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Research Article

The influence of organic solvents on estimates of genotoxicity and antigenotoxicity in the SOS chromotest

Nathalia Quintero[1], Elena E. Stashenko[2] and Jorge Luis Fuentes[1,2]

[1]*Laboratorio de Microbiología y Mutagénesis Ambiental, Escuela de Biología, Facultad de Ciencias, Universidad Industrial de Santander, Bucaramanga, Colombia.*
[2]*Centro de Investigación en Biomoléculas, Centro de Investigación de Excelencia, Universidad Industrial de Santander, Bucaramanga, Colombia.*

Abstract

In this work, the toxicity and genotoxicity of organic solvents (acetone, carbon tetrachloride, dichloromethane, dimethylsulfoxide, ethanol, ether and methanol) were studied using the SOS chromotest. The influence of these solvents on the direct genotoxicity induced by the mutagens mitomycin C (MMC) and 4-nitroquinoline-1-oxide (4-NQO) were also investigated. None of the solvents were genotoxic in *Escherichia coli* PQ37. However, based on the inhibition of protein synthesis assessed by constitutive alkaline phosphatase activity, some solvents (carbon tetrachloride, dimethylsulfoxide, ethanol and ether) were toxic and incompatible with the SOS chromotest. Solvents that were neither toxic nor genotoxic to *E. coli* (acetone, dichloromethane and methanol) significantly reduced the genotoxicity of MMC and 4-NQO. When these solvents were used to dissolve vitamin E they increased the antigenotoxic activity of this compound, possibly through additive or synergistic effects. The relevance of these results is discussed in relation to antigenotoxic studies. These data indicate the need for careful selection of an appropriate diluent for the SOS chromotest since some solvents can modulate genotoxicity and antigenotoxicity.

Key words: antigenotoxicity, genotoxicity, interference, mitomycin C, 4-nitro-quinoline-1-oxide, SOS chromotest, solvents, vitamin E.

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Introduction

Antimutagens are agents that reduce the number of spontaneous and/or induced mutations in cells. These agents are classified based on their mechanism of action (Wattenberg, 1985). A practical classification is one that separates antimutagens into des-mutagens and bio-antimutagens (Kada and Shimoi, 1987), with des-mutagens being compounds that inactivate mutagens before they can attack the DNA molecule whereas bio-antimutagens interfere with cellular processes that repair DNA damage, *e.g.*, DNA replication and/or repair mechanisms (Kuroda and Inoue, 1988).

Antimutagens may help to prevent mutations involved in cancer and aging by regulating cellular mutability. Consequently, antimutagens can be classified into several categories and subcategories, depending on the stage in which they interfere with mutagenesis and carcinogenesis and on how they modulate host defense mechanisms; these points of interference allow the rational implementation of chemoprevention strategies for controlling cancer and degenerative diseases (De Flora, 1998; De Flora and Ramel, 1988; De Flora and Ferguson, 2005).

Send correspondence to Jorge Luis Fuentes. Laboratorio de Microbiología y Mutagénesis Ambiental, Escuela de Biología, Facultad de Ciencias, Universidad Industrial de Santander, A.A. 678, Bucaramanga, Colombia. E-mail: jfuentes@uis.edu.co, jlfuentes2005@yahoo.es.

The assessment of natural antimutagens has involved mainly the use of short-term bacterial assays because their sensitivity, simplicity and flexibility to different experimental settings facilitates the study of antimutagenesis mechanisms (De Flora *et al.*, 1992a,b). Among the *in vitro* assays used in antimutagen studies, the SOS chromotest (Quillardet *et al.*, 1982) is one of the most widely used. This assay, which is based on a colorimetric assessment of SOS transcriptional gene fusion in *Escherichia coli* PQ37, allows one to estimate primary DNA damage produced by chemical and physical mutagens by measuring the expression of a reporter gene, β-galactosidase (Quillardet and Hofnung, 1993). Compared to other methods, the SOS chromotest allows greater throughput when screening natural compounds (Fuentes *et al.*, 2006a).

The use of *in vitro* assays to study antimutagens/antigenotoxins can be limited by the toxicity, *e.g.*, cytotoxic and cytostatic effects and inhibition of macromolecule synthesis, of the compounds being investigated (Zeiger, 2007). Secondary interactions between the solvent used to dissolve the test compounds or extracts and the mutagen may result in antagonistic effects in the cells, depending on the compounds or mixtures involved (Donnelly *et al.*, 1998).

For example, dimethylsulfoxide (DMSO, a commonly used solvent for many test compounds) reduces the mutagenicity of benzo[a]pyrene, cisplatin derivatives, dimethylnitrosamine, dimethylcarbamylchlorides and diethyl-carbamylchlorides in the *Salmonella*/microsome reversion assay (Yahagi *et al.*, 1977; Hermann *et al.*, 1978; Maron *et al.*, 1981; Uno and Morita, 1993), attenuates the genotoxicity of 2-aminoanthracene and 2-nitrofluorene in the microscreen prophage-induction assay (DeMarini *et al.*, 1991) and attenuates cisplatin genotoxicity in the SOS chromotest (Gebel and Koenig, 1999). Other solvents, such as acetone and ethanol, also reduce benzo[a]pyrene mutagenicity in the *Salmonella*/microsome reversion assay (Maron *et al.*, 1981) whereas methanol inhibits 2-aminoanthracene and 2-nitrofluorene genotoxicity in the microscreen prophage-induction assay (DeMarini *et al.*, 1991). Since the inhibition of toxicity and genotoxicity by solvents can lead to overestimates of antigenotoxicity it is essential to determine which solvents are appropriate for screening antigenotoxins with the SOS chromotest.

We have previously shown that the antigenotoxicity of certain compounds is influenced by the solvent used to dissolve essential oils (Vicuña *et al.*, 2010; López *et al.*, 2011). We have observed that the use of DMSO leads to overestimation of the antigenotoxicity of essential oils when compared with results obtained using distilled water as the diluent (unpublished data). In the present study, we used a series of approaches to examine the extent to which organic solvents can affect estimates of antigenotoxicity in the SOS chromotest. First, we examined the direct toxicity and genotoxicity of several solvents (acetone, carbon tetrachloride, dichloromethane, DMSO, ether, ethanol, and methanol) in *E. coli* PQ-37. Second, solvents that were neither toxic nor genotoxic to *E. coli* (acetone, dichloromethane and methanol) were assayed for their ability to interfere with the genotoxic effect of the directly acting mutagens mitomycin C (MMC) and 4-nitro-quinoline-1-oxide (4-NQO). MMC is an alkylating agent that produces adducts and strand crosslinking in DNA (Tomasz and Palom, 1997) while 4-NQO produces mainly guanine adducts (Fronza *et al.*, 1994). Both mutagens are powerful inducers of the SOS response in *E. coli* (Quillardet and Hofnung, 1993). Third, solvent concentrations that did or did not interfere (non-interfering) with direct genotoxic activities were assayed to assess whether they increased the antigenotoxicity of α-tocopherol (vitamin E). Vitamin E was used because this compound protects against oxidative mutagenesis (Odin, 1997), the primary mechanism by which MMC and 4-NQO damage DNA.

