

Table 2. Cell growth inhibition test of 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene in cultured CHL cells
-The continuous treatment method-

Test Substance	Concentration ($\mu\text{g/mL}$)	Treated for 24 hr		
		No. of cells ($\times 10^4/\text{plate}$)	Survival ratio ^{a)} (%)	IC ₅₀ ($\mu\text{g/mL}$)
Negative control (Dimethyl sulfoxide)	—	65	100	—
	4.69	62	95	
	9.38	59	91	
	18.8	44	68	
	37.5	23	35	
1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene	75	0	0	23.9
	150	0	0	
	300*	0	0	
	600*	0	0	
	1200*	0	0	
	2400*	0	0	

a): (1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene treated group / negative control) $\times 100$.

*: White oily precipitations were noted at the time of application of the test solution and clean oily precipitations were noted on completion of the incubation.

Table 3. Chromosomal aberration test of 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene in cultured CHL cells
— The short treatment method —

Test substance	Concentration ($\mu\text{g/mL}$)	With (+) or without (-) S9 mix	No. of metaphase examined	Numerical aberration				Structural aberrations										Survival ratio ^{e)} (%)		
				No. of Polyploid cells	No. of endoreduplication cells	Incidence ^{a)} (%)	Judgement ^{b)}	Types ^{c)} and numbers (cumulative)						No. of cells with chromosome aberration		Incidence ^{d)} (%)	Judgement ^{b)}			
								gap	ctb	csb	cte	cse	frg	(+g)	(-g)				(+g)	(-g)
Negative control	—	+	200	2	0	1.0	—	0	1	0	0	0	0	0	1	1	0.5	0.5	—	100
	12.5	+	200	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	—	98
1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene	25	+	200	1	0	0.5	—	0	1	0	0	0	0	1	1	0.5	0.5	—	90	
	50	+	200	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0	—	71
	100	+	200	2	0	1.0	—	0	1	0	1	0	0	1	1	0.5	0.5	—	49	
	200	+	200	2	0	1.0	—	0	2	0	1	0	0	3	3	1.5	1.5	—	30	
Dimethylnitrosamine	500	+	200	1	0	0.5	—	0	66	0	107	0	0	137	137	68.5	68.5	+	83	
Negative control	—	—	200	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	—	100	
	6.25	—	200	0	0	0	—	0	0	0	0	0	0	0	0	0	0	—	98	
1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene	12.5	—	200	0	0	0	—	0	2	0	0	0	0	2	2	1.0	1.0	—	98	
	25	—	200	1	0	0.5	—	0	2	0	0	0	0	2	2	1.0	1.0	—	82	
	50	—	200	0	0	0	—	0	0	0	1	0	0	1	1	0.5	0.5	—	53	
	100	—	200	1	0	0.5	—	0	3	0	0	0	0	3	3	1.5	1.5	—	31	
Mitomycin C	0.1	—	200	0	0	0	—	0	57	0	72	0	0	105	105	52.5	52.5	+	89	

Negative control: Dimethyl sulfoxide.

a): (Numerical aberration cells / observed metaphase cells) \times 100.

b): Judged on the basis of incidence as; —: negative (less than 5.0%) ; \pm : equivocal (5.0% or higher to less than 10.0%) ; +: positive (10.0% or higher) .

c): ctb: chromatid break; csb: chromosome break; cte: chromatid exchange; cse: chromosome exchange; frg: fragmentation.

d): (Cells with structural chromosome aberration / observed metaphase cells) \times 100.

e): {1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene treated group or positive control / negative control} \times 100.

(+g): Total aberrant cells including the gap; (-g): total aberrant cells excluding the gap.

