

Table 5 Synergistic effects of sodium nitrite and catechol on cytogenetic toxicity in Chinese hamster cells

Treatment		No. of cells scored	Polyploid cells	No. of cells with structural chromosome aberrations										Mitotic index (%)	Relative mitotic index (%)
NaNO ₂ (mg/mL)	Catechol (µg/mL)			Gap	Chromatid type			Chromosome type		Fragmen-tation		Others			
				ctb	cte	csb	cse					+g	-g		
0	0 (control)	200	1 (0.5)	0	1	0	1	0	0	0	0	3 (1.5)	2 (1.0)	5.1	100
	12.5	200	0 (0)	3	4	1	1	0	0	0	0	9 (4.5)	7 (3.5)	6.2	122
	25	200	2 (1.0)	3	5	3	0	0	0	1	0	10 (5.0)	9 (4.5)	6.2	122
	50	200	1 (0.5)	5	11	1	2	0	0	0	0	15 (7.5)	15 (7.5)**	3.1	61
	100	200	1 (0.5)	7	18	4	1	1	1	6	0	31 (15.5)	28 (14.0)***	1.5	29
	200	200	18 (9.0)***	3	4	1	2	0	0	3	0	21 (10.5)	12 (6.0)*	1.1	22
5.0	0 (control)	200	1 (0.5)	3	5	2	1	0	0	0	0	11 (5.5)	10 (5.0)	6.7	100
	12.5	200	3 (1.5)	1	6	2	1	0	0	0	0	11 (5.5)	8 (4.0)	5.9	88
	25	200	3 (1.5)	10	25	6	1	2	0	0	0	35 (17.5)	34 (17.0)***	4.9	73
	50	200	3 (1.5)	12	32	4	2	1	2	2	0	42 (21.0)	41 (20.5)***	2.5	37
	100	200	7 (3.5)	8	26	2	0	3	8	8	0	42 (21.0)	40 (20.0)***	1.7	25
	200	200	9 (4.5)*	7	12	2	2	0	0	0	0	24 (12.0)	18 (9.0)***	2.1	31

NaNO₂, sodium nitrite

ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; +g, including gaps ; -g, excluding gaps
 The figures shown in parentheses are percentages.

*, **, *** : Significantly different from the control at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

Table 6 DNA damage in CHL cells after 1h treatment with sodium nitrite

Sampling time (h)	Substance	Dose (mg/mL)	% Migrated DNA		Tail Length		Olive Tail Moment	
			Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
1	Control (1%saline)	0	4.69	± 1.15	5.88	± 0.78	0.37	± 0.08
		1.25	4.81	± 0.80	4.50	± 1.08	0.40	± 0.08
		2.5	4.87	± 0.44	5.27	± 1.11	0.40	± 0.07
	Sodium nitrite ^{a)}	5	4.93	± 0.31	6.59	± 1.33	0.39	± 0.02
		2 μM	47.75	*** ± 1.86	45.92	*** ± 1.95	13.31	*** ± 1.44
		4NQO ^{b)}						

4NQO: 4-nitroquinoline 1-oxide

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Welch test.

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 7 DNA damage in CHL cells after 1h treatment with (-)-epigallocatechin-gallate

Sampling time (h)	Substance	Dose (μ M)	% Migrated DNA		Tail Length		Olive Tail Moment		Cytotoxicity %
			Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	
1	Control (1%saline)	0	6.32	± 1.74	5.91	± 3.72	0.55	± 0.24	0.00
		100	4.66	± 0.58	4.72	± 0.31	0.38	± 0.05	-5.62
	EGCG ^{a)}	200	5.03	± 1.01	5.43	± 2.18	0.44	± 0.11	12.03
		400	5.66	± 1.19	8.79	± 1.26	0.53	± 0.11	46.55
	4NQO ^{b)}	2	54.67	*** ± 5.46	50.55	*** ± 5.20	17.77	** ± 2.97	

EGCG: (-)-epigallocatechin-gallate

4NQO: 4-nitroquinoline 1-oxide

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Welch test.

