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Genetic Toxicology and Environmental Mutagenesis

Mutagenicity testing of (±)-camphor, *1,8*-cineole, citral, citronellal, (−)-menthol and terpineol with the *Salmonella*/microsome assay

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Abstract

The essential oils and their monoterpenoid constituents have been widely used as fragrances in cosmetics, as flavouring food additives, as scenting agents in a variety of household products, as active ingredients in some old drugs, and as intermediates in the synthesis of perfume chemicals. The present study was undertaken to investigate the mutagenic potential of six monoterpenoid compounds: two aldehydes (citral and citronellal), a ketone ((±)-camphor), an oxide (*1,8*-cineole, also known as eucalyptol), and two alcohols (terpineol and (−)-menthol). It is part of a more comprehensive toxicological screening of monoterpenes under way at our laboratory. Mutagenicity was evaluated by the *Salmonella*/microsome assay (TA97a, TA98, TA100 and TA102 tester strains), without and with addition of an extrinsic metabolic activation system (lyophilized rat liver S9 fraction induced by Aroclor 1254). In all cases, the upper limit of the dose interval tested was either the highest non-toxic dose or the lowest dose of the monoterpene toxic to TA100 strain in the preliminary toxicity test. No mutagenic effect was found with (±) camphor, citral, citronellal, *1,8*-cineole, and (−)-menthol. Terpineol caused a slight but dose-related increase in the number of *his*$^+$ revertants with TA102 tester strain both without and with addition of S9 mixture. The results from this study therefore suggest that, with the exception of terpineol, the monoterpenoid compounds tested are not mutagenic in the Ames test.

Keywords: Monoterpene; Essential oil; Genotoxicity; *Salmonella typhimurium*; Ames test

1. Introduction

Monoterpenes are the main chemical constituents of the essential oils which are mixtures of odoriferous principles obtained from a large variety of plants [1,2]. The essential oils and their monoterpenoid constituents are widely used as fragrances in cosmetics, as flavouring additives in food and beverages, as scenting agents in a variety of household products (e.g., detergents, soaps, room-air-fresheners, and insect repellents), as constituents of some old over-

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the-counter drugs (e.g., menthol, cineole, camphor) and as intermediates in the manufacture of perfume chemicals [1–3].

The present study was undertaken to evaluate the mutagenic potential of (±)-camphor, citral, citronellal, *1,8*-cineole, (−)-menthol and terpineol. These monoterpenoid compounds are found in the essential oils of edible and medicinal plants and are employed in the industry for different purposes.

2. Material and methods

2.1. Chemicals

(±)-Camphor, *1,8*-cineole (also known as eucalyptol), citral, citronellal, (−)-menthol and terpineol, mitomycin C (MC), benzo[*a*]pyren (B-[*a*]-P), 4-nitroquinolone-*n*-oxide (4-NQNO), nitro-*o*-phenilene-diamine (NPD) were all purchased from Sigma Chemical (St. Louis, MO, USA). 2-Aminofluorene (2AF) and 2-aminoanthracene (2AA) were bought from Merck KGaA, and sodium azide (SA) was from Aldrich Chemical.

2.2. Metabolic activation system (S9 mixture)

Lyophilized rat liver S9 fraction induced by Aroclor 1254 was purchased from Moltox (Molecular Toxicology, Annapolis, USA). The S9 mixture [21 ml] was prepared for use as follows: 7.0 ml of distilled water; 10.5 ml of 100 mM phosphate buffer pH 7.4; 0.84 ml of 100 mM NADP; 0.105 ml of 1 M glucose-6-phosphate; 0.420 ml of 1.65 M KCl + 0.4 M $MgCl_2$ salt solution and 2.1 ml of lyophilized S9 fraction reconstituted with distilled water.

2.3. Bacterial strains

TA102, TA100, TA98 and TA97a tester strains of *Salmonella typhimurium* were kindly supplied by Dr B.N. Ames. The bacterial strains were checked for their genetic markers immediately before and at the end of the study. For all assays overnight fresh cultures were prepared from frozen permanents (200 μl of the culture to 20 ml of Oxoid Nutrient Broth No. 2) and incubated at 37°C with shaking until a concentration of approximately 1.2×10^9 bacteria per milliliter was obtained.

