Bull. Environ. Contam. Toxicol. (1999) 63:438-443

Bulletin of Environmental Contamination and Toxicology

Influence of River Water in the Detection of Cr(VI) Mutagenicity by the Ames Test

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Received: 3 February 1999/Accepted: 16 July 1999

Hazardous xenobiotics in the aquatic environment can interact with microorganisms, other chemicals and solar irradiation to produce derivatives often characterized by an enhanced bioavailability compared to parental compounds. Another important mechanism of interaction is represented by the adsorption/desorption phenomena between the compounds and suspended particulate matter (SPM) or sediments. SPM has a central role as a transport vehicle for toxic pollutants (McElroy et al.1989). These mechanisms are expected to affect their toxicological properties, in the sense of either activation or, more often, detoxification.

The outcome of physical and chemical interactions with other pollutants as well as with normal chemical components of the ecosystems is hardly predictable. In this context the identification by chemical analysis of a compound as a carcinogen or mutagen is not sufficient to predict specific toxicological hazards. The genotoxicity of the complex mixture must be studied with a biological system (Hermann 1981; Haugen and Peak 1983). Assessment of genotoxic risk is based in part upon results of short term *in vitro* assays. One of such test, the *Salmonella* /mammalian microsome assay (Ames test) has been used for over two decades to predict the mutagenic potential of pure compounds and complex mixtures (Maron and Ames 1983). This assay uses a set of histidine-requiring strains of the bacterium *Salmonella typhimurium* to detect mutations induced by a test agent.

Recently, a great number of studies on the mutagenicity of environmental samples have considered the possibility of adsorption/desorption of chemicals to particles (White et al. 1996; Vahl et al. 1997). Less is known about how the activity of a genotoxic xenobiotic is modified by other abiotic factors present in river waters (Abe and Urano 1994).

This paper deals specifically with the interference of sterilized river water samples on the genotoxicity detection of a well known environmental mutagen, potassium dichromate (IARC 1990). Chromium was chosen for this study as an environmental genotoxic pollutant because its toxic and mutagenic properties are well understood (Bianchi et al. 1980; Leonard and Lauwerys 1980) and it represents an important river water pollutant in Argentina. In this country, industrial effluents from tanneries and electroplating industries, which contain chromium salts (Noyes 1993) are discharged in many cases only slightly treated, or even untreated, into the rivers.

MATERIALS AND METHODS

River water samples were obtained from the Rio de la Plata in the Buenos Aires city area. This river is an estuary with an area of 35,000 km^2, 320 km long and from 22 to 222 km wide. One of its main characteristics is the high concentration (20-200 mg/L) of particulate matter (AGOSBA-OSN-SIHN 1994; Garcia Arguijo 1994). The river also

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receives a great load of sewage, untreated industrial effluents and input from extremely polluted streams. Table 1 provides a gross chemical and physical characterization of the contamination in this area.

Water samples with high levels of SPM (85-145 mg/L) were taken in-one liter flasks from the Rio de La Plata at different points in the Buenos Aires area. Prior to arrival at our laboratory the samples were mixed in a 5 L amber glass bottle and stored in the dark at 5° C (USEPA 1986) during no more than 2 hours. Within 3 hours after arriving at the laboratory, the samples were sterilized alternatively by filtration, UV irradiation or autoclaving as was previously described (Lopez and Moretton 1997). Filtration of the river water with 0.22 pm membranes removes particulate matter. UV-irradiation and autoclaving preserves both the SPM and the soluble fraction but these components may be altered by the treatment. Distilled water plus river particles was prepared as follows: 50 mL of autoclaved river water was centrifuged in plastic tubes for 10 min at 5000g. The pellet was suspended in sterile distilled water and centrifuged under the same conditions. This operation was repeated once. Finally the pellet was suspended in 50 ml of sterile distilled water.

Mutagenicity was measured by using the *Salmonella* /microsome assay (Ames test) following the standard plate incorporation technique (Maron and Ames 1983). The *Salmonella typhimurium* strain TA100 was kindly provided by Dr. Bruce Ames (University of California, Berkeley, CA. USA). For this study, TA100 strain was chosen because mutagenicity of potassium dichromate was demonstrated for this strain without microsome activation (Bianchi et al. 1980). The experiments, were performed with three plates per concentration and the criterions of positive results were those defined by Maron and Ames (1983): (a) two fold or greater increase in the number of revertants exposed to the test material over spontaneous reversion rates and (b) a reproducible dose-response relationship.