Table 4. Chromosomal aberration test of 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene in cultured CHL cells
— The continuous treatment method —

Test substance	Concentration ($\mu\text{g/mL}$)	Time of treatment (hr)	No. of metaphase examined	Numerical aberration				Structural aberrations										Survival ratio ^{e)} (%)		
				No. of Polyploid cells	No. of endoreduplication cells	Incidence ^{a)} (%)	Judgement ^{b)}	Types ^{c)} and numbers (cumulative)						No. of cells with chromosome aberration		Incidence ^{d)} (%)	Judgement ^{b)}			
								gap	ctb	csb	cte	cse	fig	(+g)	(-g)				(+g)	(-g)
Negative control	—	24	200	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	—	100
	3.13	24	200	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0	—	98
1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene	6.25	24	200	1	0	0.5	—	0	0	0	1	0	0	1	1	0.5	0.5	—	100	
	12.5	24	200	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	—	82	
	25	24	200	0	0	0	—	0	0	0	1	0	0	1	1	0.5	0.5	—	48	
	50	24	200	1	0	0.5	—	0	2	0	0	0	0	2	2	1.0	1.0	—	27	
	Mitomycin C	0.05	24	200	0	0	0	—	0	47	0	67	0	0	93	93	46.5	46.5	+	85

Negative control: Dimethyl sulfoxide.

a): (Numerical aberration cells / observed metaphase cells) \times 100.

b): Judged on the basis of incidence as; —: negative (less than 5.0%) ; \pm : equivocal (5.0% or higher to less than 10.0%) ; +: positive (10.0% or higher) .

c): ctb: chromatid break; csb: chromosome break; cte: chromatid exchange; cse: chromosome exchange; fig: fragmentation.

d): (Cells with structural chromosome aberration / observed metaphase cells) \times 100.

e): {1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene treated group or positive control / negative control} \times 100.

(+g): Total aberrant cells including the gap; (-g): total aberrant cells excluding the gap.

