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The *Salmonella* mutagenicity assay in a surface water quality monitoring program based on a 20-year survey

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Abstract

Since 1979, the Environmental Agency of São Paulo State in Brazil, CETESB, has been using the *Salmonella* mutagenicity assay to assess the quality of natural waters. This paper is a compilation of data obtained during the last 20 years from more than a thousand samples. Potencies up to 30,000 revertants/l were observed in 137 positive samples. The *Salmonella typhimurium* strain TA98 was more sensitive than TA100; 79% of the mutagenicity was detected by this strain, regardless of the presence of S9-mix. A classification of the mutagenic response was proposed to facilitate in the dissemination of the information to the public. The classification was *low*, *moderat*e, *high* and *extreme* for samples with mutagenic potency (revertants/l equivalent) of <500, 500–2500, 2500–5000 and >5000, respectively. As a result of this effort to standardize methodologies, compile and classify the mutagenic effect of water pollution, in 1998, the *Salmonella* mutagenicity assay was officially and systematically included in the São Paulo State Water Quality Monitoring Program. This assay has proven to be a useful tool in the identification of important pollution sources. Correction and prevention actions in Water Pollution Control Programs were generated as a result.

Keywords: Water quality; Water monitoring program; *Salmonella* assay; Mutagenicity; Brazil

1. Introduction

The quality of water in the State of São Paulo, one of the more developed and industrialized states in Brazil, is regulated by both Federal and State Laws. These laws are based on the same physical and chemical standards, covering toxic metals and some organic toxic substances such as benzene, benzo(a)pyrene and pesticides. But, several other important toxic and carcinogenic substances are not regulated under these laws, such as other polycyclic aromatic hydrocarbons and aromatic amines.

Although since 1977, physical and chemical analyses were performed routinely by the São Paulo environmental agency — CETESB, other tools seemed to be necessary to complement those analyses. In order to address more adequately the water quality, the *Salmonella* mutagenicity assay (Ames test) has been used in various studies, with different objectives, usually aiming to characterize a site of study and/or develop and validate analytical methodologies. In 1998, because there were sufficient data and validated

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methodologies to prepare and to test water samples, the *Salmonella* assay was officially and systematically included in the State Water Quality Monitoring Program at sites where water was used as source for drinking water, after treatment. Even though disinfection processes can generate by-products with mutagenic activity [1–5], the presence of mutagens in source waters could enhance the levels of mutagenicity in finished water, and subsequently the risk of cancer in the exposed population [5,6].

This work shows a compilation of the data that generated support for the inclusion of the *Salmonella* mutagenicity assay in the monitoring program, and the establishment of a classification system that was deemed necessary to facilitate the dissemination of the information in terms that are familiar to the general public. It also shows that, as a result of the systematic mutagenicity testing, we could identify a contaminated site, find the pollution source and the appropriate actions were taken to prevent the distribution of the water to the population.

2. Materials and methods

2.1. Sample collection and concentration procedures

Between 1979 and 1999, different surface water samples were collected according to American Public Health Association Standard Methods for the Examination of Water and Wastewater [7]. More than thousand samples that were part of many different projects and political demands were analyzed, and the analytical methodologies were developed, improved, and validated.

Different methodologies were used to extract and/or concentrate the samples for the *Salmonella* mutagenicity tests (Table 1). All the eluates were reduced to 2–3 ml using an evaporator, transferred to small vials, evaporated to dryness with a gentle stream of nitrogen just before testing, and then resuspended in dimethylsulfoxide (DMSO). For in situ concentration samples were sterilized by filtration, and tested using top agar in higher concentrations.

2.2. Salmonella mutagenicity assay — Ames test

Salmonella mutagenicity tests were performed using the standard plate incorporation method [8] with the *Salmonella typhimurium* strains TA98 and TA100, with and without S9-derived metabolic activation. The S9-mix was freshly prepared before each test using Aroclor-1254-induced rat liver S9 fraction, that was purchased (lyophilized) from Moltox — Molecular Toxicology Inc.