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 8 *In vitro* comet assay - Sodium nitrite + EGCC

Sodium nitrite (mg/ml)	EGCC (μ M)	% Tail DNA		Tail length		Olive tail moment		Cell viability %
		Mean	\pm S.D.	Mean	\pm S.D.	Mean	\pm S.D.	
0	0	4.32	\pm 0.97	10.39	\pm 1.88	0.44	\pm 0.11	100
1.25	100	6.10	* \pm 0.49	16.82	** \pm 2.58	0.75	** \pm 0.03	90
2.5	100	6.16	* \pm 1.11	16.61	** \pm 0.72	0.77	** \pm 0.11	74
5	100	9.45	** \pm 0.50	23.01	** \pm 0.78	1.60	** \pm 0.16	57

Data were statically analyzed by Dunnett's test following one-way ANOVA. Significantly different from control: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 9 *In vitro* comet assay – Effect of post-treatment with sodium nitrite on EGCG

1st treatment EGCG (μ M)	2nd treatment Sodium nitrite (mg/ml)	% Tail DNA		Tail length		Olive tail moment		Statistics
		Mean \pm S.D.	S.D.	Mean \pm S.D.	S.D.	Mean \pm S.D.	S.D.	
0	0	4.53 \pm 0.66		8.17 \pm 2.37		0.46 \pm 0.07		-
100	1.25	4.75 \pm 1.17		9.11 \pm 4.28		0.47 \pm 0.13		
100	2.5	4.83 \pm 0.95		7.01 \pm 0.70		0.46 \pm 0.09		a
100	5	5.05 \pm 1.72		9.68 \pm 2.23		0.50 \pm 0.16		
100	0	4.31 \pm 0.99		9.32 \pm 1.89		0.42 \pm 0.10		b

One hour later EGCG treatment, CHL cells were rinsed twice with PBS and treated with Sodium nitrite for one hour.

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Welch test.

Table 10 *In vitro* comet assay – Effect of pre-treatment with sodium nitrite on EGCG

1st treatment Sodium nitrite (mg/ml)	2nd treatment EGCG (μ M)	% Tail DNA		Tail length		Olive tail moment		Statistics
		Mean	S.D.	Mean	S.D.	Mean	S.D.	
0	0	4.19	± 0.84	8.00	± 0.52	0.53	± 0.26	-
1.25	100	4.24	± 0.92	8.35	± 1.26	0.40	± 0.08	
2.5	100	5.15	± 0.33	9.78	± 0.67	0.50	± 0.06	a
5	100	5.54	± 0.79	12.00 *	± 3.21	0.69	± 0.14	
0	100	5.26	± 0.65	11.47 *	± 2.06	0.54	± 0.10	b

One hour later Sodium nitrite treatment, CHL cells were rinsed twice with PBS and treated with EGCG for one hour.

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Welch test.

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 11 Reverse mutation test (-S9 mix) (pH7.4)

Test substance : Sodium nitrite + EGCG

EGCG Dose ($\mu\text{g}/\text{plate}$)	No. of revertant colonies/plate			
	TA100		WP2 <i>uvrA</i> /pKM101	
	Pure water	Sodium nitrite 5000 $\mu\text{g}/\text{plate}$	Pure water	Sodium nitrite 5000 $\mu\text{g}/\text{plate}$
Solvent control (Pure water)	114	499	84	205
	105	453	95	212
	108	445	91	241
	(109 \pm 5)	(466 \pm 29)	(90 \pm 6)	(219 \pm 19)
39.1	117	466	98	212
	121	447	102	228
	115	439	95	235
	(118 \pm 3)	(451 \pm 14)	(98 \pm 4)	(225 \pm 12)
78.1	119	432	106	228
	131	442	115	235
	122	432	101	231
	(124 \pm 6)	(435 \pm 6)	(107 \pm 7)	(231 \pm 4)
156	109	412	127	251
	118	408	136	265
	133	431	110	221
	(120 \pm 12)	(417 \pm 12)	(124 \pm 13)	(246 \pm 22)
313	121	464	103	214
	117	416	100	184
	126	433	113	179
	(121 \pm 5)	(438 \pm 24)	(105 \pm 7)	(192 \pm 19)
625	108	445	95	205
	124	401	85	219
	113	379	112	241
	(115 \pm 8)	(408 \pm 34)	(97 \pm 14)	(222 \pm 18)
1250	119	318 *	108	212 *
	99	314 *	106	194 *
	109	353 *	85	187 *
	(109 \pm 10)	(328 \pm 21)	(100 \pm 13)	(198 \pm 13)
2500	109 *	0 *	57 *	94 *
	124 *	0 *	74 *	86 *
	98 *	0 *	55 *	90 *
	(110 \pm 13)	(0 \pm 0)	(62 \pm 10)	(90 \pm 4)
Positive control	AF-2		AF-2	
$\mu\text{g}/\text{plate}$	0.01		0.01	
Revertants/plate	498		1191	
	496		1151	
	501		1093	
	(498 \pm 3)		(1145 \pm 49)	

() : Mean \pm SD

* : Toxicity to the tester strains was observed.

