollution. Water environments vary widely in their characteristics. This may be due to the region, water depth and circulation, the existence of aquatic vegetation, the amount of suspended solid material, the type of sediment, pH, solar radiation and type of biota. These variables affect diversely the effects of the herbicides in the ecosystem and their final destination may be accounted for through their adsorption by sediment and suspended solid material and by water plants and algae (Valério, 1998).

Insecticides, herbicides and fungicides have been intensively used during the last decades to protect plants against blights that damage crops and post liabilities to farmers. Agricultural toxics are frequently applied to crops and soil without any technical prescription. When washed away by rains, they gradually reach the rivers, lakes and the sea. Even at low concentrations these compounds, mainly non-biodegradable or cumulatively contaminated items (organic chlorides) may be dangerous. Biocumulative phenomenon may affect entire food chains, starting from low toxic concentrations. This may occur when water microorganisms retain a low chloride concentration derived from a toxic product for agricultural use. Small fish preying on these microorganisms extend the poisoning, since they increase the compounds' concentration in their body. Bigger fish that prey upon smaller ones also contaminate themselves and increase toxic concentration. As a consequence, birds and even man may receive a higher toxic concentration when they feed on contaminated fish, with serious health consequences (Tomé, 2003).

The agriculture has been one of the main economic activities of Paraná. The emergence of new plagues has been driving the farmers to the use higher and higher quantities of agricultural defensives, such as herbicides, fungicides and insecticides. The rains cart the superficial layers of the soil to the bed of the rivers, contaminating the water with residues of the pesticides that were applied in the cultures. The area of Ubitatã is agricultural, presenting soybean and cotton, both cultures with a massive use of pesticides. Many of these substances proved mutagenic and cancerous, risking the health of the rural populations.

The well water analyzed in this work, close to the river Fichta, is consumed by the locals. River

Ficha, running some 5km distant from a small village, has dense riparian vegetation and is surrounded by soybean, corn and cotton plantations. River Minas Gerais, about 2km from the same village and also surrounded by soybean, maize and cotton plantations, has scanty riparian vegetation and small pasture area. Water collections were done near the river mouths during January, after a big rain. No sewage or other wastes were detected nearby.

Due to these increasing problems, water analysis near agriculture sites is a must. Well water and water from the rivers Ficha and Minas Gerais, near the municipality of Ubiratã, State of Paraná, southern Brazil, were evaluated for their cytotoxic and mutagenic effects on the system of bone marrow cells of Wistar rats treated subchronically with the aforementioned water, via gavage.

Material and methods

The water from the rivers Ficha and Minas Gerais, near Ubiratã, state of Paraná, was collected in disinfected plastic bottles, which were covered and kept in refrigerator. The water was cloudy, due to rain.

Wistar rats, *Rattus norvegicus*, with body-weight (bw) of approximately 100g, were obtained from the vivarium of Universidade Estadual de Maringá, Paraná state, Brazil. Three males and three females were used for negative and positive controls and for treatment with different types of water.

Rats were fed for seven days, in the same time (subchronic treatment) with 1mL of experimental water/100g bw, via gavage. Tap water used in negative controls was similarly administered and in the same quantity. 1.5mg of the clastogenic compound cyclophosphamide/1mL water/100g bw, intraperitoneally and acutely (24h), was dispensed to positive control animals. Feed was given *ad libitum*. On the eighth day the rats were put down.

An hour and a half before being killed, the rats were injected with 0.5mL/100g bw of colchicine 0.16%. Bone marrow cells were obtained according to Ford and Hamerton (1956), with modifications.

Metaphases were analyzed by "blind" test, under an immersion lens 100X-optic microscope. One hundred metaphases per animal were analyzed, totalling 600 per group. Gap, breaks and other chromosomal changes were thus assessed. Mitotic index (MI) was calculated at 5,000 cells per sex, totalling 10,000 cells per group of rats. Statistic analysis was undertaken by Chi-square (χ^2) test ($\alpha=0.05$).