2.4. Salmonella / microsome reverse mutation assay

The *Salmonella* mutagenicity test was performed by the plate incorporation method [4]. Briefly, 2 ml of top-agar was mixed with 100 μl of an overnight grown culture of *S. typhimurium*, 100 μl of the test substance (diluted in ethanol analytical grade, Merck, KGaA), the negative control, or the positive control (PC) and 500 μl of the phosphate buffer or the S9 mixture. The doses of the monoterpenes ranged from 1 μg up to 2500 μg per plate. Ethanol served as the negative (solvent) controls, while the positive control substances were: SA (0.5 μg/plate), NPD (1 μg/plate), MC (0.5 μg/plate), 4-NQNO (1 μg/plate), 2AF (10 μg/plate), 2AA (0.5 or 1 μg/plate), and B-[*a*]-P (50 μg/plate). SA and MC were dissolved in distilled water and dimethyl sulfoxide [DMSO] was used as vehicle for the other positive controls. Plates were incubated at 37°C for 72 h in the dark and then scored for revertant his^+ bacteria colonies. Every determination was made in triplicate and each experiment was repeated at least once in order to check the reproducibility of the results.

3. Results and discussion

Toxicity of monoterpenoid compounds to *S. typhimurium* was investigated in a preliminary test carried out with TA100 strain without and with addition of S9 mixture (Table 1). In all subsequent assays, the upper limit of the dose interval tested was either the highest non-toxic dose or the lowest toxic dose determined in this preliminary assay. Toxicity was apparent either as a reduction in the number of revertants, and or as an alteration of the auxotrophic background growth (i.e., background lawn). It is of note that the six monoterpenoid compounds tested were different regarding their toxicity towards *S. typhimurium* tester strains. Citronellal was by far the most toxic monoterpene tested (highest non-toxic dose = 200 μg/plate). Citral and (−)-menthol (highest non-toxic dose = 600–700 μg/plate) were less toxic than citronellal, but clearly more toxic than

Table 1
Toxicity of monoterpenoid compounds to *S. typhimurium* TA100 strain

Dose (μg/plate)	Citral		Citronellal		(−)-Menthol		Terpineol		(±)-Camphor		*1,8*-Cineole	
	− S9	+ S9	− S9	+ S9	− S9	+ S9	− S9	+ S9	− S9	+ S9	− S9	+ S9
3000	–	–	–	–	–	–	–	$0/3^{+}$	$69 \pm 14*$	$76 \pm 44*$	–	$101*/1^{+}$
2750	–	–	–	–	–	–	–	–	$94 \pm 40*$	–	–	–
2500	–	–	–	–	–	–	–	$180 \pm 11*$	$96 \pm 8*$	$135 \pm 9*$	–	$102 \pm 14*$
2000	–	–	–	–	–	–	$68/0/0^{+}$	184 ± 8	146 ± 7	152 ± 2	$104 \pm 46*$	$110 \pm 30*$
1500	–	–	–	–	–	–	126 ± 35	–	–	132 ± 13	150 ± 8	147 ± 0
1250	–	–	–	–	–	–	145 ± 6	–	–	–	–	–
1000	–	–	–	–	–	–	164 ± 5	–	–	–	172 ± 6	–
900	–	–	–	–	–	$101*/0/0^{+}$	–	–	–	–	–	–
800	$7 \pm 5*$	–	–	–	$93 \pm 39*$	$167*/0/0^{+}$	–	–	–	–	–	–
700	$155*^{+}$	$12/0^{+}$	–	–	$134 \pm 29*$	$77 \pm 6*$	–	–	–	–	–	–
600	165 ± 23	$102 \pm 6*$	–	–	159 ± 14	$120 \pm 10*$	–	–	–	–	–	–
500	94 ± 12	148 ± 12	$1/0/0^{+}$	–	165 ± 12	166 ± 17	–	–	–	–	–	–
400	143 ± 28	150 ± 21	$52/3/0^{+}$	$3/3^{+}$	–	–	–	–	–	–	–	–
300	–	–	$82/29/0^{+}$	$154/75*/0^{+}$	–	–	–	–	–	–	–	–
200	–	–	139 ± 12	179 ± 23	–	–	–	–	–	–	–	–
0	178 ± 24	173 ± 14	174 ± 15^{a}	184 ± 26^{b}	174 ± 15^{a}	184 ± 26^{b}	167 ± 6	166 ± 2	145 ± 6	184 ± 26^{b}	191 ± 20	180 ± 21
PC	728 ± 83	886 ± 58	924 ± 41^{a}	591 ± 69^{b}	924 ± 41^{a}	591 ± 69^{b}	913 ± 65	464 ± 35	934	591 ± 69^{b}	842 ± 63	742 ± 13