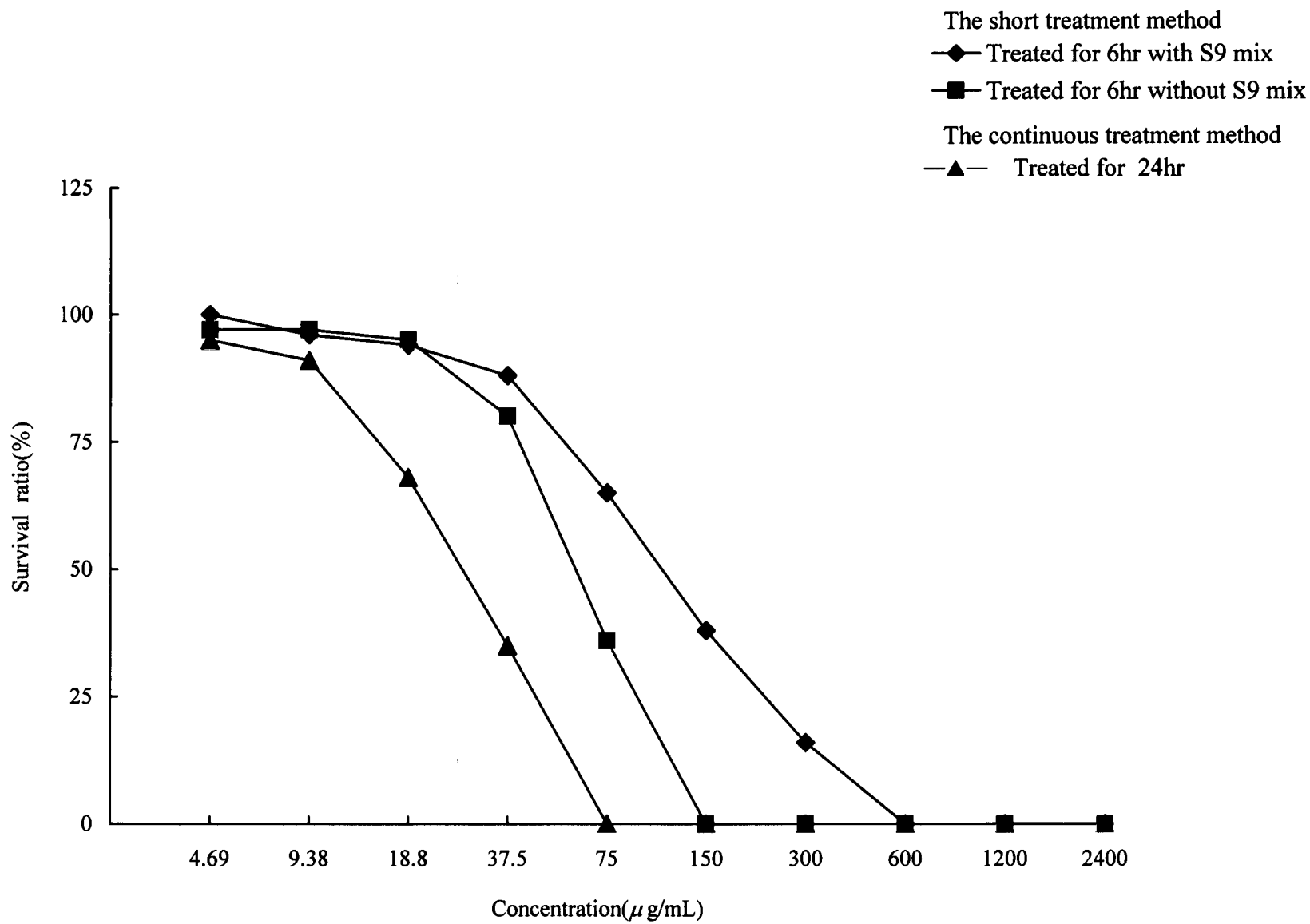


Figure 1. Cell growth inhibition test of 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl)bisbenzene in cultured CHL cells.

Appendix 1-1. Chromosomal aberration test of 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene in cultured CHL cells
-The short treatment method-

Test substance	Concentration ($\mu\text{g/mL}$)	With (+) or without (-) S9 mix	No. of metaphase examined	Numerical aberration				Structural aberrations										Survival ratio ^{e)} (%)			
				No. of Polyploid cells	No. of endoreduplication cells	Incidence ^{a)} (%)	Judgement ^{b)}	Types ^{c)} and numbers (cumulative)						No. of cells with chromosome aberration		Incidence ^{d)} (%)			Judgement ^{b)}		
								gap	ctb	csb	cte	cse	frg	(+g)	(-g)	(+g)	(-g)				
Negative control (Dimethyl sulfoxide)	—	+	100	1	0	1.0	—	0	0	0	0	0	0	0	0	0	0	0.5	0.5	—	100
			100	1	0			0	1	0	0	0	0	0	1	1	0	0			
781 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene	12.5	+	100	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0	—	98
			100	0	0			0	0	0	0	0	0	0	0	0	0				
	25	+	100	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0.5	0.5	—	90
			100	0	0			0	1	0	0	0	1	1	0	0					
	50	+	100	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0	0	—	71
			100	0	0			0	0	0	0	0	0	0	0	0					
	100	+	100	1	0	1.0	—	0	0	0	0	0	0	0	0	0	0	0.5	0.5	—	49
			100	1	0			0	1	0	1	0	0	1	1	0	0				
	200	+	100	2	0	1.0	—	0	1	0	0	0	0	1	1	1	1	1.5	1.5	—	30
			100	0	0			0	1	0	1	0	0	2	2	0	0				
	Dimethylnitrosamine	500	+	100	1	0	0.5	—	0	24	0	48	0	0	62	62	68.5	68.5	+	83	
				100	0	0			0	42	0	59	0	0	75	75	0	0			

a): (Numerical aberration cells / observed metaphase cells) \times 100.

b): Judged on the basis of incidence as; —: negative (less than 5.0%) ; \pm : equivocal (5.0% or higher to less than 10.0%) ; +: positive (10.0% or higher) .

c): ctb: chromatid break; csb: chromosome break; cte: chromatid exchange; cse: chromosome exchange; frg: fragmentation.

d): (Cells with structural chromosome aberration / observed metaphase cells) \times 100.

e): {1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene treated group or positive control / negative control} \times 100.

(+g): Total aberrant cells including the gap; (-g): total aberrant cells excluding the gap.

