Research Articles

Comparison of the Salmonella/Microsome Microsuspension Assay with the new Microplate Fluctuation Protocol for Testing the Mutagenicity of Environmental Samples

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The objective of this study was to compare the responses of the Salmonella/microsome microsuspension assay with the new microplate fluctuation protocol (MPF) for the evaluation of the mutagenic activity of environmental samples. Organic extracts of total particulate atmospheric air samples, surface waters, and effluents were tested in dose–response experiments. The assays were performed with strain TA98 in the absence and presence of S9 mix. Both protocols produced similar results, despite the fact that the maximum score of the MPF is limited to 48 wells, whereas in the regular plate assay it is possible to count up to 1,500 colonies using an automatic counter. Similar sensitivities based on the lowest dose that resulted in a positive response were obtained for both assays. The MPF procedure is less laborious (e.g., all-liquid format, use of multichannel pipettors) and allows for automation of the pipetting and dispensing steps, thus, reducing time of the analysis which is particularly important in environmental quality monitoring programs or in effect-directed analysis. The results show that the MPF procedure is a promising tool to test environmental samples for mutagenic activity. Environ. Mol. Mutagen. 00:000–000, 2009. © 2009 Wiley-Liss, Inc.

Key words: mutagenicity; monitoring; Salmonella/microsome assay; ames MPF; microsuspension; microplate fluctuation test

INTRODUCTION

The Salmonella/microsome assay has been widely used for testing chemicals and environmental samples. A recent review of the mutagenicity of environmental samples showed that the assay is the most widely used for testing surface waters (37%) [Ohe, 2004], aquatic sediments (41%) [Chen and White, 2004], soil (38%) [White and Claxton, 2004], and atmospheric samples [Claxton et al., 2004]. In addition, the use of the assay for environmental regulatory purposes [CONSEMA, 2006], in water quality monitoring programs [Umbuzeiro et al., 2001; Arimoto-Kobayashi et al., 2007], and effect-directed analysis (EDA) [Marvin and Hewitt, 2007] are increasing. Simpler protocols and automation could provide important tools to the effective use of the Salmonella/microsome assay around the world. The microsuspension version of the Salmonella/microsome assay was developed by Kado et al. [1983] to test urine samples and has been frequently applied to test environmental samples because it requires less sample quantity when compared with the regular plate or preincubation assay.

The Ames microplate fluctuation protocol (MPF) assay kits from Xenometrix are a liquid microplate modification of the traditional Salmonella fluctuation method [Green et al., 1976; Gee at al., 1998; Flückiger-Isler et al., 2004]. The use of these kits reduces sample consumption and hands-on time, and increases the throughput as compared with the traditional plate test method. The kits contain

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ready-to-use media and performance-tested Salmonella tester strains that are phenotyped (*uvr*B, *rfa*, Δ*bio*, Ampicillin resistance) and sequenced to confirm their respective *his*$^-$ genotypes.

The aim of the present study was to compare the responses of the Salmonella/microsome microsuspension assay, which has been extensively used to test environmental samples, with the new MPF-microplate format protocol for the evaluation of the mutagenic activity of different environmental samples.

MATERIALS AND METHODS

Sampling and Sample Preparation Procedures

Two total particulate atmospheric air samples (Air 1 and Air 2) were collected in Sao Paulo city with glass fiber filters using a 24-hr high-volume sampler [Umbuzeiro et al., 2008]. Four samples of river surface water (Water 1–4) and three samples of different industrial effluents (Effluent 1–3) were collected, transported to the laboratory protected from light, and stored refrigerated for a maximum of 14 days before extraction. Effluent 1 was from a dye manufactory industry, Effluent 2, from a textile dyeing plant, and Effluent 3, from a petrochemical facility.

Atmospheric samples were extracted according to Sato et al. [1995]. Briefly, the sample filters or a clean filter (blank) were extracted three times by ultrasonication with methylene chloride. The extracts were filtered through Teflon membranes, the volume was reduced using a rotary evaporator, dried under a gentle stream of pure nitrogen gas, and resuspended in dimethylsulfoxide (DMSO) just before testing. The extractable organic matter (EOM) was obtained for each sample by gravimetric analysis.