Results

Table 1 shows results of mitotic index, number and types of alterations in metaphases of Wistar rats in negative and positive controls and treatments with well water and water from rivers Ficha and Minas Gerais.

Chi-square test ($\alpha=0.05$) showed that there was no induction of statistically significant chromosome alterations in bone marrow cells of Wistar rats, in all types of water, when compared to negative control results. Alterations statistically significant in cell division index did not occur in treatment with well water and with water from the two rivers when results from both controls were compared.

Table 1. Total mean mitotic index (MI), total and types of chromosomal alterations and total metaphases analyzed in Wistar rats, negative control/CO⁻ (1mL water/100g bw), positive control/CO⁺ (1.5mg cyclophosphamide/1mL water/100g bw) and subchronic treatment with well water and water from rivers Ficha and Minas Gerais, both near the town of Ubiratã, Paraná, Brazil (1mL water/100g bw), with gavage subchronic treatment.

Groups	MI %	Alterations Total (%)	Chromosome Alterations			Metaphases Total
			Gap	Break	Others	
CO ⁺	1.32	79 (13.2)* (12.2)*#	6ct	46ct	27af	600
CO ⁻	1.41	2 (0.3)	-	2ct	-	600
Well water	1.35	4 (0.6) (0.3)#	2ct	-	2mi	600
River Ficha	1.42	1 (0.6)	-	-	1mi	600
River Minas Gerais	1.04	0	-	-	-	600

ct: chromatidic; af: acentric fragment; mi: minute. *Statistically significant result when compared to its respective negative control. #Result without gaps.

Discussion

There are many and continuous challenges that the atmosphere promotes to the stability of the genetic information. The must keep going until the recognition and the regulation of environmental mutagenic agents, which is urgent, due to the accumulation of data suggesting that mutagenicity is a reasonable prognostic for carcinogenicity (Ribeiro *et al.*, 2003).

Water pollution is the introduction of chemical, physical and biological materials that destroy the water quality, affecting live beings organisms. That process goes from simple paper bags to the most dangerous poisonous pollutant, as pesticides, heavy metals (mercury, chrome, lead) and detergents (Moraes, 2002).

The water of a river is considered of good quality when it presents less than a thousand fecal coliformes and less than ten pathogenic microorganisms per liter (as those verminoses cause cholera, esquistossomose, typhoid fever, hepatitis, leptospirose, poliomyelitis, etc.). Therefore, for

maintaining water in those conditions, contamination by residues should be avoided, not mattering if they are agricultural (of chemical or organic origin), sewers, industrial residues, garbage or sediments caused by erosion (Zampierom and Vieira, 2003).

In the current research, the mutagenic activity of well water and water from the rivers Fica and Minas Gerais, near Ubatã, was evaluated. Comparison of positive and negative controls and of treatments showed that the different waters, when administered in small doses to Wistar rats (*Rattus norvegicus*), via gavage or by mouth, failed to cause any statistically significant difference in cell division cycle of bone marrow cells analyzed by Chi-square test.

We have verified that seven-day subchronic treatment did not cause any statistically significant increase in chromosome alterations when these were compared to negative control data. In fact, no clastogenic effect was detected in that water samples when tested *in vivo* in Wistar rats within the limits of type and time of treatments.

Local pollution of aquifers by various oil products, industrial solvents and cleaning products are also a source of pollution (Falkenmark and Allard, 1991). Alterations in water will gradually occur when the flow and normal direction are disturbed by human activities in the soil and vegetation.

Research work (Fiskejö, 1985; Sudhakar et al., 1988; Smaka-Klinc et al., 1996) indicated that water toxicity may be easily measured by root tip cells growth inhibition. Smaka-Klinc et al. (1996) have shown that water samples from non-treated urban and industrial wastes are highly toxic.