Materials and Methods

Chemicals

Luria-Bertani (LB) medium, antibiotics (ampicillin, MMC and tetracycline), vitamin E and 4-NQO were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). The substrates for β-galactosidase (*o*-nitrophenyl-β-d-galactopyranoside or ONPG) and alkaline phosphatase (*p*-nitrophenylphosphate or PNPP) were purchased from Merck (Darmstadt, Germany). The solvents (acetone, carbon tetrachloride, dichloromethane, DMSO, ether, ethanol and methanol) and the other chemicals were obtained from different commercial suppliers (Table 1).

Bacterial strain and culture

Escherichia coli strain PQ37 [F^- *thr leu his-4 pyrD thi galE galK* or *galT lacΔU169 srl300::Tn10 rpoB rpsL uvrA rfa trp::Muc*$^+$ *sfiA::Mud(Ap,lac)ts*] which has been recommended for the detection of genotoxic carcinogens (Quillardet *et al.*, 1982) was used in this work. This strain carries the *sulA::lacZ* fusion gene as a reporter gene for primary DNA damage induced during the SOS response. The cells were grown overnight at 37 °C and shaken at 100 rpm in LB medium (10 g tryptone/L, 5 g yeast extract/L, 10 g sodium chloride/L, pH 7.4) supplemented with ampicillin (50 μg/mL) and tetracycline (17 μg/mL).

Genotoxicity assay

Genotoxicity assays were done using the SOS chromotest as described by Quillardet *et al.* (1982), with small modifications. Briefly, overnight cultures were grown in fresh LB medium to an optical density (OD_{600nm}) of 0.4, diluted 10-fold in double strength LB medium (20 g tryptone/L, 10 g yeast extract/L, 20 g sodium chloride/L, pH 7.4) and mixed (v/v) with the test substances of interest (mutagens and solvents). A negative control (distilled water) was always included in each assay. The bacteria were exposed to different concentration ranges, depending on the test substance, for 30 min at 8 °C and then cultured for 2 h at 37 °C.

β-Galactosidase and alkaline phosphatase were assayed in 96-well plates (Brand GMBH, Germany). For β-galactosidase activity, cell membranes were disrupted by mixing 142 μL of Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM Mg_2SO_4, 0.1% SDS and 40 mM β-mercaptoethanol, pH 7.0) with 15 μL of cell culture for 20 min at room temperature. The reaction was started by adding 30 μL of ONPG (4 mg/mL in T buffer: 1 M Tris-HCl, pH 8.8). After 40 min, the enzymatic reaction was stopped by adding 50 μL of 1 M Na_2CO_3. After five min, 50 μL of 2 M Tris was added to restore the color. For alkaline phosphatase activity, cell membranes were disrupted by adding 130 μL of T buffer, 7 μL of chloroform and 5 μL of 0.1% sodium dodecyl sulfate (SDS) solution to 15 μL of cell culture followed by mixing vigorously for 20 min at room temperature. The enzyme reaction was started by adding 30 μL of PNPP solution (4 mg/mL in T buffer). After 40 min, the reaction was stopped by adding 50 μL of 2 M HCl. After 5 min, 50 μL of 2 M Tris was

Table 1 - Data for the solvents assayed in this study.

Solvent	Molecular structure	Commercial supplier	Reference	Molecular mass	Purity (%)	Density (g/mL)	Molarity (M)	Polarity	Water solubility
Acetone		Merck	1.00014.4000	58.08	99.8	0.79	13.56	Polar	Miscible
Carbon tetrachloride		Merck	1.02222.2500	153.82	99.8	1.59	10.32	Apolar	0.1 g/100 mL
Dichloromethane		J.T. Baker	9324-03	84.93	99.5	1.32	15.44	Apolar	1.3 g/100 mL, 20 °C
Dimethylsulfoxide		Sigma-Aldrich	D5879-1L	78.13	99.5	1.10	14.01	Polar	Miscible
Ethanol		J.T. Baker	9014-03	46.07	99.9	0.79	17.11	Polar	Miscible
Ether		J.T. Baker	9240-03	74.12	99.9	0.71	9.61	Apolar	1.5 g/100 mL, 25 °C
Methanol		J.T. Baker	9070-03C	32.04	98.8	0.80	24.67	Polar	Totally miscible

added to restore the color. The final absorbances of the β-galactosidase and alkaline phosphatase assays were measured at 420 nm using an iMark microplate reader (BioRad, Hercules, CA, USA). β-Galactosidase and alkaline phosphatase activities were calculated using the relationship: enzyme units = (1000 x A_{420})/t (Quillardet and Hofnung, 1985), where A_{420} is the optical density at 420 nm and t is the length of incubation (min) with substrate (ONPG or PNPP). The ratio of β-galactosidase units to alkaline phosphatase units (R = (β - galactosidase units)/alkaline phosphatase units) reflects the induction of the *sulA* gene even when there is some inhibition of protein synthesis.

The genotoxicity criterion used was the SOS induction factor (I) that represents the normalized induction data of the *sulA* gene in each treatment (mutagen, solvent, etc.) and was therefore considered to be an indirect measure of the primary DNA damage (genotoxicity) induced by the treatments. This parameter was calculated as: $I = R_t/R_{nt}$, where t and nt are the treated and non-treated cells, respectively. A substance was classified as not genotoxic if I was < 1.5, inconclusive if I was between 1.5 and 2.0, and genotoxic if I was > 2.0 and a clear concentration-response relationship was observed.

Antigenotoxicity assay

Antigenotoxicity was assayed using a co-incubation procedure described by Fuentes *et al.* (2006b). The antigenotoxicity assay was done essentially as the genotoxicity protocol described above, except that the cells were co-treated with different concentrations of the test substances (solvents, vitamin E or both) and mutagens (0.187 μM MMC or 2.34 μM 4-NQO), simultaneously. Co-treatments with solvent and mutagens allowed us to determine the solvent concentrations that interfered with MMC and 4-NQO activities. To determine whether the solvents also affected the antigenotoxicity of vitamin E, a stock solution (8 mM) of this substance was prepared as indicated by Aiub *et al.* (2009). For the SOS chromotest, vitamin E (0.02-2 mM; antigenotoxic concentration range) was prepared by dissolving the compound in compatible solvents (acetone, dichloromethane and methanol) at a final solvent concentration that did or did not interfere with the genotoxic activities of MMC and 4NQO. The interfering solvent concentration in co-treatments was always 25% while the non-interfering solvent concentration varied as follows: MMC/acetone = 0.78%, 4-NQO/acetone = 1.56%, MMC/dichloromethane = 3.12%, 4-NQO/dichloromethane = 6.25%, MMC/methanol = 3.12% and 4-NQO/methanol = 3.12%. These non-interfering solvent concentrations were selected based on their insignificant effect in the solvent/mutagen co-treatments. A control assay in which vitamin E was dissolved in distilled water was included in all experiments for comparison.

