

Figure 1. Growth inhibition of CHL cells treated with 2,6-bis(1,1-dimethylethyl)-4-ethylphenol [Short-term treatment]

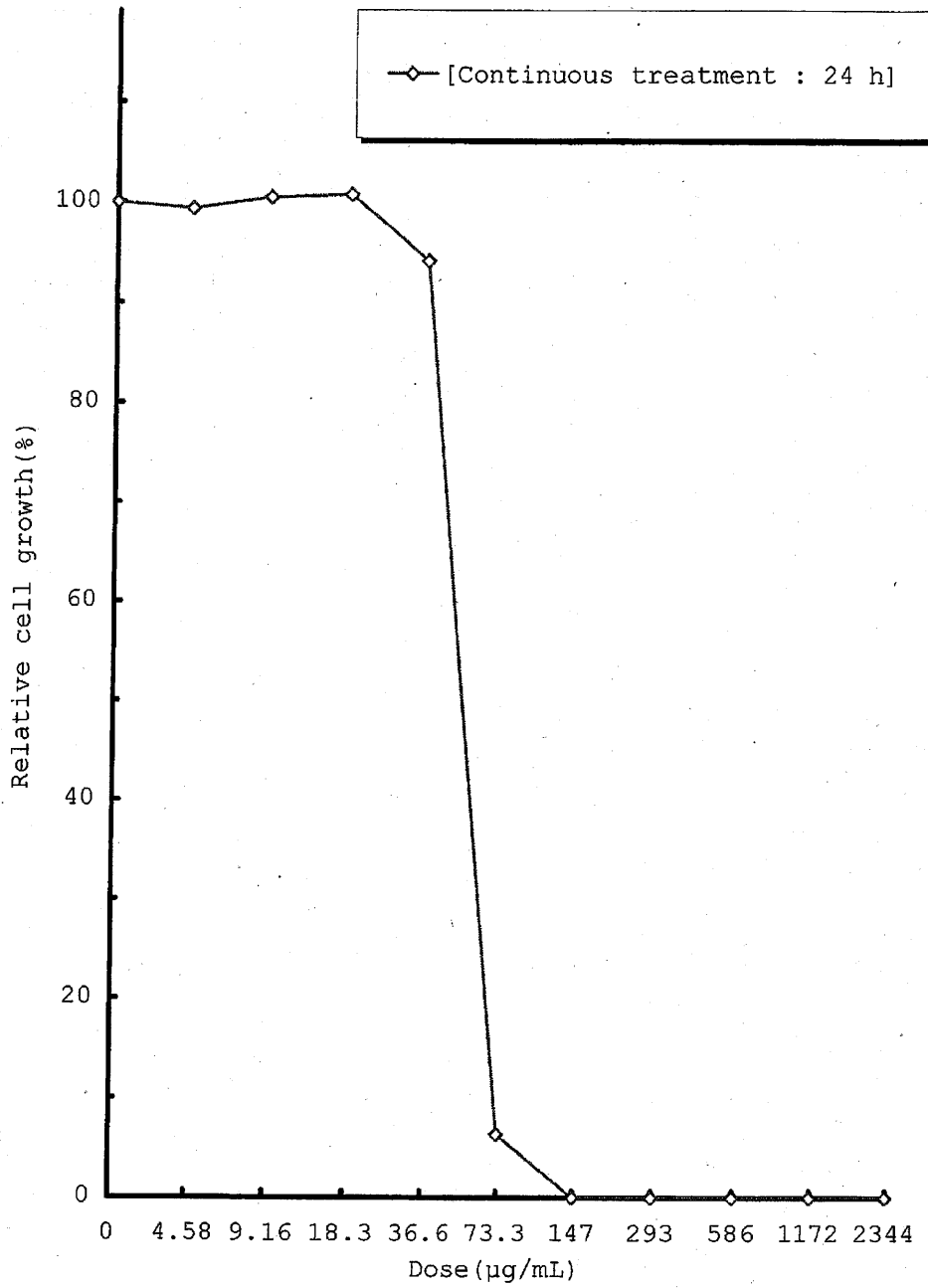


Figure 2. Growth inhibition of CHL cells treated with 2,6-bis(1,1-dimethylethyl)-4-ethylphenol [Continuous treatment]