Table 12 Reverse mutation test (-S9 mix) (pH6.0)

Test substance : Sodium nitrite + EGCG

EGCG Dose ($\mu\text{g}/\text{plate}$)	No. of revertant colonies/plate			
	TA100		WP2 <i>uvrA</i> /pKM101	
	Pure water	Sodium nitrite 5000 $\mu\text{g}/\text{plate}$	Pure water	Sodium nitrite 5000 $\mu\text{g}/\text{plate}$
Solvent control (Pure water)	120	458	66	368
	124	497	81	373
	156	445	88	399
	(133 \pm 20)	(467 \pm 27)	(78 \pm 11)	(380 \pm 17)
39.1	113	469	67	264
	118	429	83	295
	101	407	73	275
	(111 \pm 9)	(435 \pm 31)	(74 \pm 8)	(278 \pm 16)
78.1	130	452	80	244
	131	420	82	220
	107	375	77	242
	(123 \pm 14)	(416 \pm 39)	(80 \pm 3)	(235 \pm 13)
156	106	455	77	247
	143	426	78	247
	136	422	91	268
	(128 \pm 20)	(434 \pm 18)	(82 \pm 8)	(254 \pm 12)
313	131	446	88	265
	123	475	87	266
	122	496	89	232
	(125 \pm 5)	(472 \pm 25)	(88 \pm 1)	(254 \pm 19)
625	121	460	82	256
	137	431	74	236
	128	377	87	205
	(129 \pm 8)	(423 \pm 42)	(81 \pm 7)	(232 \pm 26)
1250	117	385	87	281
	152	421	72	318
	108	388	58	309
	(126 \pm 23)	(398 \pm 20)	(72 \pm 15)	(303 \pm 19)
2500	100 *	247 *	54 *	178 *
	110 *	260 *	51 *	208 *
	89 *	252 *	40 *	151 *
	(100 \pm 11)	(253 \pm 7)	(48 \pm 7)	(179 \pm 29)
Negative control (Pure water) (pH7.4)	127	521	61	190
	125	463	85	218
	132	410	74	199
	(128 \pm 4)	(465 \pm 56)	(73 \pm 12)	(202 \pm 14)
Positive control	AF-2		AF-2	
$\mu\text{g}/\text{plate}$	0.01		0.01	
Revertants/plate	439		757	
	422		869	
	445		857	
	(435 \pm 12)		(828 \pm 61)	

() : Mean \pm SD

* : Toxicity to the tester strains was observed.

Table 13 Reverse mutation test (-S9 mix) (pH5.0)

Test substance : Sodium nitrite + EGCG

EGCG Dose ($\mu\text{g}/\text{plate}$)	No. of revertant colonies/plate	
	WP2 <i>uvrA</i> /pKM101	
	Pure water	Sodium nitrite 313 $\mu\text{g}/\text{plate}$
Solvent control (Pure water)	81	871
	77	866
	70	962
	(76 \pm 6)	(900 \pm 54)
39.1	68	997
	50	929
	73	904
	(64 \pm 12)	(943 \pm 48)
78.1	84	992
	73	998
	78	972
	(78 \pm 6)	(987 \pm 14)
156	79	918
	73	928
	83	1031
	(78 \pm 5)	(959 \pm 63)
313	76	1130
	73	1203
	80	1175
	(76 \pm 4)	(1169 \pm 37)
625	66	1052
	68	1097
	68	1102
	(67 \pm 1)	(1084 \pm 28)
1250	64	597 *
	46	574 *
	38	541 *
	(49 \pm 13)	(571 \pm 28)
2500	44 *	143 *
	46 *	142 *
	31 *	179 *
	(40 \pm 8)	(155 \pm 21)
Solvent control (Pure water) (pH7.4)	71	
	77	
	77	
	(75 \pm 3)	
Positive control	AF-2	
$\mu\text{g}/\text{plate}$	0.01	
Revertants/plate	1027	
	1034	
	991	
	(1017 \pm 23)	

() : Mean \pm SD

* : Toxicity to the tester strains was observed.