Antigenotoxicity (the ability of the test substance to protect DNA against damage) was measured as a signifi-

cant reduction in the induction factor (*I*) in the combined treatments (substance + mutagens) and expressed as a percentage of the genotoxicity inhibition (%*GI*):

$$\%GI = 1 - \frac{I_{CO} - I_{BASAL}}{I_{MUT} - I_{BASAL}} \times 100$$

where I_{CO} is the SOS induction factor in co-treated cells (substance + mutagen), I_{BASAL} is the basal SOS induction factor and I_{MUT} is the SOS induction factor in mutagen-treated cells. Negative values of %*GI* were considered as zero, which meant that this parameter ranged from a minimum of 0% to a maximum of 100%.

Statistical analysis

The β-galactosidase and alkaline phosphatase activities and the induction factor (*I*) values were expressed as the mean±SEM. The data normality was tested using the Kolmogorov-Smirnov test and the homogeneity of variances was assessed by analysis of variance (ANOVA). Statistical comparisons were done using Student's *t*-test. Product-moment (Pearson) correlation analysis was used to examine the concentration-response relationship in the genotoxicity experiments. A value of $p < 0.05$ indicated significance in all cases. All data analyses were done using the STATISTICA software package v. 6 (StatSoft Inc., Tulsa, OK, USA).

Results

Induction kinetics of the *sul*A gene by standard mutagens

Figure 1 shows the induction kinetics of the *sulA* gene in *E. coli* PQ37 following treatment with mutagens MMC and 4-NQO. The extent and kinetics of *sulA* gene induction by MMC and 4-NQO varied with the mutagen concentration, with the highest levels of induction occurring at 0.187 μM and 2.34 μM, respectively. At these concentrations, there was no significant reduction in protein synthesis as indicated by the constitutive alkaline phosphatase activity. These concentrations were therefore used for co-treatments in antigenotoxicity assays.

Solvent toxicity and genotoxicity in *E. coli* PQ37

Solvent toxicity was measured indirectly based on the inhibition of constitutive alkaline phosphatase activity in *E. coli* PQ37 (Table 2). Acetone, dichloromethane and methanol did not significantly reduce protein synthesis over the concentration range studied. In contrast, carbon tetrachloride, DMSO, ethanol and ether significantly reduced alkaline phosphatase activity at concentrations = 161, 438, 266 and 150 mM, respectively. The inhibition of protein synthesis by these solvents was also evidenced by the decrease in β-galactosidase activity (data not shown).

The genotoxicity of the organic solvents was assayed before their modulating effect was investigated (Table 2). Acetone, dichloromethane, DMSO, ethanol, ether and methanol did not significantly increase the *I* values in *E. coli* PQ37, indicating that these compounds do not induce the SOS response in *E. coli*. Carbon tetrachloride significantly increased the *I* values at the highest concentration (5158 mM), although correlation analysis revealed no concentration-response relationship for this solvent (data not shown). Only solvents that were neither toxic nor genotoxic to *E. coli* PQ37 (acetone, dichloromethane and methanol) were considered for further assays.

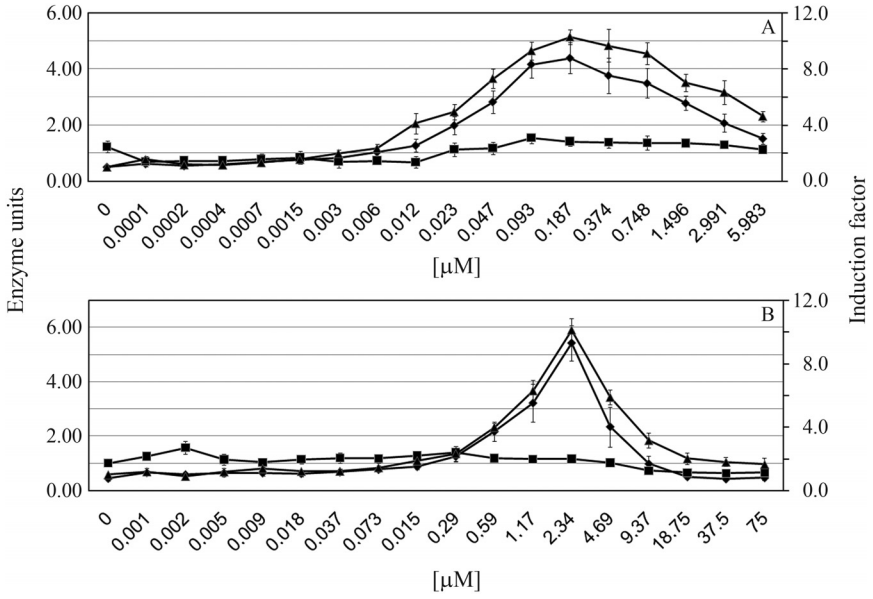

Figure 1 - Induction kinetics of the *sulA* gene in response to mutagens MMC (A) and 4-NQO (B) in *E. coli* PQ37 cells. (■) alkaline phosphatase, (◆) β-galactosidase and (▲) SOS induction factor.

Table 2 - Alkaline phosphatase (AP) activities and the SOS induction factor (*I*) in *E. coli* PQ37 cells treated with organic solvents.