Table 1. Results of growth inhibition test of 2,6-bis(1,1-dimethylethyl)-4-ethylphenol
 [Short-term treatment]

Exp. No. 9049 (115-200)

Compound	[Short-term treatment : -S9]		[Short-term treatment : +S9]	
	Dose (µg/mL)	Survival (%) [Mean]	Dose (µg/mL)	Survival (%) [Mean]
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	100.0 [100.0]	0 a)	100.0 [100.0]
	4.58	101.3 [102.6]	4.58	98.7 [95.8]
	9.16	97.7 [97.5]	9.16	96.3 [94.3]
	18.3	101.2 [101.2]	18.3	93.0 [90.4]
	36.6	105.4 [100.8]	36.6	41.2 [45.3]
	73.3	38.6 [37.4]	73.3	5.3 [5.3]
	147 d)	0.0 [0.0]	147 d)	1.3 [2.4]
	293 d)	0.0 [0.0]	293 d)	0.7 [1.1]
	586 d)	0.0 [0.0]	586 d)	0.8 [0.6]
	1172 d)	0.0 [0.0]	1172 d)	0.0 [1.0]
	2344 d)	0.0 [0.0]	2344 d)	2.0 [2.8]
		0.0		3.6

50% Growth inhibition dose was as follows:

[Short-term treatment : -S9] ——— 71.0 (µg/mL)

[Short-term treatment : +S9] ——— 34.3 (µg/mL)

a): Negative control (Dimethyl sulfoxide, 10 µL/mL)

d): Visible precipitation was observed at the end of exposure period.

Table 2. Results of growth inhibition test of 2,6-bis(1,1-dimethylethyl)-4-ethylphenol
[Continuous treatment]

[Continuous treatment : 24 h]		
Compound	Dose (µg/mL)	Survival (%) [Mean]
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	100.0 [100.0]
		100.0
	4.58	98.5 [99.4]
		100.3
	9.16	98.9 [100.5]
		102.1
	18.3	100.6 [100.8]
		100.9
	36.6	103.8 [94.2]
		84.5
	73.3	5.4 [6.4]
		7.3
	147 d)	0.0 [0.0]
		0.0
	293 d)	0.0 [0.0]
	0.0	
586 d)	0.0 [0.0]	
	0.0	
1172 d)	0.0 [0.0]	
	0.0	
2344 d)	0.0 [0.0]	
	0.0	

50% Growth inhibition dose was as follows:

[Continuous treatment : 24 h] — 52.0 (µg/mL)

a): Negative control (Dimethyl sulfoxide, 10 µL/mL)

d): Visible precipitation was observed at the end of exposure period.

Table 3. Chromosome aberration test in CHL cells treated with 2,6-bis(1,1-dimethylethyl)-4-ethylphenol
 [Short-term treatment: -S9] Exp. No. 9049 (115-200)

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of analyzed cells	Number of cells with structural aberrations				Number of cells analyzed with aberrations		Number of polyploid cells (%)			
					gap	ctb	cte	csb	cse	oth		aberrations -gap(%)	for polyploid	
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	6	100.0	200	1	2	0	0	0	2	(1.0)	200	0	(0.0)
	35.0	6	130.6	200	0	1	2	1	0	0	(2.0)	200	1	(0.5)
	55.0	6	120.0	200	0	1	2	0	0	3	(1.5)	200	1	(0.5)
	75.0 d)	6	49.0	200	1	0	1	0	0	1	(0.5)	200	1	(0.5)
85.0 d)	6	20.1	NA											
MMC b)	0.1	6	78.6	200	12	34	54	0	0	76	(38.0) *	200	2	(1.0)

Abbreviation: ctb; chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, oth: others
 -gap: total number of cells with aberrations except gap
 * : Significant difference from control (Fisher's exact test): $p \leq 0.025$
 NA: Not analyzed
 a): Negative control (Dimethyl sulfoxide, 10 µL/mL)
 b): Positive control (Mitomycin C)
 d): Visible precipitation was observed at the end of exposure period.