For single-dose assays, 20 ml equivalent of water per plate were used, and for multiple dose assays, doses ranged from 25 to 200 ml equivalent of water. For the in situ concentration method, doses varied from 0.1 to 2.0 ml of water per plate. Positive controls were 4-nitroquinoline-1-oxide (4NQO) without metabolic activation, and 2-aminoanthracene (2AA) with S9. Results were statistically analyzed using Salmonel or Salanal computer programs, with Bernstein et al. model [9], and expressed as revertants/l equivalent of water.

Table 1
Sample preparation methodologies and *Salmonella* test conditions employed in this study

Sampling period	Number of samples	Volume extracted (l)	Organic extraction method	Extraction solvent	Ames test conditions	Reference
1979–1986	256	1	Liquid/liquid	Methylene chloride	20 ml equivalent per plate (single dose)	[27]
1987–1999	594	10–20	XAD resin	(N/B)[a] methylene chloride/methanol, (H^+)[b] ethyl acetate	10–200 ml equivalent per plate (multiple dose)	[28]
1993–1999	157	1	Filtration 0.45 or 0.22 μm	None	0.1–2.0 ml[c] per plate (multiple dose)	[29]

[a] (N/B): neutral/basic extraction.
[b] (H^+): acidic extraction.
[c] For liquid samples, the maximum volume used in the regular Ames test is 200 μl per plate. In order to test the sample *in natura*, the top agar is prepared in higher concentrations and is used to dilute the sample during the test [29].

3. Results and discussion

The *Salmonella* assay has been widely used throughout the world to detect the mutagenic activity of complex environmental mixtures. The test has been proven to be sensitive with many classes of mutagenic compounds, such as polycyclic aromatic hydrocarbons, aromatic amines, nitroarenes and some chlorinated compounds [10–14].

Since the late seventies, CETESB has been using the *Salmonella* mutagenicity assay — Ames test — to assess the mutagenic activity in water samples. Optimization of the mutagenic response was achieved through modifications of testing procedures and sample preparation techniques throughout the years.

In the last 20 years, 1007 samples of surface water were analyzed by CETESB, and 137 (14%) showed mutagenic activity. The incidence of mutagenicity according to the main uses of the water is presented in Fig. 1. Of the 525 source water samples analyzed, 18% showed mutagenic response. This percentage was higher than the ones observed for recreational water samples (6.5%) and for waters where no uses were given (11%).

Among the positive samples, 56 were tested at single-dose, and responses given in terms of presence or absence of mutagenic activity. A total of 81 samples were analyzed using multiple doses, where slopes were calculated. The results are listed according to their mutagenic potencies: up to 500 revertants/l (Table 2a), 500–2500 revertants/l (Table 2b), 2500–5000 revertants/l (Table 2c) and more than 5000 revertants/l (Table 2d). Pollution sources were shown as well, when available.

For surface source waters the mutagenic potencies in *Salmonella* observed in our data varied from 12 to 3625 revertants/l, which are on the same order of magnitude of the results obtained in other countries, where values ranged from 10 to 3600 revertants/l [5,15–17]. Although source waters gave more percentage of mutagenic response, when compared to other surface waters, the majority of them presented potencies below 500 revertants/l (Fig. 2). The results obtained for source waters indicate the need for monitoring and controlling actions to guarantee a good quality of the waters that after treatment will be served to the population.