C (%)	Treatment	AP activity	*I*
	Distilled water	0.51 ± 0.18	1.0 ± 0.2
	MMC (0.187 µM)	0.41 ± 0.10	8.6 ± 2.0*
	4-NQO (2.34 µM)	0.61 ± 0.14	13.6 ± 2.8*
0.19	Acetone (26 mM)	0.66 ± 0.15	1.0 ± 0.5
0.39	Acetone (53 mM)	0.50 ± 0.09	0.9 ± 0.1
0.78	Acetone (106 mM)	0.48 ± 0.08	1.1 ± 0.4
1.56	Acetone (212 mM)	0.47 ± 0.13	1.4 ± 0.6
3.12	Acetone (424 mM)	0.42 ± 0.09	0.8 ± 0.3
6.25	Acetone (848 mM)	0.41 ± 0.15	1.0 ± 0.3
12.5	Acetone (1697 mM)	0.46 ± 0.11	1.0 ± 0.3
25.0	Acetone (3393 mM)	0.65 ± 0.12	0.9 ± 0.1
50.0	Acetone (6787 mM)	0.79 ± 0.17	0.8 ± 0.3
	Distilled water	0.60 ± 0.08	1.0 ± 0.1
	MMC (0.187 µM)	0.48 ± 0.11	8.7 ± 2.0*
	4-NQO (2.34 µM)	0.60 ± 0.08	14.1 ± 2.1*
0.19	Carbon tetrachloride (20 mM)	0.80 ± 0.12	0.8 ± 0.2
0.39	Carbon tetrachloride (40 mM)	0.78 ± 0.17	0.9 ± 0.2
0.78	Carbon tetrachloride (81 mM)	0.54 ± 0.08	1.2 ± 0.2
1.56	Carbon tetrachloride (161 mM)	0.46 ± 0.06**	1.2 ± 0.2
3.12	Carbon tetrachloride (322 mM)	0.37 ± 0.09**	1.3 ± 0.2
6.25	Carbon tetrachloride (645 mM)	0.32 ± 0.10**	1.1 ± 0.3
12.5	Carbon tetrachloride (1289 mM)	0.35 ± 0.08**	0.9 ± 0.2
25.0	Carbon tetrachloride (2579 mM)	0.43 ± 0.07**	0.9 ± 0.1
50.0	Carbon tetrachloride (5158 mM)	0.42 ± 0.03**	3.1 ± 0.6*
	Distilled water	0.52 ± 0.15	1.0 ± 0.1
	MMC (0.187 µM)	0.37 ± 0.08	6.9 ± 0.4*
	4-NQO (2.34 µM)	0.56 ± 0.17	14.2 ± 3.6*
0.19	Dichloromethane (30 mM)	0.67 ± 0.29	0.9 ± 0.5
0.39	Dichloromethane (60 mM)	0.38 ± 0.12	1.3 ± 0.4
0.78	Dichloromethane (120 mM)	0.36 ± 0.09	1.0 ± 0.2
1.56	Dichloromethane (240 mM)	0.45 ± 0.21	1.0 ± 0.4
3.12	Dichloromethane (480 mM)	0.48 ± 0.14	0.9 ± 0.3
6.25	Dichloromethane (970 mM)	0.77 ± 0.27	0.4 ± 0.1
12.5	Dichloromethane (1930 mM)	0.63 ± 0.20	0.5 ± 0.2
25.0	Dichloromethane (3860 mM)	0.55 ± 0.21	0.8 ± 0.1
50.0	Dichloromethane (7720 mM)	0.73 ± 0.27	0.8 ± 0.2
	Distilled water	0.59 ± 0.12	1.0 ± 0.1
	MMC (0.187 µM)	0.38 ± 0.13	9.9 ± 2.1*
	4-NQO (2.34 µM)	0.45 ± 0.12	16.1 ± 4.4*
0.19	DMSO (27 mM)	0.60 ± 0.12	1.1 ± 0.1
0.39	DMSO (55 mM)	0.42 ± 0.13	1.3 ± 0.4
0.78	DMSO (109 mM)	0.45 ± 0.13	1.0 ± 0.3
1.56	DMSO (219 mM)	0.54 ± 0.14	1.1 ± 0.2
3.12	DMSO (438 mM)	0.33 ± 0.10**	1.6 ± 0.7
6.25	DMSO (875 mM)	0.29 ± 0.12**	1.6 ± 0.5
12.5	DMSO (1751 mM)	0.24 ± 0.09**	1.5 ± 0.4
25.0	DMSO (3502 mM)	0.29 ± 0.09**	1.4 ± 0.6
50.0	DMSO (7004 mM)	0.27 ± 0.08**	0.8 ± 0.2
	Distilled water	0.60 ± 0.08	1.0 ± 0.1
	MMC (0.187 µM)	0.52 ± 0.08	7.3 ± 1.0*
	4-NQO (2.34 µM)	0.50 ± 0.07	14.5 ± 2.2*
0.19	Ethanol (33 mM)	0.54 ± 0.09	1.2 ± 0.2
0.39	Ethanol (67 mM)	0.70 ± 0.11	0.9 ± 0.1
0.78	Ethanol (133 mM)	0.61 ± 0.13	1.0 ± 0.1
1.56	Ethanol (266 mM)	0.48 ± 0.08**	1.0 ± 0.2
3.12	Ethanol (532 mM)	0.32 ± 0.08**	1.3 ± 0.2
6.25	Ethanol (1065 mM)	0.45 ± 0.15**	1.2 ± 0.3
12.5	Ethanol (2130 mM)	0.26 ± 0.04**	1.4 ± 0.4
25.0	Ethanol (4260 mM)	0.32 ± 0.07**	1.4 ± 0.2
50.0	Ethanol (8520 mM)	0.28 ± 0.05**	1.1 ± 0.2
	Distilled water	0.67 ± 0.08	1.0 ± 0.0
	MMC (0.187 µM)	0.43 ± 0.07	9.6 ± 1.0*
	4-NQO (2.34 µM)	0.57 ± 0.08	13.9 ± 3.2*
0.19	Ether (19 mM)	0.78 ± 0.15	0.9 ± 0.1
0.39	Ether (37 mM)	0.59 ± 0.08	0.9 ± 0.1
0.78	Ether (75 mM)	0.65 ± 0.08	0.8 ± 0.1
1.56	Ether (150 mM)	0.53 ± 0.09**	1.0 ± 0.1
3.12	Ether (300 mM)	0.47 ± 0.12**	0.9 ± 0.1
6.25	Ether (600 mM)	0.27 ± 0.05**	1.3 ± 0.4
12.5	Ether (1200 mM)	0.29 ± 0.09**	0.9 ± 0.3
25.0	Ether (2400 mM)	0.34 ± 0.08**	1.0 ± 0.4
50.0	Ether (4800 mM)	0.35 ± 0.07**	1.1 ± 0.2
	Distilled water	0.68 ± 0.19	1.0 ± 0.3
	MMC (0.187 µM)	0.96 ± 0.34	4.2 ± 1.0*
	4-NQO (2.34 µM)	1.15 ± 0.38	6.5 ± 1.0*
0.19	Methanol (45 mM)	0.64 ± 0.12	1.3 ± 0.6
0.39	Methanol (90 mM)	0.44 ± 0.05	1.4 ± 0.3
0.78	Methanol (190 mM)	0.69 ± 0.15	1.0 ± 0.2
1.56	Methanol (380 mM)	0.62 ± 0.21	1.1 ± 0.2
3.12	Methanol (770 mM)	0.70 ± 0.20	1.0 ± 0.2
6.25	Methanol (1540 mM)	0.67 ± 0.28	1.2 ± 0.5
12.5	Methanol (3080 mM)	0.82 ± 0.17	0.5 ± 0.1
25.0	Methanol (6170 mM)	0.86 ± 0.44	0.7 ± 0.2
50.0	Methanol (12330 mM)	0.91 ± 0.34	0.4 ± 0.2

C (%): Solvent concentration calculated from the percentage purity of each solvent indicated in Table 1. The values are the mean ± SEM of three independent experiments with four replicates each. * and ** $p < 0.05$ for *I* values and alkaline phosphatase activity compared to the negative control (Student's *t*-test).

Influence of organic solvents on the activities of MMC and 4-NQO

Table 3 shows the capacity of the solvents to interfere with mutagen genotoxicity. All of the solvents assayed (acetone, dichloromethane and methanol) interfered with MMC- and 4NQO -induced genotoxicity. Dichloromethane and methanol concentrations > 6.25% significantly reduced the *I* values in co-treated cells compared to the positive control whereas concentrations ≤ 3.12% did not. In contrast, acetone reduced the *I* values in co-treatment with MMC and 4NQO at concentrations ≥ 1.56% and ≥ 3.12%, respectively.