Volumes of 10 L of the surface water samples were extracted according to Umbuzeiro et al. [2004]. Briefly, the samples were serially extracted with XAD4 resin at neutral and acidic pH using methanol and methylene chloride (1:4), and methanol and ethylacetate (1:4), respectively. Both extracts were combined, the volume reduced in a rotary evaporator, dried in a gentle stream of pure nitrogen gas, and resuspended in DMSO just before testing. A blank of the extraction procedure was performed using ultrapure water.

For the effluent samples, 1.5 L of each sample were extracted with methanol and methylene chloride in a proportion of 1:2.5 at neutral, basic and acidic pH as described by Umbuzeiro et al. [2004]. The different pH extracts were combined, the volume reduced in a rotary evaporator, dried in a gentle stream of pure nitrogen gas, and resuspended in DMSO just before testing. A blank of the extraction procedure was performed using ultrapure water.

Salmonella/Microsome Microsuspension Assay

Samples were tested in the microsuspension Salmonella/microsome assay using *Salmonella typhimurium* TA98 (*HisD*3052, *rfa*, Δ*bio*, *uvr*B, pKM101) kindly provided by Dr. Larry Claxton, from United States Environmental Protection Agency (USEPA). The assays were performed using five doses and triplicate plates/dose, both in the presence and absence of S9 using preincubation of 90 min at 37°C [Kado et al., 1983].

TABLE I. Comparison of the MPF and Microsuspension Protocols for Testing Atmospheric Sample Extracts with *S. typhimurium* Strain TA98 Without S9

	MPF protocol (–S9)				Microsuspension protocol (–S9)				
	Positive wells per microplate				Revertants per plate				
Sample	Concentration (μg EOM)	Mean	SD	FIB	Concentration (μg EOM)	Mean	SD	MR	*P*
Air 1	0	1.33	0.58		0	22.2	3.03	0	
					0.5	28.3	2.52	1.3	
	1	1.67	1.15	0.87	1	26.0	1.00	1.2	
	5	5.00	1.73	**2.62**	5	58.0	5.29	**2.6**	**
	10	17.33	4.04	**9.07**	10	113.7	4.04	**5.1**	**
	25	20.67	3.51	**10.82**	25	225.7	25.8	**10.2**	**
	50	25.67	3.06	**13.43**	50	217.0	15.0	**9.8**	**
Air 2	0	1.00	1.00		0	20.2	4.60		
					0.5	32.3	4.04	1.6	*
	1	2.67	1.53	1.33	1	50.3	7.02	**2.5**	**
	2.5	4.00	0.00	**2.00**					
	5	5.33	1.53	**2.67**	5	75.7	19.5	**3.8**	*
	10	8.67	5.69	**4.33**	10	105.7	4.51	**5.2**	**
	25	16.33	4.73	**8.17**	25	292.0	30.5	**14.5**	**
	50	23.33	2.31	**11.67**	50	664.7	87.7	**32.9**	**
Air	0	1.67	1.53		0	21.0	2.45		
blank	0.1	0.67	0.58	0.21	0.1	17.0	0.00	0.8	
	0.5	1.33	1.53	0.42	0.5	19.0	5.66	0.9	
	1	1.00	1.00	0.31	1	21.5	6.36	1.0	
	2.5	0.67	0.58	0.21	2.5	17.5	7.78	0.8	
	5	2.00	1.73	0.63	5	20.5	2.12	1.0	
	10	1.33	1.53	0.42	10	20.5	2.12	1.0	

Values in bold indicates FIB greater than 2.

*$P < 0.05$, **$P < 0.01$.

EOM = extractable organic material; FIB = fold induction over baseline (baseline = mean zero-dose control + 1 SD); SD = standard deviation; MR= mutagenic ratio.