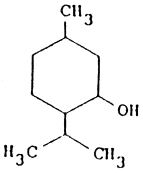
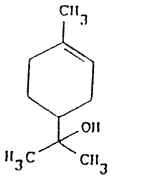
(*) Toxicity apparent as an alteration of the background lawn.
(^{a,b}) Toxicity assays carried out concomitantly shared the same solvent- and positive controls.
(–) Dose not tested.
($/^{+}$) Mutant counts of individual plates.
Data are shown as mutant counts (mean ± SD) of three plates.

Table 2
Mutagenicity testing of the monoterpenoid aldehydes citral (3,7-dimethyl-2,6-octadienal) and citronellal (3,7-dimethyl-6-octenal) in the *Salmonella* / microsome assay [TA100, TA98, TA97a and TA102 tester strains]

MONOTERPENE	DOSE	NUMBER OF REVERTANTS (Mean ± S.D.)							
		TA 100		TA 98		TA 97a		TA 102	
	(μg/plate)	- S 9	+ S 9	- S 9	+ S 9	- S 9	+ S 9	- S 9	+ S 9
CITRAL	700	177 ± 31	-	46 ± 1	64 ± 4	132 ± 11	193 ± 14	-	-
	600	174 ± 2	186 ± 14	36 ± 2	45 ± 6	144 ± 3	221 ± 9	-	-
	500	164 ± 14	175 ± 15	40 ± 9	59 ± 12	124 ± 8	210 ± 4	-	-
	400	170 ± 15	167 ± 8	39 ± 4	52 ± 8	138 ± 9	242 ± 16	-	544 ± 136*
	300	181 ± 22	211 ± 19	46 ± 5	60 ± 7	138 ± 15	236 ± 6	492 ± 93	894 ± 76
	200	-	178 ± 30	41 ± 5	-	149 ± 1	211 ± 7	626 ± 64	922 ± 124
	100	-	195 ± 10	-	-	-	-	672 ± 24	861 ± 18
	50	-	-	-	-	-	-	693 ± 37	895 ± 154
	25	-	-	-	-	-	-	638 ± 50	874 ± 137
	10	-	-	-	-	-	-	648 ± 37	884 ± 169
geranial (citral a) neral (citral b)	5	-	-	-	-	-	-	696 ± 51	776 ± 82
	0	168 ± 14	201 ± 16	51 ± 6	64 ± 1	171 ± 14	214 ± 18	687 ± 47	839 ± 124
	PC	633 ± 42	1165 ± 151	204 ± 26	479 ± 11	767 ± 103	870 ± 140	2837 ± 544	1921 ± 107
CITRONELLAL	300	-	-	-	47 ± 14*	-	-	-	-
	200	131 ± 12	172 ± 11	29 ± 3	54 ± 1	-	174 ± 25	-	645 ± 50*
	150	151 ± 11	-	34 ± 11	-	125 ± 8	-	-	706 ± 55
	100	152 ± 10	199 ± 12	42 ± 1	57 ± 6	146 ± 19	218 ± 30	-	655 ± 41
	50	167 ± 4	196 ± 18	31 ± 2	61 ± 4	153 ± 13	181 ± 31	548 ± 62	846 ± 28
	30	156 ± 11	-	-	-	-	-	674 ± 70	-
	25	-	203 ± 9	-	-	167 ± 16	225 ± 2	-	751 ± 66
	20	-	-	-	-	-	-	631 ± 35	-
	10	164 ± 14	205 ± 14	32 ± 6	70 ± 8	152 ± 16	187 ± 16	599 ± 81	787 ± 66
	5	186 ± 12	201 ± 14	36 ± 6	62 ± 11	158 ± 18	211 ± 19	612 ± 20	752 ± 26
	1	-	-	-	-	-	-	646 ± 56	-
	0	182 ± 14	201 ± 16	49 ± 16	70 ± 6	157 ± 2	212 ± 17	628 ± 12	771 ± 37
	PC	907 ± 63	1165 ± 151	208 ± 1	312 ± 33	749 ± 25	1002 ± 101	5351 ± 98	2024 ± 182