Investigations carried out by Rehana et al. [19] in surface waters from three sites in the Ganges river in India, suggested that the mutagenic activity (3600–10,000 revertants/l) was mainly due to the presence of pesticides. Helma et al. [20] find industrial discharges to be responsible for the mutagenicity found in rivers from Poland and Austria, with mutagenic potencies ranging from 200 to 2000 revertants/l. Since our data usually came from projects with different objectives, we did not have the necessary

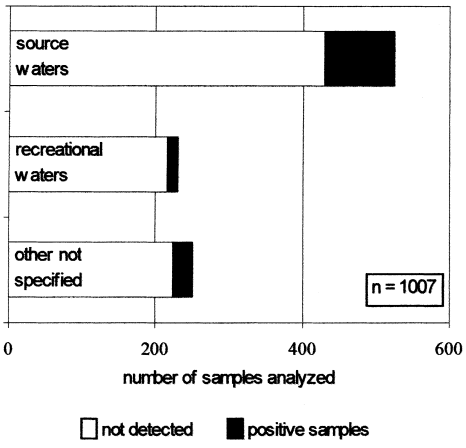

Fig. 1. Distribution of the number of samples analyzed and incidence of mutagenic activity according to the uses of the water.

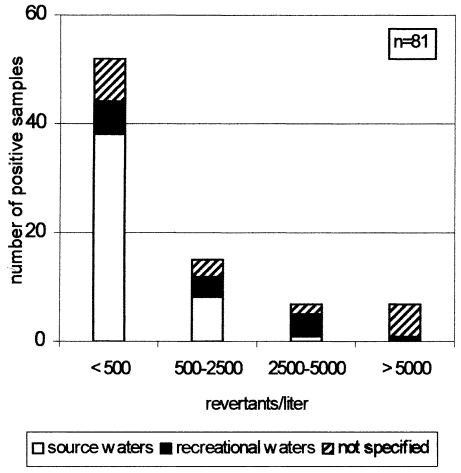

Fig. 2. Distribution of the mutagenic potencies of the 81 positive water samples where slopes could be calculated from the 1007 samples analyzed for the *Salmonella* assay.

Table 2
Mutagenic potencies of surface water samples analyzed by the *Salmonella* assay[a]

Sample #	Revertants/l				Sampling date	Uses of water[b]	Possible pollution source	Reference[c]
	TA98 − S9	TA98 + S9	TA100 − S9	TA100 + S9				
(a) Up to 500 revertants/l								
1	39	–	–	–	April 1988	sw	Industrial[d]	[30]
2	51	–	–	–	August 1988	sw	Industrial	[30]
3	53	354	–	–	April 1988	sw	Industrial (food)	[30]
4	57	477	–	–	January 1988	sw	Heavily industrial	[30]
5	76	–	–	–	August 1991	ns	Industrial (petrochemical)	
6	80	110	–	–	July 1999	sw	Unknown	
7	83	–	467	–	December 1987	sw	Heavily industrial	[30]
8	84	–	–	–	April 1990	sw	Industrial	
9	87	–	–	–	February 1988	sw	Industrial	[30]
10	90	100	–	–	February 1999	ns	Industrial (steel)	
11	100	–	256	–	December 1987	sw	Industrial (paper), rural	[30]
12	101	–	–	–	February 1988	sw	Rural	[30]
13	104	–	–	–	December 1988	sw	Industrial (plastic)	[31]
14	116	91	–	–	August 1991	ns	Industrial (petrochemical)	
15	130	60	–	–	February 1999	ns	Industrial (steel)	
16	153	43	–	–	August 1991	ns	Industrial (petrochemical)	
17	191	–	–	–	October 1993	rw	Industrial, untreated domestic sludge	
18	210	–	–	–	April 1995	ns	Oil spill	
19	230	–	–	–	April 1989	sw	Industrial (plastic)	[30]
20	232	112	–	–	February 1988	sw	Industrial (paper), rural	[30]
21	240	330	–	–	October 1998	sw	Industrial (textile)	
22	248	–	–	–	October 1993	rw	Industrial, untreated domestic sludge	
23	266	320	–	353	October 1987	sw	Heavily industrial	[30]
24	290	340	–	–	July 1999	sw	Industrial (textile)	
25	324	228	–	–	October 1993	rw	Industrial, untreated domestic sludge	
26	355	234	–	–	February 1988	sw	Industrial, rural	[30]
27	394	122	–	–	February 1988	sw	Rural	[30]
28	408	–	–	–	April 1989	sw	Industrial (plastic), rural	[30]
29	429	–	–	–	October 1993	rw	Industrial, untreated domestic sludge	
30	500	–	–	–	May 1996	ns	Industrial	