Table 14 Chromosome aberrations in CHL cells treated with a combination of sodium nitrite and EGCG for 3 h (-S9 mix)

S9 mix	Treatment		No. of cells scored	Polyploid cells	No. of cells with structural chromosome aberrations										Mitotic index (%)	Relative mitotic index (%)
	Sodium nitrite (mg/mL)	EGCG (μ M)			Gap	Chromatid type			Chromosome type		Fragmentation	Others	Total			
						ctb	cte	csb	cse	+g			-g			
-	0	0 (control)	200	2 (1.0)	2	1	0	0	0	0	0	0	3 (1.5)	1 (0.5)	5.6	100
		50	200	0 (0.0)	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	3.9	70
		100	200	0 (0.0)	1	1	0	0	0	0	0	0	2 (1.0)	1 (0.5)	3.8	68
		200	200	3 (1.5)	0	1	1	0	0	0	0	0	2 (1.0)	2 (1.0)	2.2	39
-	0.5	400	Toxic	-	-	-	-	-	-	-	-	-	-	-	-	-
		50	200	4 (2.0)	1	9	2	0	0	0	0	1	12 (6.0)	11 (5.5)**	3.8	68
		100	200	2 (1.0)	3	12	13	0	0	0	0	1	26 (13.0)	23 (11.5)***	2.9	52
		200	100	2 (2.0)	1	12	15	0	0	1	0	0	22 (22.0)	21 (21.0)***	0.6	11
-	5	400	Toxic	-	-	-	-	-	-	-	-	-	-	-	-	-
		50	200	3 (1.5)	3	10	23	0	0	0	0	7	36 (18.0)	34 (17.0)***	3.0	54
		100	200	4 (2.0)	5	14	17	0	1	0	0	0	32 (16.0)	30 (15.0)***	2.4	43
		200	100	0 (0.0)	3	35	47	0	1	1	0	0	61 (61.0)	60 (60.0)***	1.1	20
-	400	Toxic	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Toxic	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; +g, including gaps ; -g, excluding gaps

EGCG: (-)-epigallocatechin-gallate

The figures shown in parentheses are percentages.

,* : Significantly different from the concurrent control at $p \leq 0.01$ and $p \leq 0.001$, respectively.

Table 15 Chromosome aberrations in CHL cells treated with a combination of sodium nitrite and EGCG for 3 h (+S9 mix)

S9 mix	Treatment		No. of cells scored	Polyploid cells	No. of cells with structural chromosome aberrations										Mitotic index (%)	Relative mitotic index (%)	
	Sodium nitrite (mg/mL)	EGCG (μ M)			Gap		Chromatid type		Chromosome type			Others	Total				
					ctb	cte	ctb	cse	Fragmentation	+g	-g						
+	0	0 (control)	200	1 (0.5)	0	1	0	0	0	0	0	0	0	1 (0.5)	1 (0.5)	7.0	100
		50	200	5 (2.5)	0	5	1	0	2	0	0	0	0	8 (4.0)	8 (4.0)*	5.5	79
		100	200	0 (0.0)	3	6	1	0	1	0	0	0	0	11 (5.5)	8 (4.0)*	6.2	89
		200	200	1 (0.5)	1	5	2	0	0	0	0	0	0	7 (3.5)	6 (3.0)	5.3	76
		400	200	0 (0.0)	2	28	11	0	0	0	1	2	39 (19.5)	37 (18.5)***	6.0	86	
	0.5	50	200	1 (0.5)	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	8.8	126
		100	200	1 (0.5)	1	0	0	0	0	0	0	0	1 (0.5)	0 (0.0)	6.6	94	
		200	200	2 (1.0)	0	3	11	0	0	0	0	0	13 (6.5)	13 (6.5)**	6.7	96	
		400	200	2 (1.0)	1	7	16	5	1	1	2	27 (13.5)	26 (13.0)***	2.0	29		
		50	200	6 (3.0)	0	10	24	0	0	1	3	30 (15.0)	30 (15.0)***	5.5	79		
5	100	200	3 (1.5)	1	18	34	0	0	0	7	45 (22.5)	45 (22.5)***	3.5	50			
	200	200	6 (3.0)	2	27	48	0	1	1	3	67 (33.5)	67 (33.5)***	4.2	60			
	400	200	5 (2.5)	5	34	37	0	1	1	4	67 (33.5)	65 (32.5)***	3.9	56			

ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; +g, including gaps ; -g, excluding gaps

EGCG: (-)-epigallocatechin-gallate

The figures shown in parentheses are percentages.