Table 3 - Influence of organic solvents on mitomycin C (MMC)- and 4-nitro-quinoline-1-oxide (4-NQO)-induced DNA damage in *Escherichia coli* PQ37 cells.

C (%)	Cell treatment	*I* (%GI)	Cell treatments	*I* (%GI)
	Distilled water	1.0 ± 0.1	Distilled water	1.0 ± 0.1
	MMC	7.4 ± 0.9	4-NQO	9.8 ± 0.8
0.19	Acetone + MMC	9.1 ± 2.5 (0)	Acetone + 4-NQO	13.7 ± 3.0 (0)
0.39	Acetone + MMC	8.9 ± 2.4 (0)	Acetone + 4-NQO	11.1 ± 3.3 (0)
0.78	Acetone + MMC	6.2 ± 1.0 (18)	Acetone + 4-NQO	11.2 ± 3.4 (0)
1.56	Acetone + MMC	5.1 ± 1.2 (36) *	Acetone + 4-NQO	8.8 ± 2.2 (11)
3.12	Acetone + MMC	1.5 ± 0.3 (92) *	Acetone + 4-NQO	1.6 ± 0.5 (93) *
6.25	Acetone + MMC	1.1 ± 0.1 (98) *	Acetone + 4-NQO	1.2 ± 0.2 (98) *
12.5	Acetone + MMC	1.1 ± 0.1 (99) *	Acetone + 4-NQO	1.0 ± 0.2 (100) *
25.0	Acetone + MMC	0.7 ± 0.2 (100) *	Acetone + 4-NQO	0.9 ± 0.1 (100) *
50.0	Acetone + MMC	0.8 ± 0.1(100) *	Acetone + 4-NQO	0.8 ± 0.1 (100) *
	Distilled water	1.0 ± 0.1	Distilled water	1.0 ± 0.2
	MMC	5.8 ± 0.9	4-NQO	9.0 ± 2.8
0.19	Dichloromethane + MMC	6.2 ± 1.9 (0)	Dichloromethane + 4-NQO	10.5 ± 3.9 (0)
0.39	Dichloromethane + MMC	9.3 ± 2.2 (0)	Dichloromethane + 4-NQO	10.4 ± 2.6 (0)
0.78	Dichloromethane + MMC	8.5 ± 2.4 (0)	Dichloromethane + 4-NQO	10.6 ± 2.7 (0)
1.56	Dichloromethane + MMC	6.9 ± 1.3 (0)	Dichloromethane + 4-NQO	12.0 ± 2.1 (0)
3.12	Dichloromethane + MMC	5.1 ± 0.6 (14)	Dichloromethane + 4-NQO	10.9 ± 2.9 (0)
6.25	Dichloromethane + MMC	3.3 ± 0.8 (52) *	Dichloromethane + 4-NQO	6.4 ± 2.1 (32)
12.5	Dichloromethane + MMC	1.4 ± 0.2 (92) *	Dichloromethane + 4-NQO	3.4 ± 1.2 (70) *
25.0	Dichloromethane + MMC	0.8 ± 0.2 (100) *	Dichloromethane + 4-NQO	0.5 ± 0.1 (100) *
50.0	Dichloromethane + MMC	0.7 ± 0.2 (100) *	Dichloromethane + 4-NQO	0.5 ± 0.1 (100) *
	Distilled water	1.0 ± 0.1	Distilled water	1.0 ± 0.1
	MMC	6.9 ± 1.9	4-NQO	10.8 ± 1.2
0.19	Methanol + MMC	7.8 ± 1.0 (0)	Methanol + 4-NQO	14.2 ± 2.7 (0)
0.39	Methanol + MMC	7.8 ± 0.7 (0)	Methanol + 4-NQO	15.6 ± 4.8 (0)
0.78	Methanol + MMC	7.6 ± 1.2 (0)	Methanol + 4-NQO	16.3 ± 2.7 (0)
1.56	Methanol + MMC	6.6 ± 1.0 (6)	Methanol + 4-NQO	12.8 ± 3.3 (0)
3.12	Methanol + MMC	5.4 ± 0.8 (25)	Methanol + 4-NQO	11.0 ± 2.6 (0)
6.25	Methanol + MMC	1.2 ± 0.3 (97) *	Methanol + 4-NQO	1.7 ± 0.5 (93) *
12.5	Methanol + MMC	0.7 ± 0.4 (100) *	Methanol + 4-NQO	1.3 ± 0.2 (97) *
25.0	Methanol + MMC	0.6 ± 0.3 (100) *	Methanol + 4-NQO	1.2 ± 0.3 (98) *
50.0	Methanol + MMC	0.5 ± 0.2 (100) *	Methanol + 4-NQO	0.7 ± 0.1 (100) *

C (%): Solvent concentration in the mixture calculated from the percentage purity of each solvent indicated in Table 1. MMC and 4-NQO were assayed at concentrations of 0.187 μM and 2.34 μM, respectively. The values are the mean ± SEM of three independent experiments with four replicates each. The percentage of genotoxicity inhibition (%GI) was calculated as described in the Methods. *$p < 0.05$ compared to the corresponding positive control (Student's *t*-test).

Influence of organic solvents on vitamin E antigenotoxicity

We hypothesized that the solvent concentrations that interfered with the genotoxic activities of MMC- and 4-NQO could increase the antigenotoxicity of vitamin E against these mutagens, resulting in overestimates of antigenotoxicity. To assess this hypothesis, we assayed the acetone, dichloromethane and methanol concentrations that did and did not interfere with the genotoxic activities of MMC and 4-NQO (Tables 4 and 5). A control assay in

Table 4 - Influence of solvents on vitamin E antigenotoxicity against MMC-induced DNA damage in *E. coli* PQ37 cells.