TABLE II. Comparison of the MPF and Microsuspension Protocols for Testing Atmospheric Sample Extracts with *S. typhimurium* Strain TA98 With S9

	MPF protocol (+S9)				Microsuspension protocol (+S9)				
	Positive wells per microplate				Revertants per plate				
Sample	Concentration (μg EOM)	Mean	SD	FIB	Concentration (μg EOM)	Mean	SD	MR	*P*
Air 1	0	1.00	1.00		0	30.3	4.35		
					0.5	26.3	5.13	0.9	
	1	1.00	1.00	0.50	1	32.0	4.58	1.0	
	5	1.67	2.08	0.83	5	35.7	6.11	1.2	
	10	9.67	1.15	**4.83**	10	44.3	14.3	1.5	
	25	16.67	2.52	**8.33**	25	46.3	5.51	1.5	*
	50	21.33	2.52	**10.67**	50	210.7	49.2	**7.0**	**
Air 2	0	2.27	1.53		0	23.6	4.93		
					0.5	29.3	2.31	1.2	
	1	2.67	0.58	1.33	1	27.7	2.89	1.2	
	2.5	4.33	1.15	**2.17**					
	5	6.67	0.58	**3.33**	5	26.0	1.00	1.1	
	10	13.33	3.51	**6.67**	10	31.0	1.00	1.3	
	25	26.33	3.21	**13.17**	25	48.3	6.35	**2.1**	*
	50	35.33	4.04	**17.67**	50	41.0	8.50	**1.7**	**
Air blank	0	1.33	1.15		0	26.6	5.66		
	0.1	2.00	1.00	0.80	0.1	27.0	6.36	1.0	
	0.5	1.00	1.00	0.40	0.5	25.5	4.24	1.0	
	1	3.00	1.00	1.21	1.0	27.0	0.71	1.0	
	2.5	1.00	1.00	0.40	2.5	20.5	0.71	0.8	
	5	1.67	0.58	0.67	5	17.5	10.6	0.7	
	10	2.33	1.15	0.94	10	25.5	8.50	1.0	

Values in bold indicates FIB greater than 2.
$^{*}P < 0.05$, $^{**}P < 0.01$.
EOM = extractable organic material; FIB = fold induction over baseline (baseline = mean zero-dose control + 1 SD); SD = standard deviation; MR= mutagenic ratio.

The dose–response experiments were performed with maximum doses of 50 μg of EOM for air, 50 mL equivalent for surface water, and 5 mL equivalent for effluent samples. The S9 mix was freshly prepared according to Maron and Ames [1983] before each test using lyophilized Aroclor-1254-induced rat liver S9 fraction (Moltox, Boone, NC), resulting in 4% v/v of S9 fraction in the mixture. Colonies were counted using an automatic counter (AccuCount, Biologics, Manassas, VA). The results were analyzed with the Salanal program kindly provided by Dr John Mayers from Research Triangle Institute, Research Triangle Park, NC.

Toxicity was evaluated by careful inspection of the background using a stereomicroscope (10 × magnification). A sample was considered positive when there was a significant positive dose response, a significant statistical difference between the tested doses and the negative control (ANOVA), and the mutagenic ratio was >2. Mutagenic ratio was calculated by dividing the mean of the revertants obtained in each tested dose by the concurrent negative control.

The positive controls were 4-nitroquinoline-1-oxide (Sigma) at 0.125 μg per plate without metabolic activation and 2-aminoanthracene (Sigma) at 5 μg/plate with S9. DMSO was used as negative control.

Ames MPF Assay

The Ames MPF assay was performed in liquid media in 24-well plates during sample exposure and in 384-well plates for revertant growth and for scoring. Growth, Exposure and Indicator Media, as well as *S. typhimurium* strain TA98, were included in the kit from Xenometrix AG, Allschwil, Switzerland. The test procedure described in the 'Ames MPF Instructions for Use' was followed.