Dose O—Negative Control: 100 μl ethanol PA; PC—Positive Control: TA100/−S9, SA (0.5 μg/plate); TA100/+S9, 2AA (1 μg/plate); TA98/−S9, NPD (1 μg/plate); TA98/+S9; 2AA (0.5 μg/plate); TA97a/−S9, 4-NQNO (1 μg/plate); TA97a/+S9, 2AF (10 μg/plate); TA102/−S9, MC (0.5 μg/plate); TA102/+S9, B-[*a*]-P (50 μg/plate).
(–) Dose not tested.
(*) Toxicity apparent as an alteration of the background lawn.
Values are the means ± SD of three plates of one (out of two) representative experiment.

Table 3
Mutagenicity testing of the monoterpenoid alcohols (−)-menthol (5-methyl-2-(1-methyl-ethyl)cyclohexanol) and terpineol (*p*-menth-1-en-ol) in the *Salmonella*/microsome assay [TA100, TA98, TA97a and TA102 tester strains]

MONOTERPENE	DOSE	NUMBER OF REVERTANTS (Mean ± S.D.)							
		TA 100		TA 98		TA 97a		TA 102	
	(μg/plate)	- S 9	+ S 9	- S 9	+ S 9	- S 9	+ S 9	- S 9	+ S 9
(-) - MENTHOL	800	-	-	-	43 /0/0[†]	-	93 ± 4*	-	-
	700	-	168 ± 15	-	48 ± 6	86 ± 54*	154 ± 8	-	-
	600	161 ± 7	170 ± 10	41 ± 4	50 ± 10	132 ± 11	181 ± 8	-	-
	500	161 ± 10	179 ± 11	46 ± 1	64 ± 3	137 ± 6	186 ± 11	-	552 ± 114*
	400	183 ± 3	182 ± 18	40 ± 8	57 ± 12	148 ± 11	198 ± 13	312 ± 135	826 ± 21
	300	199 ± 17	182 ± 13	39 ± 8	57 ± 9	155 ± 6	209 ± 14	409 ± 152	754 ± 33
	200	205 ± 11	183 ± 7	35 ± 4	55 ± 10	169 ± 13	209 ± 16	643 ± 62	897 ± 18
	100	211 ± 12	-	42 ± 2	-	149 ± 2	-	665 ± 34	873 ± 66
	50	-	-	-	-	-	-	686 ± 35	738 ± 19
	25	-	-	-	-	-	-	574 ± 50	-
	10	-	-	-	-	-	-	648 ± 32	-
	5	-	-	-	-	-	-	708 ± 34	-
	0	219 ± 21	196 ± 18	47 ± 3	63 ± 1	160 ± 22	172 ± 6	719 ± 25	832 ± 67
	PC	860 ± 1	1003 ± 66	159 ± 16	343 ± 57	998 ± 52	844 ± 18	5967 ± 1198	1589 ± 157
TERPINEOL	2500	-	141 ± 62*	-	4 /0/0[†]	-	-	-	-
	2000	53 ± 14*	146 ± 2	-	68 ± 13	-	272 ± 34	-	833 ± 57*
	1500	165 ± 16	212 ± 28	43 ± 5	60 ± 10	225 ± 32	248 ± 12	-	1573 ± 191
	1250	158 ± 19	188 ± 23	42 ± 2	58 ± 2	224 ± 11	246 ± 6	862 ± 187	1727 ± 108
	1000	176 ± 6	208 ± 33	40 ± 8	54 ± 10	191 ± 8	254 ± 21	1004 ± 488	1429 ± 46
	750	177 ± 14	196 ± 1	36 ± 4	69 ± 10	216 ± 16	246 ± 12	1418 ± 176	1177 ± 42
	500	177 ± 12	161 ± 24	28 ± 7	53 ± 10	195 ± 13	241 ± 16	1312 ± 89	1116 ± 74
	250	186 ± 9	-	30 ± 6	-	182 ± 12	-	812 ± 166	790 ± 47
	100	-	-	-	-	-	-	924 ± 151	-
	50	-	-	-	-	-	-	737 ± 32	-
	25	-	-	-	-	-	-	611 ± 65	-
	0	176 ± 14	197 ± 7	40 ± 7	70 ± 6	158 ± 2	214 ± 18	731 ± 36	771 ± 37
	PC	898 ± 51	742 ± 13	192 ± 31	312 ± 33	1098 ± 80	870 ± 140	4875 ± 1031	2024 ± 182