*, **, *** : Significantly different from the concurrent control at $p \leq 0.05$, 0.01 and 0.001, respectively.

Table 16 *In vitro* comet assay - Sodium nitrite + EC

Sodium nitrite (mg/ml)	EC (μ M)	% Tail DNA		Tail length		Olive tail moment		Cell viability %	Statistics
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.				
0	0	5.18 \pm 0.42	15.94 \pm 3.31	0.61 \pm 0.07	100	-			
1.25	100	5.20 \pm 1.51	15.52 \pm 1.76	0.56 \pm 0.18	75				
2.5	100	5.51 \pm 0.62	16.06 \pm 2.37	0.57 \pm 0.07	67	a			
5	100	7.12 \pm 0.76	18.07 \pm 6.84	0.78 \pm 0.11	71				
0	100	7.08 \pm 1.58	16.81 \pm 4.73	0.71 \pm 0.19	101	b			

EC: (-)-epicatechin

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Welch test.

Table 17 *In vitro* comet assay - Sodium nitrite + ECG

Sodium nitrite (mg/ml)	ECG (μ M)	% Tail DNA		Tail length		Olive tail moment		Cell viability %	Statistics
		Mean \pm S.D.	S.D.	Mean \pm S.D.	S.D.	Mean \pm S.D.	S.D.		
0	0	6.61 \pm 1.69		17.64 \pm 5.08		0.72 \pm 0.18		100	-
1.25	100	5.08 \pm 0.52		16.24 \pm 2.28		0.58 \pm 0.04		76	
2.5	100	6.54 \pm 1.79		17.14 \pm 2.01		0.79 \pm 0.20		73	a
5	100	6.40 \pm 1.07		17.99 \pm 1.73		0.77 \pm 0.19		60	
0	100	6.07 \pm 1.30		16.68 \pm 4.90		0.70 \pm 0.20		102	b

ECG: (-)-epicatechin-gallate

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Weich test.

Table 18 *In vitro* comet assay - Sodium nitrite + EGC

Sodium nitrite (mg/ml)	EGC (µM)	% Tail DNA		Tail length		Olive tail moment		Cell viability %	Statistics
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.				
0	0	4.75 ± 0.86	11.61 ± 1.85	0.54 ± 0.17	100	-			
1.25	100	24.48 ** ± 2.15	41.76 ** ± 2.98	6.02 ** ± 0.77	73				
2.5	100	31.01 ** ± 5.33	46.65 ** ± 5.23	7.75 ** ± 1.45	66	a			
5	100	30.66 ** ± 1.51	48.18 ** ± 2.21	8.30 ** ± 0.50	57				
0	100	8.65 *** ± 0.62	23.76 ** ± 3.12	1.19 ** ± 0.16	104	b			

EGC: (-)-epigallocatechin

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Welch test.

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 19 DNA damage in CHL cells after 1h treatment with acrylamide

Sampling time (h)	Substance	Dose (mg/mL)	% Migrated DNA		Tail Length		Olive Tail Moment	
			Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
1	Control (10%saline)	0	3.40	± 0.92	5.08	± 1.01	0.39	± 0.17
		0.5	4.82	± 0.90	7.64	± 1.59	0.56	± 0.05
	Acrylamide ^{a)}	1.0	5.93	± 1.79	9.03	± 2.48	0.67	± 0.20
		2.0	6.62	± 2.60	14.34	** ± 4.74	0.94	± 0.51
	4NQO ^{b)}	2 μM	30.50	*** ± 3.30	36.98	*** ± 2.65	8.12	** ± 1.79

4NQO: 4-nitroquinoline 1-oxide

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Weich test.