Briefly, bacteria were grown overnight, diluted in Exposure Medium and exposed to test samples in 24-microwell plates for 90 min at 37°C with agitation in the presence or absence of 4.5% Aroclor 1254-induced rat liver S9 (Moltox). The exposed cultures were then diluted in Indicator Medium and the contents of each 24-well culture were distributed into 48 wells of a 384-well plate (50 μL per well). The Indicator Medium contains a pH indicator dye which changes from purple to yellow on bacterial growth. After 48-hr incubation at 37°C, the plates were scored by eye for yellow wells. Positive and negative controls were included as for the microsuspension assay, and all doses were done in triplicate. Note that the Ames MPF (microplate format) limits the number of positive wells to a maximum of 48 wells per sample.

The criteria used to evaluate the MPF results were the fold increase in number of positive wells over the solvent control baseline (FIB), and the dose dependency. The fold increase of revertants relative to the solvent control was determined by dividing the mean number of positive wells at each dose by the solvent control baseline. The solvent control baseline was defined as the mean number of positive wells in the solvent control plus 1 SD. All solvent controls from an experiment with identical conditions (same day, same bacterial culture, solvent and incubation conditions) were combined.

An increase of >2-fold relative to the baseline was classified as positive for that dose. Positive responses of >2-fold relative to the baseline at more than one dose with a dose–response led to the test sample being classified as positive. A test sample was classified as negative where no response >2 times the baseline and no dose–response was observed.

Positive controls used for the MPF protocol were 2-nitrofluorene (Sigma) at 2 μg/mL without metabolic activation and 2-aminoanthracene (Sigma) at 5 μg/mL with S9. DMSO was used as the negative control.

TABLE III. Comparison of the MPF and Microsuspension Protocols for Testing Water Sample Extracts with *S. typhimurium* Strain TA98 Without S9

	MPF protocol (–S9)				Microsuspension protocol (–S9)				
	Positive wells per microplate				Revertants per plate				
Sample	Concentration (mL equiv.)	Mean	SD	FIB	Concentration (mL equiv.)	Mean	SD	MR	*P*
Water 1	0	0.92	0.67		0	23.2	3.11		
	1	0.33	0.58	0.21					
	5	4.00	2.00	**2.52**	5	23.7	2.52	1.0	
	10	2.33	1.15	1.47	10	24.7	2.31	1.1	
	25	2.67	2.08	1.68	25	28.0	1.73	1.2	
	50	3.33	1.53	**2.10**	50	33.3	4.04	1.4	*
Water 2	0	0.92	0.67		0	37.4	6.58		
	1	2.00	2.00	1.26					
	5	2.33	1.53	1.47	5	42.7	4.16	1.1	
	10	4.00	1.73	**2.52**	10	53.3	1.53	1.4	*
	25	5.67	4.73	**3.57**	25	82.0	7.00	**2.2**	**
	50	9.00	5.29	**5.68**	50	53.7	6.03	1.4	
Water 3	0	0.92	0.67		0	22.8	3.27		
	1	2.00	1.00	1.26					
	5	1.67	1.15	1.05	5	24.3	0.58	1.1	
	10	3.00	0.00	1.89	10	23.3	1.53	1.0	
	25	4.33	1.15	**2.73**	25	46.7	5.03	**2.1**	**
	50	7.00	2.65	**4.42**	50	50.7	6.11	**2.2**	**
Water 4	0	1.00	1.00		0	28.0	2.65		
	1	0.33	0.58	0.17					
	5	1.33	0.58	0.67	5	28.7	3.21	1.0	
	10	2.33	2.52	1.17	10	26.3	5.69	0.9	
	25	3.33	0.58	1.67	25	35.3	4.62	1.3	
	50	4.33	2.31	**2.17**	50	43.3	6.11	1.6	*
Water	0	1.00	1.00		0	28.0	2.65		
blank	1	0.67	0.58	0.33					
	5	1.33	0.58	0.67	5	28.6	4.16	1.0	
	10	4.33	1.53	2.17	10	27.0	6.08	1.0	
	25	2.33	2.08	1.17	25	23.0	3.61	0.8	
	50	5.00	0.00	**2.50**	50	27.0	6.00	1.0	

Values in bold indicates FIB greater than 2.
*$P < 0.05$, **$P < 0.01$.
EOM = extractable organic material; FIB = fold induction over baseline (baseline = mean zero-dose control + 1 SD); SD = standard deviation; MR= mutagenic ratio.