Dose O—Negative Control: 100 μl ethanol PA; PC—Positive Control: TA100/−S9, SA (0.5 μg/plate); TA100/+S9, 2AA (1 μg/plate); TA98/−S9, NPD (1 μg/plate); TA98/+S9; 2AA (0.5 μg/plate); TA97a/−S9, 4-NQNO (1 μg/plate); TA97a/+S9, 2AF (10 μg/plate); TA102/−S9, MC (0.5 μg/plate); TA102/+S9, B-[*a*]-P (50 μg/plate).
(–) Dose not tested.
(*) Toxicity apparent as an alteration of the background lawn.
(/[+]) mutant counts of individual plates.
Values are the means ± SD of three plates of one (out of two) representative experiment.

(±)-camphor, *1,8*-cineole and terpineol (highest non-toxic dose = 2000–2500 μg/plate). In all cases, TA102 proved to be the most susceptible tester strain.

Table 2 shows the results of the *Salmonella* mutagenicity assay with the aldehydes citral (a mixture of isomers neral and geranial) and citronellal. Citral and citronellal did not induce any increase in the number of revertant *his*$^+$ colonies over negative control values obtained for strains TA97a, TA98, TA100 and TA102, either in the presence or in the absence of extrinsic metabolic activation (S9 mix). The negative results presented in Table 2 were confirmed by a second experiment (results not shown).

Citral was regarded as a possible carcinogen/mutagen due to its α,β-unsaturated aldehyde function. However, a recent prediction for citral carcinogenicity made on the basis of computer tests (COMPACT: Computer Optimised Molecular Parametric Analysis for Chemical Toxicity) was negative [5]. The absence of citral-induced mutagenicity was previously reported by Ishidate et al. [6] with the *Salmonella*/microsome assay (strains TA92; TA1535, TA100, TA1537, TA94 and TA98) without and with S9 mixture. Citral was also tested for its potential to increase the frequencies of sister chromatid exchanges and chromosome aberrations in V79 cells (without and with addition of S9 mix) and negative results were found [7]. Kamasaki et al. [8] evaluated the mutagenic potential of citronellal with the *Salmonella* assay (TA 98 and TA100). Negative results were obtained by these authors with the two *S. typhimurium* strains but they also reported that citronellal induced an increase in the frequency of chromosome aberrations in Chinese hamster B241 cells [8]. Our results also including the TA97a and TA102 tester strains therefore confirm literature data suggesting that the monoterpenoid aldehydes citral and citronellal are not mutagenic compounds.