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 20 DNA damage in CHL cells treated with a combination of sodium nitrite and acrylamide

Sampling time (h)	Substance (Concentration)	Acrylamide (mg/mL)	% Migrated DNA		Tail Length		Olive Tail Moment	
			Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
1	Control (10%saline)	0	3.74	± 1.00	7.37	± 3.22	0.47	± 0.18
		0.5	4.28	± 0.78	5.54	± 0.35	0.46	± 0.08
		1.0	3.06	± 1.18	5.63	± 2.38	0.36	± 0.14
	Sodium nitrite ^{a)} (5mg/mL)	2.0	4.63	± 1.56	8.44	± 2.87	0.55	± 0.20
		0	34.50	*** ± 4.23	35.53	*** ± 5.76	8.73	** ± 1.73
		4NQO ^{b)} (2µM)						

4NQO: 4-nitroquinoline 1-oxide

a: Data were statically analyzed by Dunnett's test following one-way ANOVA.

b: Data were statically analyzed by Aspin-Welch test.

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$.

Table 21 Chromosome aberrations in CHL cells treated with a combination of sodium nitrite and acrylamide for 3 h (-S9 mix)

S9 mix	Treatment		No. of cells scored	Polyploid cells	Gap	No. of cells with structural chromosome aberrations										Mitotic index (%)	Relative mitotic index (%)	
	Sodium nitrite (mg/mL)	Acrylamide (mg/mL)				Chromatid type		Chromosome type	Fragmentation	Others	Total		Mitotic index (%)	Relative mitotic index (%)				
						ctb	cte				csb	cse			+g			-g
0	0	0 (control)	200	2 (1.0)	2	1	0	0	0	0	0	0	0	3 (1.5)	1 (0.5)	5.6	100	
		0.25	200	4 (2.0)	0	1	0	0	0	0	0	0	0	1 (0.5)	1 (0.5)	4.5	80	
		0.5	200	7 (3.5)	0	1	0	0	0	0	0	0	0	1 (0.5)	1 (0.5)	4.4	79	
		1	200	21 (10.5) ***	0	5	3	1	0	0	0	0	0	8 (4.0)	8 (4.0) *	3.6	64	
		2	200	11 (5.5) *	5	19	20	0	0	0	0	1	0	38 (19.0)	34 (17.0) ***	3.1	55	
		4	Toxic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		0.25	200	1 (0.5)	0	1	0	0	0	0	0	0	0	1 (0.5)	1 (0.5)	5.8	104	
0.5	0.5	0.5	200	6 (3.0)	3	0	0	0	0	0	0	0	3 (1.5)	0 (0.0)	5.4	96		
		1	200	12 (6.0) *	1	6	5	0	0	0	0	0	10 (5.0)	9 (4.5) *	3.6	64		
		2	200	12 (6.0) *	2	10	11	0	0	1	1	0	23 (11.5)	22 (11.0) ***	3.1	55		
5	5	0.25	200	2 (1.0)	0	0	4	0	0	0	0	0	4 (2.0)	4 (2.0)	4.2	75		
		0.5	200	4 (2.0)	1	6	8	0	0	0	0	0	10 (5.0)	9 (4.5) *	3.3	59		
		1	200	7 (3.5)	1	16	33	0	0	0	0	0	39 (19.5)	38 (19.0) ***	2.8	50		
2	200	5 (2.5)	4	21	17	0	0	0	0	2	39 (19.5)	35 (17.5) ***	2.7	48				

ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; +g, including gaps ; -g, excluding gaps
 The figures shown in parentheses are percentages.

*, *** : Significantly different from the concurrent control at $p \leq 0.05$ and $p \leq 0.001$, respectively.