Table 4. Chromosome aberration test in CHL cells treated with 2,6-bis(1,1-dimethylethyl)-4-ethylphenol
 [Short-term treatment : +S9] Exp. No. 9049 (115-200)

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of cells analyzed	Number of cells with structural aberrations					Number of cells with aberrations -gap(%)		Number of cells analyzed for polyploid	Number of polyploid cells (%)	
					gap	ctb	cte	csb	cse	oth	aberrations -gap(%)			#
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	6	100.0	200	2	2	1	0	0	0	3	3	200	1 (0.5) #
	25.0	6	88.1	200	2	3	1	0	0	0	4	4	200	3 (1.5)
	35.0	6	89.0	200	2	5	7	0	0	0	11	11	200	11 (5.5) *
	45.0	6	50.2	200	4	10	10	1	0	0	17	17	200	10 (5.0) *
	55.0	6	37.9	200	3	6	14	0	0	0	16	16	200	10 (5.0) *
CP b)	12.5	6	118.9	200	5	27	64	0	0	0	77	77	200	0 (0.0)

Abbreviation: ctb; chromatid break, cte; chromatid exchange, csb: chromosome break, cse: chromosome exchange, oth: others
 -gap: total number of cells with aberrations except gap
 * :Significant difference from control (Fisher's exact test):p≤0.025
 # :Significant difference from control (Choctran-Armitage trend test):p≤0.025
 a): Negative control (Dimethyl sulfoxide, 10 µL/mL)
 b): Positive control (Cyclophosphamide)

Table 5. Chromosome aberration test in CHL cells treated with 2,6-Bis(1,1-dimethylethyl)-4-ethylphenol (Confirmative examination) [Short-term treatment : +S9] Exp. No. 9049 (115-200)

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of cells analyzed	Number of cells with structural aberrations					Number of cells analyzed with aberrations -gap(%) for polyploid		Number of polyploid cells (%)			
					gap	ctb	cte	csb	cse	oth	aberrations -gap(%)		#		
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	6	100.0	200	1	1	0	0	0	0	1 (0.5)	#	200	0 (0.0)	#
	15.0	6	81.0	200	1	1	0	0	0	0	1 (0.5)		200	1 (0.5)	
	25.0	6	71.9	200	0	0	0	0	0	0	0 (0.0)		200	2 (1.0)	
	35.0	6	65.0	200	1	0	2	0	0	0	2 (1.0)		200	14 (7.0)	*
	45.0	6	36.4	200	3	5	11	0	0	0	12 (6.0)	*	200	16 (8.0)	*
	55.0	6	22.9	200	0	3	8	0	0	0	9 (4.5)	*	200	14 (7.0)	*
	65.0	6	6.1	Toxic											
CP b)	12.5	6	119.1	200	4	18	86	0	1	0	94 (47.0)	*	200	0 (0.0)	

Abbreviation: ctb; chromatid break; cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, oth: others
 -gap: total number of cells with aberrations except gap
 * : Significant difference from control (Fisher's exact test): $p \leq 0.025$
 # : Significant difference from control (Chochran-Armitage trend test): $p \leq 0.025$
 a): Negative control (Dimethyl sulfoxide, 10 µL/mL)
 b): Positive control (Cyclophosphamide)

Table 6. Chromosome aberration test in CHL cells treated with 2,6-bis(1,1-dimethylethyl)-4-ethylphenol
 [Continuous treatment : 24 h] Exp. No. 9049 (115-200)

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of analyzed cells	Number of cells with				Number of cells with		Number of polyploid cells (%)		
					ctb	cte	csb	cse	oth	aberrations -gap(%)		for polyploid	
2,6-Bis(1,1- dimethylethyl) -4-ethylphenol	0 a)	24	100.0	200	2	1	1	0	0	0	2 (1.0)	200	0 (0.0)
	35.0	24	102.5	200	0	2	1	0	0	0	3 (1.5)	200	1 (0.5)
	55.0	24	104.7	200	0	3	0	0	0	0	3 (1.5)	200	3 (1.5)
	75.0 d)	24	35.6	200	2	0	0	0	0	0	0 (0.0)	200	0 (0.0)
MMC b)	0.05	24	138.5	200	12	18	46	0	0	0	54 (27.0) *	200	0 (0.0)