The results of the mutagenicity assay with the monoterpenoid alcohols (−)-menthol and terpineol are shown in Table 3. (−)-Menthol was tested in doses up to 600–700 μg/plate (TA97a, TA98 and TA100) and 200–400 μg/plate (TA102), and no increase in the number of revertant colonies was observed without and with addition of S9 mixture. Terpineol, tested in doses up to 1500–2000 μg/plate, in the absence as well as in the presence of metabolic activation, also gave negative results in three of the four strains used. In contrast to the absence of mutagenicity towards TA97a, TA98 and TA100 strains, terpineol produced a dose-related increase in the number of TA102 revertants. The maximum effects were a 2.0-fold increase in the number TA102 revertants at doses as high as 750 μg/plate in the absence of S9 mixture, and a 2.2-fold increase at doses as high as 1250 μg/plate in the presence of S9 mixture. The negative as well as the positive results presented in Table 3 were reproduced by a confirmatory experiment. As shown in Table 4, terpineol also induced a dose related increase in the number of TA102 revertants in this second experiment.

Menthol, a constituent of the peppermint oil, had been previously evaluated in the *Salmonella*/microsome assay with the tester strains TA92, TA1535, TA100, TA1537, TA94 and TA98 and no mutagenic effect was found [6,9]. The present study findings adding TA97a and TA102 strains to the testing battery, confirmed that menthol is not mutagenic in the Ames test.

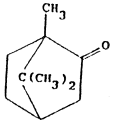
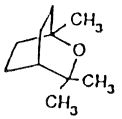
No previous study of the mutagenicity of terpineol was found in the literature. The present study showed that terpineol was weakly mutagenic with TA102 strain and that exogenous metabolic activation is not required for this effect. This positive

Table 4
Mutagenicity of terpineol to TA102 tester strain in the confirmatory experiment

Dose (μg/plate)	−S9	+S9
2000	–	572 ± 28 *
1500	–	1562 ± 123
1250	–	1526 ± 219
1000	1050 ± 38	1114 ± 562
750	1088 ± 32	1057 ± 74
500	786 ± 58	824 ± 6
250	742 ± 24	–
100	634 ± 26	–
50	600 ± 32	–
25	630 ± 54	–
0	613 ± 24	790 ± 120
PC	5820 ± 311	1627 ± 260

Dose O—negative control (100 μl ethanol PA); PC—positive controls, −S9 (MC: 0.5 μg/plate); +S9 (B-[*a*]-P: 50 μg/plate). (*) Toxicity apparent as an alteration of the background lawn. Values are revertant counts (mean ± SD of three plates).

Table 5
Mutagenicity testing of the monoterpenoid compounds (±)-camphor (1,7,7-trimethyl-bicyclo[2.2.1]-heptan-2-one) [ketone] and *1,8*-cineole (*1,8*-epoxy-*p*-menthane) [oxide] in the *Salmonella*/microsome assay [TA100, TA98, TA97a and TA102 tester strains]