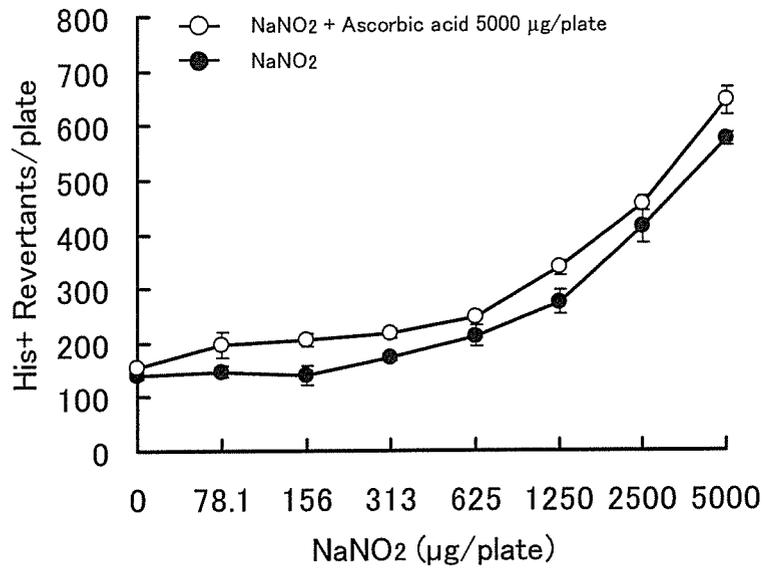
Table 22 Chromosome aberrations in CHL cells treated with a combination of sodium nitrite and acrylamide for 3 h (+S9 mix)

S9 mix	Treatment		No. of cells scored	Polyploid cells	Gap	No. of cells with structural chromosome aberrations							Mitotic index (%)	Relative mitotic index (%)		
	Sodium nitrite (mg/mL)	Acrylamide (mg/mL)				Chromatid type		Chromosome type		Fragmentation	Others	Total				
						ctb	cte	csb	cse			+g			-g	
+	0	0 (control)	200	1 (0.5)	0	1	0	0	0	0	0	0	1 (0.5)	1 (0.5)	7.0	100
		0.25	200	4 (2.0)	1	0	0	0	0	0	0	0	1 (0.5)	0 (0.0)	6.9	99
		0.5	200	4 (2.0)	0	2	1	0	0	0	0	0	3 (1.5)	3 (1.5)	5.5	79
	0.5	1	200	26 (13.0)***	1	2	0	0	0	0	0	0	3 (1.5)	2 (1.0)	6.8	97
		2	200	14 (7.0)**	2	13	23	0	0	0	0	3	34 (17.0)	32 (16.0)***	6.7	96
		0.25	200	0 (0.0)	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	6.2	89
5	0.5	0.5	200	2 (1.0)	1	0	0	0	0	0	0	1 (0.5)	0 (0.0)	5.7	81	
		1	200	22 (11.0)***	0	1	1	0	0	0	0	1 (0.5)	1 (0.5)	5.8	83	
		2	200	6 (3.0)	2	24	66	0	0	0	6	82 (41.0)	81 (40.5)***	5.0	71	
5	0.25	0.25	200	2 (1.0)	0	1	2	0	0	0	0	3 (1.5)	3 (1.5)	5.6	80	
		0.5	200	4 (2.0)	0	0	2	0	0	0	0	2 (1.0)	2 (1.0)	6.1	87	
		1	200	8 (4.0)*	1	3	10	0	0	0	0	12 (6.0)	11 (5.5)**	4.8	69	
		2	200	5 (2.5)	2	22	64	0	0	0	8	84 (42.0)	82 (41.0)***	5.2	74	

ctb, chromatid break ; cte, chromatid exchange ; csb, chromosome break ; cse, chromosome exchange ; +g, including gaps ; -g, excluding gaps
 The figures shown in parentheses are percentages.

*, **, *** : Significantly different from the concurrent control at $p \leq 0.05$, 0.01 and 0.001, respectively.