Abbreviation: ctb; chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, oth: others
 -gap: total number of cells with aberrations except gap

* : Significant difference from control (Fisher's exact test): $p \leq 0.025$

a): Negative control (Dimethyl sulfoxide, 10 µL/mL)

b): Positive control (Mitomycin C)

d): Visible precipitation was observed at the end of exposure period.

Appendix 1. Chromosome aberration test of 2,6-bis(1,1-dimethylethyl)-4-ethylphenol [Short-term treatment : -S9] Exp. No. 9049 (115-200)

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of cells analyzed	Number of cells with structural aberrations						Only gap (%)	Total (-gap) (%) for polyploid	Number of cells analyzed for polyploid	Polyploid cells (%)
					gap	ctb	cte	csb	cse	oth				
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	6	100.0	100	1	1	0	0	0	0	0	1.0	100	0.0
		6	100.0	100	0	1	0	0	0	0	0	0.0	100	0.0
	35.0	6	129.3	100	0	1	1	0	0	0	0	0.0	100	0.0
		6	131.8	100	0	0	1	1	0	0	0	0.0	100	1.0
	55.0	6	110.3	100	0	1	1	0	0	0	0	0.0	100	1.0
		6	129.7	100	0	0	1	0	0	0	0	0.0	100	0.0
75.0 d)	6	46.4	100	1	0	1	0	0	0	0	0.0	100	0.0	
	6	51.5	100	0	0	0	0	0	0	0	0.0	100	1.0	
85.0 d)	6	28.1	NA											
	6	12.1	NA											
MMC b)	0.1	6	71.4	100	6	16	27	0	0	0	3.0	38.0	100	1.0
		6	85.8	100	6	18	27	0	0	0	2.0	38.0	100	1.0

Abbreviation: ctb; chromatid break, cte; chromatid exchange, csb; chromosome break, cse; chromosome exchange, oth; others
 -gap: total number of cells with aberrations except gap
 a): Negative control (Dimethyl sulfoxide, 10 µL/mL)
 b): Positive control (Mitomycin C)
 d): Visible precipitation was observed at the end of exposure period.
 NA: Not analyzed

Appendix 2. Chromosome aberration test of 2,6-bis(1,1-dimethylethyl)-4-ethylphenol
 [Short-term treatment : +89]

Exp. No. 9049 (115-200)

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of cells analyzed	Number of cells with structural aberrations					Only gap (%)	Total (-gap) (%)	Number of cells analyzed for polyploid (%)	Polyploid cells (%)
					gap	ctb	cte	csb	cse				
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	6	100.0	100	1	1	0	0	0	0	1.0	2.0	1.0
		6	100.0	100	1	1	0	0	0	0	1.0	1.0	0.0
	25.0	6	71.9	100	0	2	0	0	0	0	0.0	2.0	1.0
		6	104.2	100	2	1	1	0	0	0	2.0	2.0	2.0
	35.0	6	85.7	100	0	3	4	0	0	0	0.0	6.0	7.0
		6	92.3	100	2	2	3	0	0	0	2.0	5.0	4.0
45.0	6	50.7	100	3	5	3	0	0	0	2.0	7.0	6.0	
	6	49.6	100	1	5	7	1	0	0	1.0	10.0	4.0	
55.0	6	44.7	100	2	4	7	0	0	0	2.0	8.0	8.0	
	6	31.1	100	1	2	7	0	0	0	1.0	8.0	2.0	
CP b)	12.5	6	137.3	100	2	7	29	0	0	0	1.0	34.0	0.0
		6	100.4	100	3	20	35	0	0	0	1.0	43.0	0.0

Abbreviation: ctb; chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, oth: others
 -gap: total number of cells with aberrations except gap
 a): Negative control (Dimethyl sulfoxide, 10 µL/mL)
 b): Positive control (Cyclophosphamide)