An experiment, using *Salmonella* TA68, TA100, YG1024, YG1041 and YG1042, to estimate the mutagenic activity of river waters flowing through large metropolitan areas in North America, showed that YG1024 and YG1041 were found to be more sensitive than TA68. The mutagenic potential of heterocyclic amine, regarding the genotoxicity of water from river Yodo was also estimated by Obe et al. (2003) from organic extracts obtained by the blue rayon method. Guanting Reservoir and Yongding River were also analyzed by Ma et al. (2001). Mutagenic activity of organic pollution from these waters showed that they are only scantily polluted with mutagenic, when tested by XAD-2 resin.

Humans use water environments for definite ends. These include water supply, electrical power, irrigation, navigation, fish farming and harmonic landscape (Sperling, 1993). Owing to the population increase and intense industrial development, water

environments are subjected to various changes in quality levels. According to the same author, water quality may be impaired by the occurrence of pulses that negatively alter the water's physical-chemical and hydrobiological characteristics, commonly called pollution.

Pollution of water resources by humans was originally associated with human diseases, mainly diseases caused by oxygen-requiring organisms and residues (Meybeck and Helmer, 1989). Genotoxic effects on health, such as cancer, congenital defects and reproductive anomalies, were also mentioned. An increase in gastrointestinal and bladder cancer, reproduction anomalies and congenital malformation have been detected in populations living near toxic waste deposits. Analyses have been published by various authors, mentioned by Houk (1992).

An extremely adequate method for toxic verification consists of the study of bone marrow and its possible modifications in chromosome changes and on the cell division cycle. Whereas water bio-indicators, physical-chemical, saprobiological and radiological analyses merely indicate pollution level in water, cytogenetic and genotoxic analyses show the amount of damage already present in the organism. Cytogenetic tests identify the dangerous effects of toxic compounds in different concentrations within different time exposition and estimate their influence on the organisms. Reaction of organisms to environmental and water pollution may be measured by this test system (Smaka-Klinc et al., 1996).

Mutagenic negative results may be related to the period in which the water collection occurred. It was in January, or rather, during the rainy season; therefore, after great rains, which may have affected results. Since there was an increase in water volume, possible mutagenic compounds may have been dispersed and probably collected waters did not contain any pollutants in such amount that could be detected by our test.

Since water samples were collected near the river mouth, pollution is shown to be low at this precise spot, when compared to water from the middle of the river. The results may thus have also been affected by this fact.

Analysis of mitotic index was used as a cytotoxic paradigm in our research. In fact, it has been complementary to analyses employed to estimate toxic potential of samples under analyses. The results may be related to pH, total solids and organic material conditions, which did not affect results.

Although innumerable systems have been developed to monitor genotoxic pollutants, the monitoring of low levels of genotoxic pollutants and the precise surveying of an environment or an ecosystem are difficult, due to the limitations in their applications for a wide variety of environments.

It seems that no research has been undertaken with bone marrow test system of Wistar rats to estimate pollution in river waters. The importance of our experiment is thus validated.

Chromosome alterations and change in cell division cycle may be detected by analyses of Wistar rats' bone marrow. Similar effects may also occur in humans and this test system may identify any improper water use by humans. We may, thus, conclude that water samples collected during a certain period may not be contaminated at levels that will cause cytotoxic and clastogenic damages for the type and time of evaluation and within this test system.

The rivers are a source of life. From the Antique, their waters are essential for people's life, allowing such activities as drinking, taking baths, navigating, besides other useful activities, like fishing, which makes important to continue evaluating the cytotoxic and mutagenic potential of the river waters, studying its effect in chromosomal level and on the cycle of cellular division.

Acknowledgments

The authors would like to thank Rosinete Gonçalves Mariucci for her technical assistance.