31	–	12	–	–	November 1989	sw	Industrial	
32	–	50	–	–	February 1999	ns	Industrial (steel)	
33	–	71	–	324	December 1989	sw	Heavily industrial	[30]
34	–	93	–	–	December 1989	sw	Heavily industrial	[30]
35	–	96	–	–	March 1990	sw	Heavily industrial	[30]
36	–	132	–	–	January 1988	sw	Heavily industrial	[30]
37	–	136	–	–	October 1989	sw	Heavily industrial	[30]
38	–	170	–	–	April 1997	rw	Industrial, untreated domestic sludge	
39	–	180	–	–	August 1989	sw	Unknown	[31]
40	–	271	–	195	January 1997	rw	Industrial, untreated domestic sludge	
41	–	341	–	83	April 1988	sw	Heavily industrial	[30]
42	–	347	–	–	April 1989	sw	Rural	[30]
43	–	477	–	149	April 1988	sw	Industrial	[30]
44	–	–	146	–	October 1988	sw	Industrial	[30]
45	–	–	241	–	October 1988	sw	Rural	[30]
46	–	–	382	–	April 1988	sw	Rural	[30]
47	–	–	467	–	December 1987	sw	Untreated domestic sludge	[30]
48	–	–	–	261	October 1988	sw	Industrial (plastic)	[30]
49	–	–	–	221	October 1987	sw	Heavily industrial	[30]
50	–	–	–	202	January 1988	sw	Rural	[30]
51	–	–	–	183	August 1988	sw	Industrial	[30]
52	–	–	–	164	April 1989	sw	Industrial, rural	[30]
(b) From 500 to 2500 revertants/l								
53	340	–	1400	680	March 1997	rw	Industrial, untreated domestic sludge	
54	370	570	–	–	August 1989	sw	Industrial	[31]
55	480	–	2300	–	February 1997	rw	Industrial, untreated domestic sludge	
56	612	–	–	–	February 1997	rw	Industrial, untreated domestic sludge	
57	620	–	2200	–	February 1997	rw	Industrial, untreated domestic sludge	
58	640	90	–	–	April 1995	ns	Oil spill	
59	658	–	–	–	December 1987	sw	Industrial	[30]
60	791	–	–	–	December 1987	sw	Heavily industrial	[30]

Table 2 (*Continued*)

Sample #	Revertants/l				Sampling date	Uses of water[b]	Possible pollution source	Reference[c]
	TA98 − S9	TA98 + S9	TA100 − S9	TA100 + S9				
61	932	–	–	–	December 1988	sw	Industrial, rural	[30]
62	1100	1000	–	–	April 1995	ns	Oil spill	
63	1100	1700	–	700	June 1999	sw	Industrial (textile)	
64	1141	201	–	–	February 1988	sw	Industrial, rural	[30]
65	1413	270	–	–	February 1988	sw	Industrial	[30]
66	–	530	–	–	February 1995	ns	Oil spill	
67	–	–	–	554	January 1989	sw	Rural	[30]
(c) From 2500 to 5000 revertants/l								
68	570	–	2900	890	February 1997	rw	Industrial, untreated domestic sludge	
69	650	220	2600	–	February 1997	rw	Industrial, untreated domestic sludge	
70	790	–	3100	630	February 1997	rw	Industrial, untreated domestic sludge	
71	890	–	4400	–	February 1997	rw	Industrial, untreated domestic sludge	
72	1787	3625	–	–	December 1987	sw	Industrial	[30]
73	4200	4200	–	–	March 1999	ns	Industrial (petrochemical)	
74	–	1700	2600	3500	February 1995	ns	Oil spill	
(d) More than 5000 revertants/l								
75	1400	360	6000	1400	February 1997	rw	Industrial, untreated domestic sludge	
76	1600	5400	–	3600	May 1995	ns	Oil spill	
77	2200	980	3600	7900	April 1995	ns	Oil spill	
78	3400	4000	4000	8300	May 1995	ns	Oil spill	
79	6000	2200	–	–	May 1995	ns	Oil spill	
80	22000	5300	–	30000	March 1995	ns	Oil spill	
81	–	–	–	30000	March 1999	ns	Industrial (petrochemical)	