Table 1. Water quality of Rio de la Plata in Buenos Aires area*.

PH	7.1-7.4	Nitrites	<0.1 mg/L
SPM	45-150 mg/L	Nitrates	0.5-3 mg/L
DO	2.5-5 mg/L	Sulfide	0.05-0.1 mg/L
BOD	1-5 mg/L	Cadmium	0.25-1.4 μg/mL
Alkalinity	30-50 mg/L	Chromium	<1 μg/mL
Hardness	10-15 mg/L	Arsenic	6-6.5 μg/L
Chloride	22-40 mg/L	Lead	1-17 μg/mL
Ammonium	0.3-0.6 mg/L	Phenols	0.014 mg/L
		Detergents	<0.1 mg/L

DO: dissolved oxygen; BOD: biological oxygen demand

*source Obras Sanitarias de la Provincia de Buenos Aires, Obras Sanitarias de la Nación (AGOSBA-OSN-SINH 1994).

Mean values were statistically compared using a one-way analysis of variance with SYSTAT for Windows 6.0 statistical package (SPSS Inc.). The level of statistical significance was $p < 0.05$.

RESULTS AND DISCUSSION

The results obtained when sterilized river water samples and distilled water plus SPM were tested with *the Salmonella typhimurium* assay, are shown in Table 2. In this case no positive results were obtained for any of the treatments performed.

Results of the mutagenicity of potassium dichromate dissolved in sterilized river water samples or in a mixture of distilled water plus SPM are given in Figures 1 A-D. The values obtained for the control, where distilled water was used as solvent, were similar to those reported previously by Bianchi et al. (1980). The concentrations of potassium dichromate selected for assay, were: 0,2.5, 5, 10,20,30 and 40 µg/plate.

Table 2. Mutagenicity of sterile river water samples and distilled water plus SPM.

	Salmonella typhimurium TA100*
Distilled water (control)	141±34
Autoclaved	164±40
Filtered	155±27
UV-treated	104±39
Distilled water + SPM	190± 7

*revertants per plate, average ± SD (standard deviation).

Toxicity was found for concentrations higher than 30 µg/plate. To check whether synergic and/or protective effects were induced by river water on the biological activity of chromium salts assays were performed at concentrations with no mutagenic or toxic effects as well as concentrations from the linear portion of the dose response curve. The promotive or inhibitory influence on mutagenicity was determined according to whether or not the change in the number of revertant colonies, compared with the control, was statistically significative in the corresponding portion of the curve.

The results obtained indicate that no significative differences in the number of revertants were observed for W-treated river water (Fig. 1A), autoclaved river water treatment (Fig. 1B) or distilled water plus SPM (Fig. 1D) when compared with the control. But filtered river water, (Fig 1C) produced a significative reduction in the number of revertants at 20 µg/plate of potassium dichromate.

The supernatants obtained from centrifugation of autoclaved river water and from the washing of SPM, were tested with and without chromium. The results are graphed in figure 2. An interference with the mutagenic activity could be observed when the river soluble fraction was in contact with potassium dichromate.

The results obtained clearly demonstrate, that there is an interference in the genotoxic activity of potassium dichromate due to some compound/s present in the soluble fraction of the river water. The data (Figure 1 C) obtained when filtrated river water was used as solvent without SPM showed an important decrease in the mutagenic activity of potassium dichromate.