Calculation of Potencies

The potencies (slopes) for both procedures were expressed as the number of revertants per unit, depending on the sample tested; atmospheric samples were expressed as revertants per mg of EOM and liquid samples (surface water and effluents) as revertants per milliliter equivalent. For the microsuspension assay, the slopes were calculated from the revertants per plate using the Bernstein et al. [1982] model. For the MPF assay, the slope of the linear part of the dose–response curve from the number of positive wells was calculated using the linear regression function of Microsoft Excel. The slopes of each assay were log 10-transformed and compared using the same Microsoft Excel function.

RESULTS AND DISCUSSION

The negative control values (DMSO) obtained for both assays were within the expected ranges (Tables I–VI) with one exception, the negative control of the Ames MPF in Table V, which provided an unexpectedly high spontaneous rate. All the positive controls provided the expected responses (data not shown).

Air 1 and Air 2 were clearly mutagenic in the absence and presence of metabolic activation (Tables I and II). A comparison of the lowest positive dose obtained in each test is presented in Table VII. In the absence of S9, the lowest positive dose of Air 1 was identical (5 μg of EOM) in the microsuspension and MPF assays. For Air 2, the lowest positive dose was 1 μg of EOM in the microsuspension assay and 2.5 μg of EOM in the MPF assay. Sorensen et al. [1982] compared the mutagenicity results of air atmospheric samples tested without S9 in the standard plate incorporation Salmonella/microsome assay and the fluctuation test. They also observed a slight advantage in sensitivity for the standard Salmonella assay in the absence of S9. In the presence of S9, the MPF assay was

TABLE IV. Comparison of the MPF and Microsuspension Protocols for Testing Water Sample Extracts with *S. typhimurium* Strain TA98 With S9

	MPF protocol (+S9)				Microsuspension protocol (+S9)				
	Positive wells per microplate				Revertants per plate				
Sample	Concentration (mL equiv.)	Mean	SD	FIB	Concentration (mL equiv.)	Mean	SD	MR	*P*
Water 1	0	1.58	1.16		0	28.0	3.46		
	1	1.00	1.00	0.36					
	5	2.67	1.53	0.97	5	22.7	3.06	0.8	
	10	1.67	2.89	0.61	10	24.3	5.13	0.9	
	25	3.33	1.15	1.21	25	29.7	9.50	1.1	
	50	4.67	1.15	1.70	50	32.0	3.00	1.1	
Water 2	0	1.58	1.16		0	33.0	6.07		
	1	1.00	1.00	0.36					
	5	2.67	0.58	0.97	5	48.7	6.03	1.5	
	10	2.33	1.15	0.85	10	48.3	3.79	1.5	*
	25	6.00	1.00	**2.18**	25	69.3	3.06	**2.1**	**
	50	6.00	1.73	**2.18**	50	72.0	14.11	**5.1**	*
Water 3	0	1.58	1.16		0	28.6	6.80		
	1	4.00	1.00	1.46					
	5	7.33	1.53	**2.67**	5	29.0	7.55	1.0	
	10	17.00	3.61	**6.19**	10	56.0	4.58	**2.0**	**
	25	18.67	1.15	**6.79**	25	83.7	5.86	**2.9**	**
	50	13.00	3.00	**4.73**	50	61.7	6.11	**2.2**	**
Water 4	0	2.27	1.53		0	26.0	6.06		
	1	1.67	1.53	0.44					
	5	2.00	0.00	0.53	5	27.0	4.00	1.0	
	10	4.00	1.00	1.05	10	32.7	7.57	1.3	
	25	3.00	1.00	0.79	25	34.7	6.11	1.3	
	50	10.00	1.00	**2.63**	50	41.7	4.73	1.6	*
Water	0	2.27	1.53		0	26.0	6.06		
Blank	1	3.00	3.00	0.79					
	5	2.67	3.06	0.70	5	25.3	4.73	1.0	
	10	2.67	0.58	0.70	10	24.7	4.51	1.0	
	25	4.00	1.00	1.05	25	22.7	1.15	0.9	
	50	2.00	1.73	0.53	50	26.0	7.07	1.0	

Values in bold indicates FIB greater than 2.
*$P < 0.05$, **$P < 0.01$.
EOM = extractable organic material; FIB = fold induction over baseline (baseline = mean zero-dose control + 1 SD); SD = standard deviation; MR= mutagenic ratio.

more sensitive for the air samples when compared in terms of the lowest dose per plate that produced a positive response (Tables II and VII). Negative results were obtained with the blank filters using both assays (Tables I and II).