References

- ABES. IX Semana Interamericana, II Semana da água: água e agricultura, 2002. Disponível em <http://www.abes.rs.org.br/semana_da_agua/2002/textos/exto.emater.htm>. Acesso em: 20 agosto 2003.
- CEDASPY. Cedasy: água, 2003. Disponível em <www.cedasy.com.br/rjt/agua>. Acesso em: 19 julho 2003.
- FALKENMARK, M.; ALLARD, B. Water quality and disturbances of natural freshwater. In: *The Handbook of Environmental Chemistry Part A - Water pollution*. Berlin: Hutzinger, v. 5, 1991, p. 46-78.
- FISKEJÖ, G. *Allium* test II: Assessment of a chemical's genotoxic potential by recording aberrations in chromosomes and cell divisions in root tips of *Allium cepa* L. Environmental Toxicological and Water Quality. *International Journal*, v. 9, p.235-241, 1985.
- FORD, C. E.; HAMERTON, J. L. A colchicine, hypotonic citrate, squash sequence for mammalian chromosome. *Stain Technol.*, Wilmington, v. 31, p.247-251, 1956.
- HOUK, V. S. Genetic analysis of the mitotic spindle. *Annu. Rev. Genet.*, Palo Alto, v. 30, p.7-33, 1992.
- MA, M. *et al.* Mutagenic activity of organic pollutants extracted from waters in Guanting Reservoir and Yongding River. *Wei Sheng Yan Jiu*, Beijing, v. 30, n.6, p.355-357, 2001.
- MELO, I. S.; AZEVEDO, J. L. *Microbiologia ambiental*. Jaguariúna: Embrapa, 1997.
- MEYBECK, M.; HELMER, R. The Quality of Rivers: from pristine stage to global pollution. Paleogeography, Paleoclimatology and Paleoecology. *Global and Planetary Change Section*, Amsterdam, v. 75, p.283-309, 1989.
- MORAES, L. A. *Poluição dos Rios. Belo Horizonte*, 2002. Disponível em <<http://gold.br.inter.net/luisinfo/polution.html>>. Acesso em: 08 junho 2003.
- OBE, T.; WHITE, P. A.; DEMARINI, D. M. Mutagenic characteristics of river waters flowing through large metropolitan areas in North America. *Mutat. Res.*, Amsterdam, v. 534, n.1-2, p.101-112, 2003.
- RIBEIRO, L. R.; SALVADORI, D. M. F.; MARQUES, E. K. *Mutagenese Ambiental*. Canoas: Editora da Ulbra, 2003.
- SENRA, J. B. Cultura da abundância, o mal das águas. In: FÓRUM NACIONAL DAS ÁGUAS. Poços de Caldas, 2003. p. 6-7.
- SILVA, J. *et al.* *Genética toxicológica*. Porto Alegre: Alcanço, 2003.
- SMACKA-KLINC, V. *et al.* The evaluation of the waste, surface and ground water quality using the *Allium* test procedure. *Mutat. Res.*, Amsterdam, v. 386, p.171-179, 1996.
- SPERLING, E. V. Considerações sobre a saúde de ambientes aquáticos. *Bio Engenharia Sanitária Ambiental*, São Paulo: ABES, Maio/Junho, Ano II (3), p.53-56, 1993.
- SUDHAKAR, D.; PANDA, K. K.; PANDA, B. B. Biomonitoring of low levels of mercurial derivatives in water and soil by *Allium* micronucleus assay. *Mutat. Res.*, Amsterdam, v. 203, p.11-21, 1988.
- TOMÉ, M. V. F. *Rede ambiente*, 2003. Disponível em <http://www.redeambiente.org.br/temas.asp?id_secao=2&artigo=17>. Acesso em: 27 agosto 2003.
- VALÉRIO, N. C. *Ação tóxica do Paraquat sobre o homem e o ecossistema terrestre e de água doce*. 1998. Monografia (Especialização em Ciências Biológicas) - Universidade Estadual de Maringá, Maringá, 1998.
- ZAMPIEROM, S. L. M.; VIEIRA, J. L. *Poluição das Águas*, 2003. Disponível em <http://educar.sc.usp.br/biologia/textos/m_a_txts.html>. Acesso em: 08 junho 2003.
- WATERS, M. D. *et al.* The performance of short-term in identifying potential germ cell mutagens: a qualitative and quantitative analysis. *Mutat. Res.*, Amsterdam, v. 341, p.109-131, 1994.

Received on September 25, 2003.

Accepted on February 25, 2004.