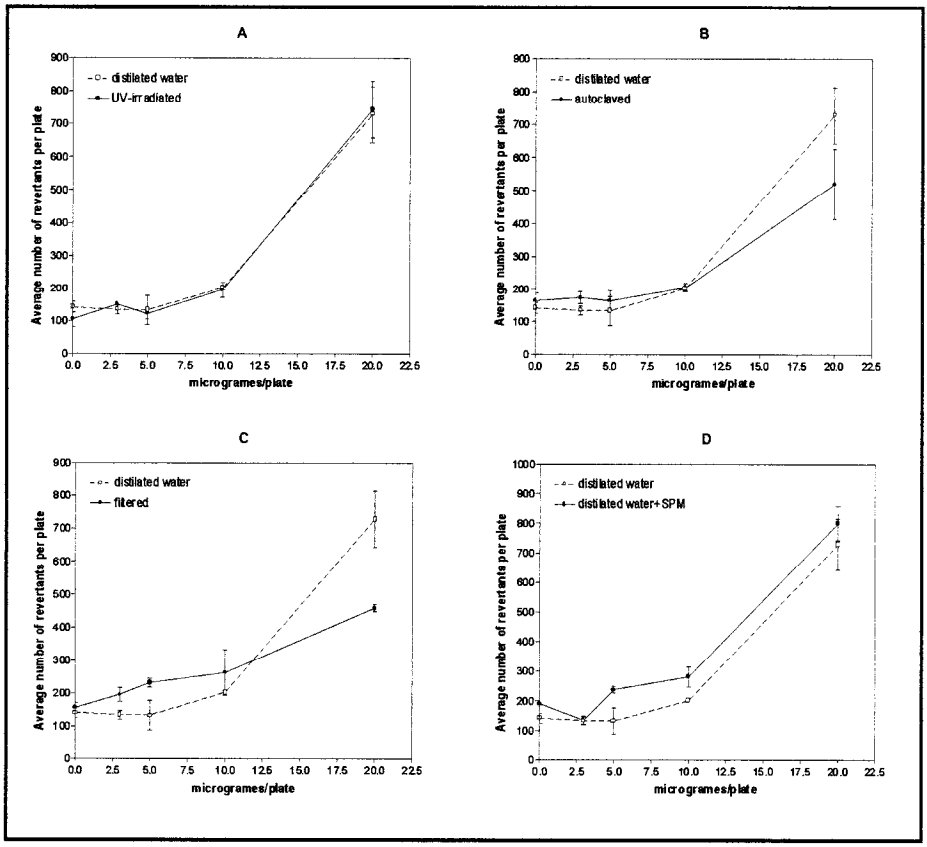

Figure 1. Mutagenicity of potassium dichromate in sterilized river water

Similar results were found when chromium salts were tested in the same conditions in the *Bacillus subtilis* Ret-assay (Lopez and Moretton 1997). On the other hand, in experiments where the supernatant fraction of centrifuged river water was tested with and without potassium dichromate, an interference with activity was detected whenever the soluble fraction was in contact with chromium salts. In the experiments where distilled water plus SPM were assayed (Figure 1D) no differences with the mutagenic activity of the control were found. This indicates that SPM was not responsible for the reduction on the mutagenic activity of potassium dichromate and is consistent with data reported in AGOSBA-OSN-SIHN (1994) where Cr(VI) was shown not to bind to SPM.

The experimental protocol used in this report allowed the detection of interference with mutagenic activity. When potassium dichromate was dissolved in different water samples, before treating the *Salmonella typhimurium* cells, the formation of chemical complexes with chromium were possible. In this situation fewer mutagenic molecules were free to reach DNA. It is important to note that the interfering substances were not affected by the temperature used to autoclave the samples. As the decrease on the mutagenicity was observed even after the heat treatment was performed.

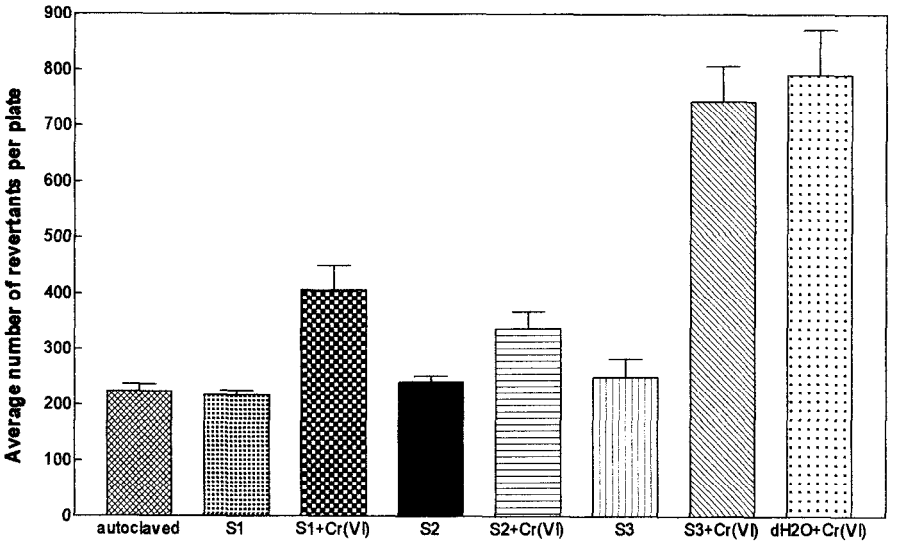

Figure 2. Supernatants with and without potassium dichromate. Control: autoclavated river water, Sl, S2, S3: supernatants from the first, second and third washing steps of SPM. Potassium dichromate was added at 20 µg/plate.