Appendix 1-2. Chromosomal aberration test of 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene in cultured CHL cells
-The short treatment method-

Test substance	Concentration ($\mu\text{g/mL}$)	With (+) or without (-) S9 mix	No. of metaphase examined	Numerical aberration				Structural aberrations										Survival ratio ^{e)} (%)		
				No. of Polyploid cells	No. of endoreduplication cells	Incidence ^{a)} (%)	Judgement ^{b)}	Types ^{c)} and numbers (cumulative)						No. of cells with chromosome aberration		Incidence ^{d)} (%)			Judgement ^{b)}	
								gap	ctb	csb	cte	cse	frg	(+g)	(-g)	(+g)	(-g)			
Negative control (Dimethyl sulfoxide)	—	—	100	0	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0	—	100
			100	1	0			0	0	0	0	0	0	0	0	0	0	0	0	
1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene	6.25	—	100	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	—	98
			100	0	0			0	0	0	0	0	0	0	0	0	0	0	0	
	12.5	—	100	0	0	0	—	0	1	0	0	0	0	1	1	1.0	1.0	—	98	
			100	0	0			0	1	0	0	0	1	1	1.0	1.0	—			
	25	—	100	0	0	0.5	—	0	2	0	0	0	0	2	2	1.0	1.0	—	82	
			100	1	0			0	0	0	0	0	0	0	0	0	0	—		
	50	—	100	0	0	0	—	0	0	0	0	0	0	0	0	0.5	0.5	—	53	
			100	0	0			0	1	0	0	1	1	0.5	0.5	—				
	100	—	100	0	0	0.5	—	0	2	0	0	0	0	2	2	1.5	1.5	—	31	
			100	1	0			0	1	0	0	1	1	1.5	1.5	—				
	Mitomycin C	0.1	—	100	0	0	0	—	0	26	0	31	0	0	46	46	52.5	52.5	+	89
				100	0	0			0	31	0	41	0	0	59	59	52.5	52.5	+	

a): (Numerical aberration cells / observed metaphase cells) \times 100.

b): Judged on the basis of incidence as; —: negative (less than 5.0%); \pm : equivocal (5.0% or higher to less than 10.0%); +: positive (10.0% or higher).

c): ctb: chromatid break; csb: chromosome break; cte: chromatid exchange; cse: chromosome exchange; frg: fragmentation.

d): (Cells with structural chromosome aberration / observed metaphase cells) \times 100.

e): {1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene treated group or positive control / negative control} \times 100.

(+g): Total aberrant cells including the gap; (-g): total aberrant cells excluding the gap.

Appendix 2. Chromosomal aberration test of 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene in cultured CHL cells
—The continuous treatment method—

Test substance	Concentration ($\mu\text{g/mL}$)	Time of treatment (hr)	No. of metaphase examined	Numerical aberration				Structural aberrations										Survival ratio ^{e)} (%)			
				No. of Polyploid cells	No. of endoreduplication cells	Incidence ^{a)} (%)	Judgement ^{b)}	Types ^{c)} and numbers (cumulative)						No. of cells with chromosome aberration		Incidence ^{d)} (%)			Judgement ^{b)}		
								gap	ctb	csb	cte	cse	frg	(+g)	(-g)	(+g)	(-g)				
Negative control (Dimethyl sulfoxide)	—	24	100	0	0	0	—	0	0	0	0	0	0	0	0	0	0	—	100		
			100	0	0			0	0	0	0	0	0	0	0	0	0			0	
793 -283 1,1'-(1,1-dimethyl-3-methylene-1,3-propanediyl) bisbenzene	3.13	24	100	0	0	0.5	—	0	0	0	0	0	0	0	0	0	0	—	98		
			100	1	0			0	0	0	0	0	0	0	0	0					
	6.25	24	100	0	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0.5	0.5	—	100
			100	1	0			0	0	1	0	0	1	1							
	12.5	24	100	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0	0	—	82
			100	0	0			0	0	0	0	0	0	0							
	25	24	100	0	0	0	—	0	0	0	1	0	0	1	1	0.5	0.5	—	48		
			100	0	0			0	0	0	0	0	0	0							
	50	24	100	1	0	0.5	—	0	1	0	0	0	0	1	1	1.0	1.0	—	27		
			100	0	0			0	0	0	0	0	1	1							
	Mitomycin C	0.05	24	100	0	0	0	—	0	23	0	36	0	0	48	48	46.5	46.5	+	85	
				100	0	0			0	24	0	31	0	0	45	45					

a): (Numerical aberration cells / observed metaphase cells) \times 100.

b): Judged on the basis of incidence as; —: negative (less than 5.0%) ; \pm : equivocal (5.0% or higher to less than 10.0%) ; +: positive (10.0% or higher) .

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