Treatment	*I* (%GI)		
	A (distilled water)	B (interfering concentration of solvent)	C (non-interfering concentration of solvent)
		Acetone (25%)	Acetone (0.78%)
Distilled water	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1
MMC (0.187 μM)	6.5 ± 1.6	4.9 ± 1.1	6.5 ± 1.9
Vitamin E (0.016 mM) + MMC	7.7 ± 1.9 (0)	0.5 ± 0.2 (100)*	6.6 ± 1.4 (0.0)
Vitamin E (0.031 mM) + MMC	6.8 ± 2.1 (0)	0.4 ± 0.1 (100)*	5.9 ± 1.1 (11)
Vitamin E (0.062 mM) + MMC	5.5 ± 0.9 (17)	0.3 ± 0.1 (100)*	3.7 ± 0.7 (51)*
Vitamin E (0.125 mM) + MMC	2.6 ± 0.8 (70)*	0.3 ± 0.0 (100)*	2.8 ± 0.6 (67)*
Vitamin E (0.250 mM) + MMC	0.9 ± 0.1 (100)*	0.2 ± 0.0 (100)*	0.6 ± 0.2 (100)*
Vitamin E (0.500 mM) + MMC	0.2 ± 0.0 (100)*	0.2 ± 0.1 (100)*	0.2 ± 0.1 (100)*
Vitamin E (1.000 mM) + MMC	0.2 ± 0.0 (100)*	0.4 ± 0.2 (100)*	0.1 ± 0.0 (100)*
Vitamin E (2.000 mM) + MMC	0.1 ± 0.0 (100)*	0.3 ± 0.1 (100)*	0.1 ± 0.0 (100)*
		Dichloromethane (25%)	Dichloromethane (3.12%)
Distilled water	1.0 ± 0.1	0.9 ± 0.2	1.0 ± 0.1
MMC (0.187 μM)	6.5 ± 1.6	5.7 ± 1.1	4.1 ± 1.0
Vitamin E (0.016 mM) + MMC	7.7 ± 1.9 (0)	0.6 ± 0.1 (100)*	1.2 ± 0.3 (94)*
Vitamin E (0.031 mM) + MMC	6.8 ± 2.1 (0)	0.5 ± 0.1 (100)*	0.6 ± 0.1 (100)*
Vitamin E (0.062 mM) + MMC	5.5 ± 0.9 (17)	0.7 ± 0.1 (100)*	0.9 ± 0.2 (100)*
Vitamin E (0.125 mM) + MMC	2.6 ± 0.8 (70)*	0.7 ± 0.1 (100)*	0.7 ± 0.3 (100)*
Vitamin E (0.250 mM) + MMC	0.9 ± 0.1 (100)*	0.8 ± 0.2 (100)*	0.5 ± 0.1 (100)*
Vitamin E (0.500 mM) + MMC	0.2 ± 0.0 (100)*	0.7 ± 0.1 (100)*	0.3 ± 0.0 (100)*
Vitamin E (1.000 mM) + MMC	0.2 ± 0.0 (100)*	0.8 ± 0.1 (100)*	0.3 ± 0.0 (100)*
Vitamin E (2.000 mM) + MMC	0.1 ± 0.0 (100)*	0.9 ± 0.2 (100)*	0.3 ± 0.1 (100)*
		Methanol (25%)	Methanol (3.12%)
Distilled water	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.2
MMC (0.187 μM)	6.5 ± 1.6	5.2 ± 1.8	8.3 ± 2.4
Vitamin E (0.016 mM) + MMC	7.7 ± 1.9 (0)	1.1 ± 0.0 (98)*	0.6 ± 0.1 (100)*
Vitamin E (0.031 mM) + MMC	6.8 ± 2.1 (0)	1.1 ± 0.2 (98)*	0.6 ± 0.3 (100)*
Vitamin E (0.062 mM) + MMC	5.5 ± 0.9 (17)	0.8 ± 0.1 (100)*	0.4 ± 0.1 (100)*
Vitamin E (0.125 mM) + MMC	2.6 ± 0.8 (70)*	0.4 ± 0.0 (100)*	0.3 ± 0.1 (100)*
Vitamin E (0.250 mM) + MMC	0.9 ± 0.1 (100)*	0.3 ± 0.1 (100)*	0.5 ± 0.3 (100)*
Vitamin E (0.500 mM) + MMC	0.2 ± 0.0 (100)*	0.2 ± 0.0 (100)*	0.2 ± 0.1 (100)*
Vitamin E (1.000 mM) + MMC	0.2 ± 0.0 (100)*	0.2 ± 0.0 (100)*	0.2 ± 0.0 (100)*
Vitamin E (2.000 mM) + MMC	0.1 ± 0.0 (100)*	0.2 ± 0.0 (100)*	0.1 ± 0.0 (100)*

The concentration of MMM in the co-treatments was always 0.187 μM. Vitamin E was dissolved in distilled water (A) and in solvent at an interfering (B) and non-interfering (C) concentration. Interfering and non-interfering solvent concentrations with mutagenic activity were chosen as indicated in Methods. The values are the mean ± SEM of at least three independent experiments with four replicates each. The percentage of genotoxicity inhibition (%GI) was calculated as described in Methods. * $p < 0.05$ compared to the respective positive control (MMC) (Student's *t*-test).

which vitamin E was dissolved in distilled water was included in all experiments for comparison.

Tables 4 and 5 show the antigenotoxicity of vitamin E against DNA damage induced by MMC and 4-NQO. When dissolved in distilled water (treatment A), vitamin E significantly inhibited the MMC and 4-NQO genotoxicities at concentrations ≥ 0.125 mM. In contrast, when dissolved in solutions containing 25% solvent (treatment B), vitamin E significantly reduced the genotoxicities of MMC and 4-NQO genotoxicities at concentrations = 0.016 mM. These

Table 5 - Influence of solvents on the antigenotoxicity of vitamin E against 4-NQO-induced DNA damage in *E. coli* PQ37 cells.