Water 1 without S9 seemed to show a weak positive response in the MPF assay, but it did not fulfill the criteria for a clear positive response: although there were two doses with a >2-fold induction over the baseline, a dose-dependent response was not observed (Table III). Such weak positive result can occur when very low spontaneous revertant levels occur. This illustrates the importance of requiring both a minimum of a >2-fold induction and a clear dose–response. Water 1 did not show >2-fold induction with metabolic activation at any dose. Therefore, this sample should be considered negative in the MPF assay (Table VII). This sample was clearly negative with and without S9 in the microsuspension assay (Tables III and IV).

Water 2 and Water 3 were positive with and without metabolic activation in both the MPF and the microsuspension assays. In both assays, Water 2 with S9 and Water 3 without S9 showed the same sensitivity in terms of the lowest dose that provided a positive response (Table VII). The MPF assay was more sensitive with Water 2 in the absence of S9 (10 vs. 25 mL equivalent) and with Water 3 with S9 (5 vs. 10 mL equivalent).

In the MPF assay, Water 4 showed a >2-fold increase over the baseline only at the highest concentration tested (Tables III and IV). Because the blank controls showed a similar response, Water 4 is likely to be negative and would need retesting at higher doses for confirmation of

TABLE V. Comparison of the MPF and Microsuspension Protocols for Testing Effluent Sample Extracts with *S. typhimurium* Strain TA98 Without S9

	MPF protocol (–S9)				Microsuspension protocol (–S9)				
	Positive wells per microplate				Revertants per plate				
Sample	Concentration (mL equiv.)	Mean	SD	FIB	Concentration (mL equiv.)	Mean	SD	MR	*P*
Effluent 1	0	9.17	4.43		0	22.7	3.10		
	0.05	17.33	4.93	1.28	0.05				
	0.1	17.33	4.04	1.28	0.1	38.0	3.61	1.7	*
	0.5	29.00	2.65	**2.13**	0.5	64.7	11.15	**2.8**	**
	1	37.33	3.06	**2.75**	1	98.7	2.52	**4.3**	**
	2.5	45.00	1.00	**3.31**	2.5	234.3	32.64	**10.3**	**
	5	48.00	0.00	**3.53**	5	339.0	32.51	**14.9**	**
Effluent 2	0	9.17	4.43		0	21.0	1.10		
	0.05	9.00	1.73	0.66	0.05	30.0	4.36	1.4	
	0.1	16.33	4.62	1.20	0.1	37.7	7.02	1.7	*
	0.5	41.00	1.73	**3.02**	0.5	107.0	3.61	**4.9**	**
	1	46.67	1.53	**3.43**	1	167.0	21.07	**7.7**	**
	2.5	47.67	0.58	**3.51**	2.5	540.3	68.25	**24.8**	**
	5	48.00	0.00	**3.53**	5	1292	119.15	**59.3**	**
Effluent 3	0	9.17	4.43		0	22.7	3.10		
	0.05	12.33	9.02	0.91	0.05				
	0.1	14.67	3.06	1.08	0.1	26.7	3.51	1.2	
	0.5	20.33	3.79	1.50	0.5	23.3	0.58	1.0	
	1	18.33	2.08	1.35	1	29.0	4.36	1.3	
	2.5	20.00	6.00	1.47	2.5	51.3	9.61	**2.3**	*
	5	26.33	2.52	1.94	5	87.0	11.14	**3.8**	**
Effluent	0	9.17	4.43		0	26.6	2.41		
blank	0.05	13.00	5.57	0.96	0.05	22.3	0.58	0.8	
	0.1	14.00	4.36	1.03	0.1				
	0.5	6.00	5.29	0.44	0.5	22.3	3.21	0.8	
	1	6.00	4.58	0.44	1				
	2.5	12.00	7.00	0.88	2.5	26.5	0.71	1.0	
	5	9.67	1.15	0.71	5	23.7	2.52	0.9	

Values in bold indicates FIB greater than 2.