MONOTERPENE	DOSE	NUMBER OF REVERTANTS (Mean ± S.D.)							
		TA 100		TA 98		TA 97a		TA 102	
	(μg/plate)	- S 9	+ S 9	- S 9	+ S 9	- S 9	+ S 9	- S 9	+ S 9
(±) - CAMPHOR	2500	-	157 ± 4	-	48 ± 8	-	60 ± 32	-	273 ± 25*
	2000	160 ± 8	159 ± 19	38 ± 5	56 ± 5	141 ± 1	139 ± 20	-	497 ± 55*
	1500	153 ± 13	151 ± 12	31 ± 6	54 ± 10	156 ± 5	146 ± 5	-	737 ± 131
	1250	159 ± 19	169 ± 18	36 ± 5	54 ± 8	125 ± 16	172 ± 14	437 ± 101	734 ± 23
	1000	171 ± 9	158 ± 15	33 ± 3	57 ± 7	139 ± 4	187 ± 6	544 ± 11	822 ± 50
	750	170 ± 12	160 ± 16	30 ± 2	57 ± 6	139 ± 9	191 ± 14	568 ± 19	740 ± 6
	500	185 ± 1	-	35 ± 6	53 ± 10	142 ± 9	205 ± 24	606 ± 38	747 ± 38
	250	-	-	-	-	-	-	654 ± 11	639 ± 21
	100	-	-	-	-	-	-	641 ± 32	-
	50	-	-	-	-	-	-	663 ± 16	-
	0	164 ± 12	196 ± 18	38 ± 5	63 ± 1	164 ± 3	185 ± 22	688 ± 13	776 ± 36
	PC	838 ± 21	1003 ± 66	210 ± 11	343 ± 57	759 ± 57	1048 ± 92	5973 ± 996	1952 ± 86
1,8 CINEOLE	2500	-	-	-	-	-	142 *[1]	-	-
	2000	98 ± 6*	157 ± 12	-	51 ± 18	146 ± 8	180 ± 4	-	514 ± 129*
	1500	149 ± 26	180 ± 12	37 ± 2	57 ± 13	170 ± 13	140 ± 11	619 ± 140	722 ± 21
	1250	164 ± 10	185 ± 14	34 ± 5	58 ± 4	168 ± 6	177 ± 7	605 ± 83	706 ± 4
	1000	183 ± 13	179 ± 13	38 ± 1	60 ± 6	166 ± 12	190 ± 40	692 ± 31	777 ± 31
	750	196 ± 10	177 ± 26	47 ± 6	59 ± 8	174 ± 19	180 ± 10	642 ± 23	698 ± 30
	500	199 ± 16	190 ± 19	41 ± 7	59 ± 2	163 ± 26	210 ± 9	753 ± 41	758 ± 26
	250	193 ± 12	-	43 ± 2	-	168 ± 17	-	686 ± 19	794 ± 32
	0	176 ± 14	196 ± 18	48 ± 5	54 ± 6	160 ± 22	185 ± 22	660 ± 91	776 ± 36
	PC	898 ± 51	1003 ± 66	204 ± 34	210 ± 34	998 ± 52	1048 ± 92	4102 ± 317	1952 ± 86

Dose O—Negative Control: 100 μl ethanol PA; PC—Positive Control: TA100/−S9, SA (0.5 μg/plate); TA100/+S9, 2AA (1 μg/plate); TA98/−S9, NPD (1 μg/plate); TA98/+S9; 2AA (0.5 μg/plate); TA97a/−S9, 4-NQNO (1 μg/plate); TA97a/+S9, 2AF (10 μg/plate); TA102/−S9, MC (0.5 μg/plate); TA102/+S9, B-[*a*]-P (50 μg/plate).
(–) Dose not tested.
(*) Toxicity apparent as an alteration of the background lawn.
(/[+]) Mutant counts of individual plates.
Values are the means ± SD of three plates of one (out of two) representative experiment.

result obtained only with the TA102 tester strain indicated that terpineol induced a base-pair substitution affecting a A–T base pair. We have added TA102 to the tester strain battery routinely used at our laboratory because it has been demonstrated that it detects a variety of oxidants and cross-linking agents as mutagens which are not detectable by other *Salmonella* strains [10].

Table 5 presents the results of mutagenicity testing of monoterpenoid compounds with two different functional groups, i.e., (±)-camphor, a ketone, and *1,8*-cineole, an oxide. (±)-Camphor and *1,8*-cineole were tested in doses up to 1500–2500 μg/plate and no mutagenic effect was observed with the four bacterial tester strains, in the absence as well as in the presence of extrinsic metabolic activation. In both cases, negative results were also obtained by an independent confirmatory experiment.

In spite of its large scale use, studies on the mutagenic potential of (±)-camphor are scarce. Kanematsu and Shibata [11] reported that endodontic products containing camphor presented a positive result in the 'rec-assay' with *B. subtilis*. On the other hand, Goel et al. [12] demonstrated that camphor had a radiomodifying effect, i.e., camphor antagonized the radiation-induced increase in SCE frequency in mice bone marrow cells.

As far as the authors are aware, no study on the mutagenicity of *1,8*-cineole has been published in the literature so far.

In conclusion, results from the present study thus suggest that citral, citronellal, (±)-camphor, (−)-menthol and *1,8*-cineole are not mutagenic in the Ames test and that terpineol is weakly mutagenic to TA102 tester strain.

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