Treatment	*I* (%GI)		
	A (distilled water)	B (interfering concentration of solvent)	C (non-interfering concentration of solvent)
		Acetone (25%)	Acetone (1.56%)
Distilled water	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.2
4-NQO (2.34 μM)	15.5 ± 4.8	18.6 ± 6.3	14.3 ± 1.8
Vitamin E (0.016 mM) + 4-NQO	16.6 ± 3.7 (0)	0.6 ± 0.2 (100 *	4.7 ± 1.9 (73) *
Vitamin E (0.031 mM) + 4-NQO	16.5 ± 4.9 (0)	0.5 ± 0.2 (100) *	4.6 ± 1.3 (74) *
Vitamin E (0.062 mM) + 4-NQO	16.2 ± 4.9 (0)	0.6 ± 0.1 (100) *	3.3 ± 1.2 (83) *
Vitamin E (0.125 mM) + 4-NQO	9.1 ± 2.6 (44) *	0.7 ± 0.6 (100) *	0.9 ± 0.1 (100) *
Vitamin E (0.250 mM) + 4-NQO	3.3 ± 1.1 (84) *	0.4 ± 0.1 (100) *	0.3 ± 0.1 (100) *
Vitamin E (0.500 mM) + 4-NQO	0.3 ± 0.1 (100) *	0.2 ± 0.1 (100) *	0.2 ± 0.1 (100) *
Vitamin E (1.000 mM) + 4-NQO	0.1 ± 0.0 (100) *	0.3 ± 0.1 (100) *	0.1 ± 0.0 (100) *
Vitamin E (2.000 mM) + 4-NQO	0.1 ± 0.0 (100) *	0.6 ± 0.3 (100) *	0.1 ± 0.0 (100) *
		Dichloromethane (25%)	Dichloromethane (6.25%)
Distilled water	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.0
4-NQO (2.34 μM)	15.5 ± 4.8	17.5 ± 3.9	11.1 ± 2.7
Vitamin E (0.016 mM) + 4-NQO	16.6 ± 3.7 (0)	0.9 ± 0.2 (100) *	4.6 ± 1.8 (65) *
Vitamin E (0.031 mM) + 4-NQO	16.5 ± 4.9 (0)	0.7 ± 0.1 (100) *	3.2 ± 1.3 (78) *
Vitamin E (0.062 mM) + 4-NQO	16.2 ± 4.9 (0)	0.9 ± 0.3 (100) *	3.7 ± 1.3 (73) *
Vitamin E (0.125 mM) + 4-NQO	9.1 ± 2.6 (44) *	1.0 ± 0.3 (100) *	1.4 ± 0.2 (96) *
Vitamin E (0.250 mM) + 4-NQO	3.3 ± 1.1 (84) *	1.3 ± 0.5 (98) *	0.4 ± 0.1 (100) *
Vitamin E (0.500 mM) + 4-NQO	0.3 ± 0.1 (100) *	1.4 ± 0.4 (98) *	0.1 ± 0.0 (100) *
Vitamin E (1.000 mM) + 4-NQO	0.1 ± 0.0 (100) *	1.5 ± 0.3 (97) *	0.1 ± 0.0 (100) *
Vitamin E (2.000 mM) + 4-NQO	0.1 ± 0.0 (100) *	1.2 ± 0.3 (99) *	0.1 ± 0.0 (100) *
		Methanol (25%)	Methanol (3.12%)
Distilled water	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1
4-NQO (2.34 μM)	15.5 ± 4.8	13.4 ± 2.1	14.3 ± 1.4
Vitamin E (0.016 mM) + 4-NQO	16.6 ± 3.7 (0)	0.4 ± 0.0 (100) *	2.0 ± 0.5 (93) *
Vitamin E (0.031 mM) + 4-NQO	16.5 ± 4.9 (0)	0.2 ± 0.1 (100) *	2.2 ± 1.0 (91) *
Vitamin E (0.062 mM) + 4-NQO	16.2 ± 4.9 (0)	0.2 ± 0.1 (100) *	1.4 ± 0.2 (97) *
Vitamin E (0.125 mM) + 4-NQO	9.1 ± 2.6 (44) *	0.2 ± 0.0 (100) *	0.9 ± 0.2 (100) *
Vitamin E (0.250 mM) + 4-NQO	3.3 ± 1.1 (84) *	0.1 ± 0.0 (100) *	0.3 ± 0.0 (100) *
Vitamin E (0.500 mM) + 4-NQO	0.3 ± 0.1 (100) *	0.2 ± 0.1 (100) *	0.2 ± 0.0 (100) *
Vitamin E (1.000 mM) + 4-NQO	0.1 ± 0.0 (100) *	1.1 ± 0.1 (99) *	0.1 ± 0.0 (100) *
Vitamin E (2.000 mM) + 4-NQO	0.1 ± 0.0 (100) *	1.2 ± 0.1 (99) *	0.1 ± 0.1 (100) *

The concentration of 4-NQO in the co-treatments was always 2.34 μM. Vitamin E was dissolved in distilled water (A) and in solvent at an interfering (B) and non-interfering (C) concentration. Interfering and non-interfering solvent concentrations with mutagenic activity were chosen as indicated in Methods. The values are the mean ± SEM of at least three independent experiments with four replicates each. The percentage of genotoxicity inhibition (%GI) was calculated as described in Methods. *$p < 0.05$ compared to the respective positive control (4-NQO) (Student's *t*-test).

findings support the hypothesis that organic solvents can enhance antigenotoxic activity, possibly through an additive effect. Surprisingly, the antigenotoxic effect of vitamin E against MMC and 4-NQO (treatment C) was also enhanced when the vitamin was dissolved in a non-interfering concentration of solvent. Only the co-treatment with MMC/vitamin E dissolved in 0.78% acetone showed similar antigenotoxicity to that of treatment A, although there was a significant reduction in the MMC genotoxicity at a lower vitamin concentration (0.062 mM). These results indicate that low, non-interfering concentrations of solvent can also influence the antigenotoxicity of vitamin E.

Discussion

In this study, we examined some of the factors that can influence the results of the SOS chromotest that is frequently used to screen for antimutagenic activity. Specifically, we investigated (1) the toxicity and genotoxicity of solvents, (2) the ability of solvents to interfere with mutagenesis, and (3) the contribution of organic solvents to overestimates of the antimutagenic activity of vitamin E (through additive or synergistic effects of the solvents).

Based on previous studies with the SOS chromotest (Von der Hude *et al.*, 1988; Quillardet and Hofnung, 1993; Mersch-Sundermann *et al.*, 1994), the solvents studied here were not genotoxic. However, some solvents such as carbon tetrachloride, DMSO, ethanol and ether were toxic to *E. coli* at high concentrations, as indicated by the inhibition of protein synthesis. In the standard procedure for the SOS chromotest (Quillardet and Hofnung, 1985) the maximum volume of solvent (DMSO or ethanol) used is 20 μL/assay (*i.e.*, for 600 μL of cell suspension), which corresponds to a final solvent concentration of 3.3% in the reaction mixture. Since there is very little information on the influence of solvents in the SOS chromotest, the data reported in Table 2 can be of practical use in choosing solvents compatible with the SOS chromotest when screening natural antigenotoxic compounds.

The ability of solvents to interfere with chemical mutagenesis has been documented, as indicated in the Introduction. For example, solvents such as DMSO can interfere with the genotoxicity of cisplatin in the SOS chromotest (Gebel and Koenig, 1999). As shown here, several solvents (including some not studied before) significantly reduced the genotoxicities of MMC and 4-NQO at high concentrations; this interference may limit the usefulness of the SOS Chromotest during antimutagenesis screening if the test substances are soluble only in organic solvents. Under these conditions, the assay can yield overestimates of antigenotoxicity for the tested compound. Acetone, dichloromethane and methanol increased the antigenotoxicity of vitamin E when the solvents were tested at a final concentration of 25%, which interfered with mutagenic activity. Surprisingly, these solvents also increased vitamin E antigenotoxicity at a concentration that did not interfere with mutagen activity, which suggested a synergistic interaction between solvents and vitamin E under these conditions. Donnelly *et al.* (1998) have previously shown that this synergic and/or additive interaction is characteristic of complex mixtures that contain mutagens.

DMSO is a cytoprotective compound frequently used as a solvent in mutagenic and anti-mutagenic studies because it has been considered to be non-genotoxic (Yu and Quinn, 1994). Indeed, several studies have used DMSO as a solvent to study the antimutagenic properties of biological extracts and/or natural compounds in the SOS chromotest (Table 6). However, DMSO can act as a free radical scavenger and can penetrate cell membranes to affect cell membrane stability (Yu and Quinn, 1998) and this in turn can interfere with the activity of antimutagens. As shown by Gebel and Koenig (1999), DMSO can also interfere with the activity of different mutagens in the SOS chromotest. Based on these findings, a critical evaluation of the antigenotoxic potential of the antimutagens indicated in Table 6 is required.