TA100



WP2uvrA/pKM101

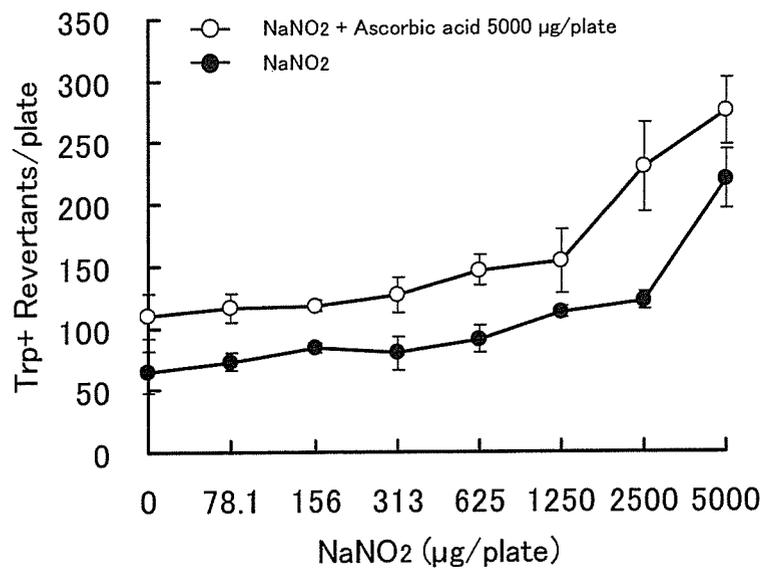


Fig. 1 Additive effects of combined treatment with NaNO₂ and ascorbic acid on bacterial mutagenicity. TA100 and WP2uvrA/pKM101 were simultaneously treated with NaNO₂ and ascorbic acid.

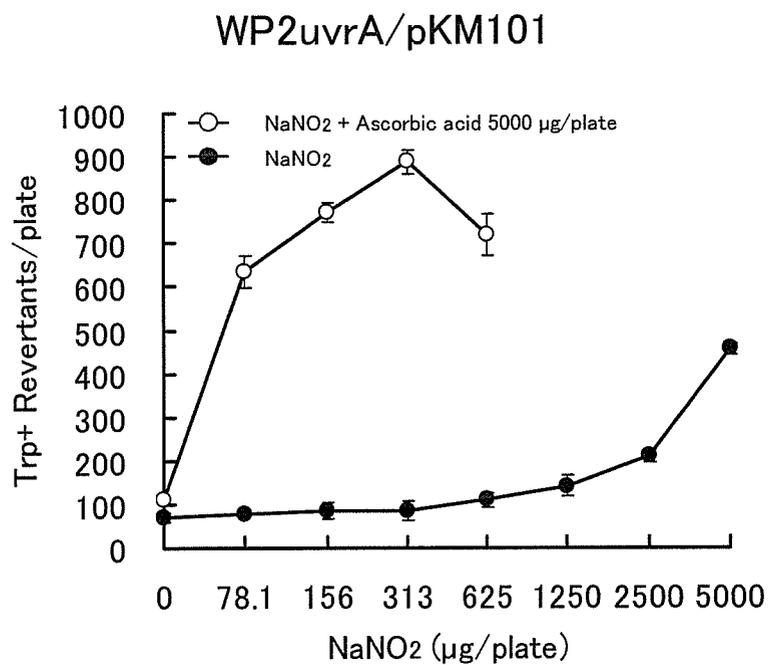
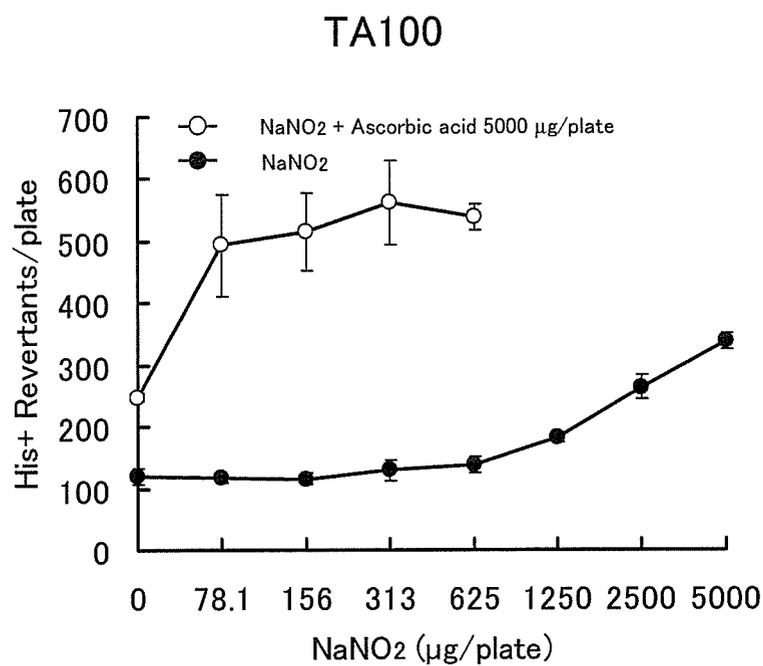


Fig. 2 Synergistic effects of combined treatment with NaNO₂ and ascorbic acid on bacterial mutagenicity. TA100 and WP2uvrA/pKM101 were simultaneously treated with NaNO₂ and ascorbic acid in pH 6.0 buffer.