[a] Mutagenic activity not detectable.

[b] Uses of the water body: sw — source waters; rw — recreational waters; ns — use not specified.

[c] References: unless specified, data not published.

[d] Industrial: unless specified, contamination comes from several industrial sources.

information to do the correlation of the mutagenic response with specific pollution sources, except when specific contamination events occurred. In that case, the mutagenicity results achieved 10^5 revertants/l (Table 2d — sample # 80), were similar to the results (10^7 revertants/l) obtained by Alzuet et al. [18] in Rio de La Plata, Argentina.

In our study, the process of obtaining the data permitted us to implement and standardize appropriate methodology for testing water samples for mutagenicity in order to apply it on a routine basis. We observed that 79% of the mutagenicity was detected with *Salmonella* strain TA98, which is in agreement with other studies performed on complex environmental mixtures [5,15–17,19–25]. TA98 without metabolic activation responded better than with activation, suggesting a prevalence of direct-acting mutagens in the surface waters analyzed. From the data listed, 38 samples were positive exclusively with one strain/condition: 17 with TA98 − S9, 10 with TA98 + S9, 4 with TA100 − S9 and 7 with TA100 + S9. It is interesting to note that if we consider only the results obtained with TA100, mutagenicity was higher with metabolic activation, suggesting that part of the indirect-acting mutagens induced base pair substitution mutations.

This 20-year survey effected the official inclusion of the *Salmonella* mutagenicity assay in the São Paulo State Water Quality Monitoring Program, beginning in October 1998. Since then, 28 sites of source waters have been monitored for mutagenic activity quarterly or bimonthly, and until October 1999, a total of 109 samples were analyzed. The results from these samples are included in this study. In one of the sites (samples # 21, 24 and 63; Table 2a and b) mutagenicity has been repeatedly detected, indicating the need for corrective actions. The potential sources were investigated and an industrial discharge with high levels of mutagenicity was identified. As a result, the industry was notified and demanded to improve the quality of the treatment in order to eliminate the mutagenicity as well as to implement pollution prevention actions. A warning was also given to the Water Treatment Plant, in order to prevent the distribution of water with those contaminants. This is a clear case that shows that without the use of the *Salmonella* mutagenicity test, the contamination would not have been detected, and the water could have been served to the population, in compliance with the Brazilian drinking water standards.

A classification of the mutagenic potency for industrial wastes and effluents was developed by Houk [26] based on information provided by studies of pure compounds and complex mixtures. Using Houk's classification as a guide, and comparing it to the distribution of mutagenic potencies found in our study (Fig. 2) we propose the following boundaries for natural water samples: up to 500 revertants/l, *low*; from 500 to 2500, *moderate*; from 2500 to 5000, *high*, and more than 5000, *extreme* mutagenic activity. This classification is mainly based upon possible occurrence, and is valid for the dataset analyzed in this 20-year survey. It can be revised in the future, depending on the evolution of the incidence and potency of the mutagenic water samples in our environment. As the data from the São Paulo State Water Quality Monitoring Program is available to the general public, this classification is a way to make the mutagenicity results more clear and understandable.

We want to emphasize that the systematic use of this strategy will, in the near future, improve the quality of the São Paulo State drinking water, if the appropriate corrective and prevention actions are implemented. The test is affordable and can be an alternative for contamination assessment in areas with high and uncontrolled levels of organic pollutants and few analytical resources.

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