We have previously proposed the SOS chromotest as a potentially useful assay for primary screening of medicinal plant extracts for antigenotoxic activity against γ-rays (Fuentes *et al.*, 2006a). With this test, we have observed that the use of DMSO as solvent resulted in overestimates of the antimutagenic activity of essential oils when compared with distilled water (unpublished data). The additive and synergistic effect of solvents on vitamin E antigenotoxicity shown here supports the use of distilled water as the ideal solvent for antimutagen screening with the SOS chromotest. When substances soluble only in organic solvents are tested it is necessary to establish adequate final solvent concentrations to avoid interference with the test results. Solvents such as acetone, dichloromethane and methanol that were neither toxic nor genotoxic to *E. coli* PQ37 cells appear to be compatible with the SOS chromotest and are potentially useful for studying antimutagens. However, their ability to interfere with the assayed mutagens needs to be evaluated in each case.

Based on the results described here, we conclude that: (1) the solvents studied were not genotoxic in *E. coli* PQ37 cells, although some of them (carbon tetrachloride, DMSO, ethanol and ether) inhibited protein synthesis, depending on their concentration, (2) organic solvents can reduce the genotoxicity of MMC and 4-NQO in the SOS chromotest in a concentration-dependent manner; water is the best solvent when screening for antigenotoxicity in this assay, and (3) a solvent concentration that interferes directly with mutagen activity can significantly increase the antigenotoxicity of vitamin E, possibly through additive and synergistic effects; this interference can result in overestimates of antigenotoxicity. Overall, these findings indicate the need to carefully determine the appropriate concentration

Table 6 - Antimutagenicity of different compounds and biological extracts detected with the SOS Chromotest.

	Species	Mutagen	Reference
Natural antioxidants			
Ascorbic acid†	-	NF, FZ	Gajewska *et al.* (1990)
		UVR, 4-NQO	Sato *et al.* (1991)
Butyl-hydroxyanisole‡	-	B[*a*]P	Potenberg *et al.* (1988)
Butyl-hydroxytoluene‡	-	B[*a*]P	Potenberg *et al.* (1988)
5-chloro-uracyl†	-	UVR, MNNG	Sato *et al.* (1991)
Dodecyl-gallate‡	-	B[*a*]P	Potenberg *et al.* (1988)
Ethoxyquin‡	-	B[*a*]P	Potenberg *et al.* (1988)
5-fluoro-uracyl†	-	UVR, 4-NQO	Sato *et al.* (1991)
		MNNG	Sato *et al.* (1991)
Gallic acid esters†	-	H_2O_2	Nakayama *et al.*, 1993
Glutathione†	-	4-NQO, MNNG	Sato *et al.* (1991)
Lignin derivates†	-	4-NQO, H_2O_2	Mikulasova and Kosikova (2003)
Octyl-gallate‡	-	B[*a*]P	Potenberg *et al.* (1988)
Propyl-gallate‡	-	B[*a*]P	Potenberg *et al.* (1988)
Sodium selenite†	-	NF	Gajewska *et al.* (1990)
Vitamin E	-	NDEA	Aiub *et al.* (2009)
Plants extract or compounds			
AE†, FF†, CE‡, EAE‡, ME‡, PEE‡	*Acacia salicina*	B[*a*]P, NFA	Bouhlel *et al.* (2007)
Norbixin†	*Bixa orellana*	UVR	Junior *et al.* (2005)
Turmeric†, curcumin†	*Curcuma longa*	4-NQO	Polasa *et al.* (1997)
AE†	*Cymbopogon citratus*	γ-radiation	Fuentes *et al.* (2006b)
AE†, EO†	*Cyperus rotundus*	AFB1, NFA	Kilani *et al.* (2005)
MCF‡, stigmasterol‡, β -sitosterol	*Gleditsia sinensis*	4-NQO, MNNG	Lim *et al.* (2005)
EO†, citral†, carvone†, limonene†	*Lippia alba*	BLM	López *et al.* (2011)
EO†, thymol†, carvacrol†	*Lippia origanoides*	BLM	Vicuña *et al*. (2010)
AE‡, FF‡, HE‡, CE‡, EAE‡, ME‡, EO‡	*Myrthus communis*	AFB1, NFA	Hayder *et al.* (2004)
Myricetin-3-*o*-galactoside‡, Myricetin-3-*o*-rhamnoside‡	*Myrthus communis*	AFB1, NFA	Hayder *et al.* (2008)
AE†	*Phyllanthus orbicularis*	γ-radiation	Fuentes *et al.* (2006b)
AE†, TT†	*Pinus caribeae*	γ-radiation	Fuentes *et al.* (2006a)
AE†,	*Pinus caribeae*	γ-radiation	Fuentes *et al.* (2006b)
Gallic acid‡, 1,2,3,4,6-pentagalloyl glucose‡	*Pistacia lentiscus*	AFB1, NFA	Abdelwahed *et al.* (2007)
EO†	*Pituranthus chloranthus*	NFA, H_2O_2	Neffati *et al.* (2009)
AE‡, emodin‡	*Polygonum cuspidatum*	1-NP	Su *et al.* (1995)
FF†, EAE‡, ME‡	*Rhamnus alaternus*	AFB1, NFA	Ammar *et al.* (2007)
AE‡, CF‡, EAF‡, BF‡, PEE‡, CE‡	*Rhamnus alaternus*	AFB1, NFA	Ammar *et al.* (2008)
Emodin derivatives‡	*Rumex acetosa*	4-NQO, MNNG	Lee *et al.* (2005)
Microbial extracts			
Probiotic preparation†	*Bacillus* sp.	4-NQO	Caldini *et al.* (2002)
Probiotic preparation†	*Lactobacillus* sp.	4-NQO, MNNG	Caldini *et al.* (2008)
Probiotic preparation†	*Lactobacillus salivarius*	4-NQO	Fang *et al.* (2008)
Probiotic preparation†	*Saccharomyces boulardii*	4-NQO, FU, NA	Toma *et al.* (2005)
Animal extracts			
BF‡, 5α-cholest-7-en-3β-ol‡	*Asterina pectinifera* (starfish)	4-NQO, MNNG	Han *et al.* (2000)

Extract definitions: AE - aqueous extract, CE - chloroform extract, EAE - ethyl acetate extract, EE - ethanol extract, EO -essential oils, HE - hexane extract, ME - methanol extracts, PEE - petroleum ether extract. Fraction definitions: BF - butanolic fraction, CF - chloroform fraction, EAF - ethyl acetate fraction, FF - flavonoid fractions, MCF - methylene chloride fraction, TT - tannin fraction. The extracts or fractions were assayed using water (†) or dimethyl sulfoxide (‡) as the diluent. Mutagen definitions: AFB1 - aflatoxin B1, B[*a*]P - benzo[amine]pyrene, BLM - bleomycin, FU - furazolidone, FZ - furazolidone, H_2O_2 - hydrogen peroxide, MNNG - N-methyl-N-nitro-N-nitrosoguanidine, NA - nalidixic acid, NDEA - N-nitrosodiethylamine, NF - nitrofurasone, NFA - nifuroxazide, NPD - 4-nitro-*o*-phenylenediamine, 1-NP - 1-nitropyrene, 4-NQO - 4-nitroquinoline-1-oxide, SA - sodium azide, γ-radiation - gamma radiation, UVR - ultraviolet radiation.

of solvent to use when screening for antigenotoxicity in the SOS chromotest.

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