*$P < 0.05$, **$P < 0.01$.

EOM = extractable organic material; FIB = Fold Induction over Baseline (baseline = mean zero-dose control + 1 SD); SD = standard deviation; MR= mutagenic ratio.

the results. Water 4 was judged negative in the microsuspension assay, although a significant ANOVA value was obtained for the highest dose tested.

Effluent 1 and Effluent 2 were clearly mutagenic in the MPF assay, both with and without metabolic activation (Tables V and VI). The lowest positive dose was 0.5 mL equivalent (Table VII). Effluent 3 did not fulfill the criteria for mutagenicity in the absence of S9 due to an unusually high spontaneous rate in this experiment. The results suggest a possible weak mutagenic activity but it would need to be confirmed. In the presence of S9, Effluent 3 was clearly mutagenic at doses >1 mL equivalent (Tables VI and VII). The responses in the microsuspension assay were very similar to those of the MPF assay: all effluents were positive including Effluent 3, which was clearly positive also in the absence of S9 (Tables V). Very similar lowest positive doses were obtained for the effluent samples in both assays (Table VII). The blank effluent control showed a clear negative response in both assays.

Potencies for all samples were calculated. The quantification of the mutagenic response (slopes of the linear part of the dose–response) is required for environmental sample testing, especially in monitoring programs, or EDA studies, where it is important to understand how the samples vary over time or within the fractions, respectively.

To compare the mutagenic potencies obtained in both assays a regression analysis was performed after potencies were log (10) transformed. A good correlation coefficient (0.84) was obtained (Fig. 1). The calculated linear equation ($y = 0.8386x - 0.1439$) allows an estimation of the potency in both assays. The potency values for the microsuspension assay were approximately 10-fold higher than in the MPF assay. This is a numerical difference that is related to the counts per plate that occur in each assay

TABLE VI. Comparison of the MPF and Microsuspension Protocols for Testing Effluent Sample Extracts with *S. typhimurium* Strain TA98 With S9

	MPF protocol (+S9) Positive wells per microplate				Microsuspension protocol (+S9) Revertants per plate				
Sample	Concentration (mL equiv.)	Mean	SD	FIB	Concentration (mL equiv.)	Mean	SD	MR	*P*
Effluent 1	0	6.42	2.11		0	25.8	4.92		
	0.05	8.00	3.46	0.94	0.05				
	0.1	7.67	2.08	0.90	0.1	28.3	2.52	1.1	
	0.5	19.67	4.04	**2.31**	0.5	38.0	4.00	1.5	
	1	28.67	2.31	**3.36**	1	53.0	7.94	**2.1**	*
	2.5	44.33	2.08	**5.20**	2.5	120.7	4.04	**4.7**	**
	5	47.67	0.58	**5.59**	5	187.7	4.16	**7.3**	**
Effluent 2	0	6.42	2.11		0	24.2	4.92		
	0.05	8.00	3.46	0.94	0.05	22.7	1.15	0.9	
	0.1	10.00	1.00	1.17	0.1	30.0	4.58	1.2	
	0.5	26.00	2.65	**3.05**	0.5	48.0	4.51	**2.0**	**
	1	34.33	2.52	**4.03**	1	65.0	2.65	**2.7**	**
	2.5	47.67	0.58	**5.59**	2.5	186.5	40.31	**7.7**	**
	5	48.00	0.00	**5.63**	5	457.7	29.14	**18.9**	**
Effluent 3	0	6.42	2.11		0	25.8	4.92		
	0.05	10.67	1.53	1.25	0.05				
	0.1	11.00	4.36	1.29	0.1	27.7	2.31	1.1	
	0.5	15.00	4.36	1.76	0.5	29.0	0.00	1.1	
	1	29.33	3.79	**3.44**	1	34.5	0.71	1.3	
	2.5	46.33	1.53	**5.43**	2.5	65.7	10.69	**2.5**	**
	5	48.00	0.00	**5.63**	5	126.7	7.23	**4.9**	**
Effluent blank	0	6.42	2.11		0	24.6	2.79		
	0.05	4.67	2.08	0.55	0.05	28.0	2.00	1.1	
	0.1	7.00	2.65	0.82	0.1				
	0.5	5.67	3.21	0.66	0.5	26.7	4.51	1.1	
	1	4.67	2.89	0.55	1				
	2.5	5.67	1.53	0.66	2.5	27.5	7.78	1.1	
	5	9.33	3.06	1.09	5	22.7	1.53	0.9	