One point that remains unclear is why in presence of SPM the interference with the mutagenic activity was not detected. Among the soluble contaminants reported in river water samples (table 1) only hardness and alkalinity showed a level of concentration high enough to interact with chromium salts. As was reported in Quality Criteria for Water an Wastewater Analysis (USEPA 1986) the toxicity of chromium salts decreases as hardness and pH of the water increase. Components of alkalinity such as carbonate and bicarbonate will complex some toxic heavy metals. The SPM are part of the loess "pampeano" composed mainly of silicon, aluminium and ferric oxides, with and a small amount (3 to 4 %) of calcium and magnesium oxides (Trelles 1972). One possible explanation of the decrease in the mutagenic effect observed might be that these components of the SPM interfere with the anionic components available in the soluble fraction of river water.

Further work is in progress in this laboratory in order to understand better the mechanisms involved in the river waters interference with the mutagenic activity of potassium dichromate.

Acknowledgments *This work was supported in part by the grant TB51 Secretaria de Ciencia y Tecnica Universidad de Buenos Aires.*

REFERENCES

Abe A., Urano K. (1994) Influence of chemicals comonly found in a water environment on the Salmonella mutagenicitytest. Sci. Total Environ. 153: 169-175

AGOSBA-OSN-SIHN. (1994) Rio de la Plata: calidad de las aguas franja costanera sur. Informe de Avance, Buenos Aires

Bianchi V, Celotti L, Lanfranchi G, Majone F, Martin G, Montaldi A, Sponza G, Tamino G, Vernier P, Zantedeschi A, Levis AG (1980) Genetic effects of chromium compounds. Mutation Res 117: 279-300

García Arguijo AR (1994) Contaminatión de1 medio ambiente acuático: introductión a los ecosistemas hídricos y evaluación de las aguas de1 Río de la Plata. Ediciones Poligrafik Proamar, Buenos Aires

Haugen DA, Peak MJ (1983) Mixtures of polycyclic aromatic compounds inhibit mutagenesis in the the *Salmonella* /micrososme assay by inhibition of metabolic activation. Mutation Res 116: 257-269

Hermann M (198 1) Synergistic effects of individual polycyclic aromatic hydrocarbons on the mutagenicity of their mixtures. Mutation Res 90: 399-409

International Agency for Research on Cancer (IARC) (1990) monographs on the evaluation of carcinogenic risk to humans. Chromium, Nickel and Welding, vol 49. World Health Organization, Lyon France.

Leonard A, Lauwerys RR (1980) Carcinogenicity and mutagenicity of chromium. Mutation Res 76: 227-239

Lopez LC, Moretton J (1997) Influencia de factores abióticos presentes en el río de la Plata sobre la genotoxicidad de1 cromo. Rev. Jnt. Contam. Ambient. 13: 7-13

McElroy AE, Farrington JW, Teal JM (1989) Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment. In: Varanasi U (ed) Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment, CRC Press, Boca Raton, Florida, p 1

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

Noyes R (1993) Pollution prevention technology handbook 1 ed Noyes publications, New Jersey

Trelles R.A. (1972) Química de las aguas de la Republica Argentina. Instituto de Ingeniería Sanitaria. Universidad de Buenos Aires, Buenos Aires

USEPA (1986) Quality criteria for water. Office of Water Regulations and Standards, Washington

Vahl HH, Karbe L, Westendorf J (1997) Genotoxicity assesment of suspended particulate matter in the Elbe river: comparison of Salmonella microsome test, arabinose resistance test, and *umu-test.* Mutation Res: 394, 81-93

White PA, Rasmussen JB, Blaise C (1996) Comparing the presence, potency, and potencial hazard of genotoxins extractes from a broad range of industrial effluents. Env Mol Mutagen 27: 116-139