Appendix 3. Chromosome aberration test of 2,6-bis(1,1-dimethylethyl)-4-ethylphenol (Confirmative examination) [Short-term treatment: +S9] Exp. No. 9049 (115-200)

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of cells analyzed	Number of cells with structural aberrations						Only gap (%)	Total (-gap) (%) for polyploid	Number of cells analyzed for polyploid	Polyploid cells (%)	
					gap	ctb	cte	csb	cse	oth					
2,6-Bis(1,1-dimethylethyl)-4-ethylphenol	0 a)	6	100.0	100	0	1	0	0	0	0	0	0.0	1.0	100	0.0
		6	100.0	100	1	0	0	0	0	0	0	1.0	0.0	100	0.0
	15.0	6	83.5	100	1	0	0	0	0	0	1.0	0.0	0.0	100	1.0
		6	78.4	100	0	1	0	0	0	0	0.0	1.0	1.0	100	0.0
	25.0	6	67.1	100	0	0	0	0	0	0	0.0	0.0	0.0	100	1.0
		6	76.6	100	0	0	0	0	0	0	0.0	0.0	0.0	100	1.0
	35.0	6	63.1	100	1	0	1	0	0	0	1.0	1.0	1.0	100	8.0
		6	66.9	100	0	0	1	0	0	0	0.0	1.0	1.0	100	6.0
	45.0	6	37.9	100	1	3	5	0	0	0	0.0	6.0	6.0	100	5.0
		6	34.9	100	2	2	6	0	0	0	1.0	6.0	6.0	100	11.0
	55.0	6	17.1	100	0	2	5	0	0	0	0.0	6.0	6.0	100	9.0
		6	28.6	100	0	1	3	0	0	0	0.0	3.0	3.0	100	5.0
CP b)	65.0	6	5.0	Toxic											
		6	7.2	Toxic											
	12.5	6	125.4	100	2	9	36	0	1	0	1.0	41.0	41.0	100	0.0
		6	112.8	100	2	9	50	0	0	0	1.0	53.0	53.0	100	0.0

Abbreviation: ctb; chromatid break, cte; chromatid exchange, csb; chromosome break, cse; chromosome exchange, oth; others
 -gap: total number of cells with aberrations except gap
 a): Negative control (Dimethyl sulfoxide, 10 µL/mL)
 b): Positive control (Cyclophosphamide)

Appendix 4. Chromosome aberration test of 2,6-bis(1,1-dimethylethyl)-4-ethylphenol
[Continuous treatment : 24 h]

Compound	Dose (µg/mL)	Time of exposure (h)	Relative Cell growth (%)	Number of cells analyzed	Number of cells with structural aberrations					Only gap (%)	Total (-gap) (%)	Number of cells analyzed for polyploid	Polyploid cells (%)
					gap	ctb	cte	csb	cse				
2,6-Bis(1,1 -dimethylethyl) -4-ethylphenol	0 a)	24	100.0	100	1	0	0	0	0	0	1.0	100	0.0
		24	100.0	100	1	1	1	0	0	0	1.0	100	0.0
	35.0	24	95.6	100	0	1	0	0	0	0	0.0	100	1.0
		24	109.4	100	0	1	1	0	0	0	0.0	100	0.0
	55.0	24	109.2	100	0	1	0	0	0	0	0.0	100	0.0
		24	100.2	100	0	2	0	0	0	0	0.0	100	3.0
	75.0 d)	24	29.6	100	2	0	0	0	0	0	2.0	100	0.0
		24	41.6	100	0	0	0	0	0	0	0.0	100	0.0
MMC b)	0.05	24	139.4	100	5	9	20	0	0	0	3.0	100	0.0
		24	137.6	100	7	9	26	0	0	0	4.0	100	0.0

Abbreviation: ctb; chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange, oth: others
-gap: total number of cells with aberrations except gap
a): Negative control (Dimethyl sulfoxide, 10 µL/mL)
b): Positive control (Mitomycin C)
d): Visible precipitation was observed at the end of exposure period.