Values in bold indicates FIB greater than 2.
*$P < 0.05$, **$P < 0.01$.
EOM = extractable organic material; FIB = fold induction over baseline (baseline = mean zero-dose control + 1 SD); SD = standard deviation; MR= mutagenic ratio.

TABLE VII. Lowest Dose Per Plate that Provided a Positive Response in Each Assay for the Tested Samples

	TA98-S9		TA98+S9	
Samples	MPF	Microsuspension	MPF	Microsuspension
Air 1	5	5	10	25
Air 2	2.5	1	2,5	25
Water 1	Negative	Negative	Negative	Negative
Water 2	10	25	25	25
Water 3	25	25	5	10
Water 4	Negative	Negative	Negative	Negative
Effluent 1	0.5	0.5	0.5	1
Effluent 2	0.5	0.5	0.5	0.5
Effluent 3	Negative[a]	2.5	1	2.5

For the air samples, the dose is expressed in μg of EOM per plate and for the liquid samples (water and effluent) in mL equivalent per plate.
[a]Sample was not classified as positive based on our evaluation criteria because it exhibited an elevated baseline.

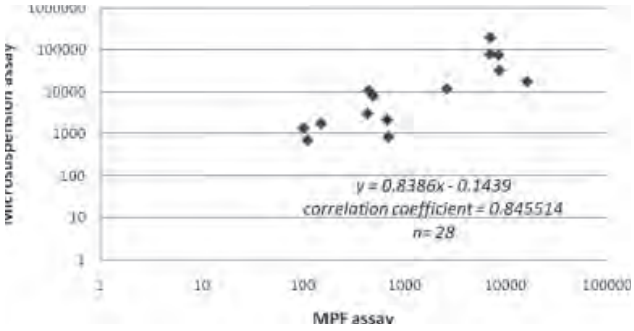

Fig. 1. Correlation of the potencies expressed in log of number of revertants per μg of EOM or mL equivalent obtained in the MPF assay and in the microsuspension Salmonella/microsome assay for the samples tested using TA98 with and without S9.

but is not related to the assay sensitivity (Table VII). The microsuspension assay counts vary from 20 to 1,500 colonies per plate, and in the MPF assay counts vary between 0 and 48 positive wells per plate. The equation shown in Figure 1 can be used to compare a result obtained with the MPF with historical results of the microsuspension assay.

CONCLUSIONS

The results from the Ames MPF and the microsuspension assays were in agreement with respect to their identification of environmental samples as positive, both in the absence or presence of metabolic activation. In the absence of S9, the Ames MPF was slightly less sensitive than the microsuspension assay with respect to the lowest mutagenic sample concentration. Conversely, in the presence of S9, the Ames MPF assay was slightly more sensitive.

The mutagenic potencies, i.e., revertants per sample unit, obtained for this set of samples correlated well when tested in both assays. Because the Ames MPF assay is easier to perform (e.g., all-liquid format, use of multichannel pipettors) and allows for automation of the pipetting and dispensing steps, it seems to be an interesting and valid alternative to the microsuspension assay especially when a large number of samples have to be tested, such as in monitoring programs and EDA studies.

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