

Table 2-1. Reverse mutation test of bumetrizole in bacteria (mutagenicity test I : -S9 mix)

Compound concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (number of colonies/plate)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 <i>uvrA</i>	TA98	TA1537
Negative control	86, 107, 123 (105 \pm 18.6)	11, 13, 15 (13 \pm 2.0)	17, 27, 28 (24 \pm 6.1)	13, 15, 15 (14 \pm 1.2)	7, 9, 16 (11 \pm 4.7)
0.61	/	/	/	17, 17, 19 (18 \pm 1.2)	2, 7, 9 (6 \pm 3.6)
1.22	/	/	/	13, 22, 25 (20 \pm 6.2)	9, 10, 11 (10 \pm 1.0)
2.44	/	/	/	19, 19, 26 (21 \pm 4.0)	10, 10, 11 (10 \pm 0.6)
4.88	/	/	/	18, 20, 28 (22 \pm 5.3)	8, 11, 13 (11 \pm 2.5)
9.77	90, 96, 112 (99 \pm 11.4)	/	/	19*, 22*, 23* (21 \pm 2.1)	7*, 11*, 13* (10 \pm 3.1)
19.5#	90, 115, 115 (107 \pm 14.4)	/	/	17*, 22*, 23* (21 \pm 3.2)	7*, 7*, 15* (10 \pm 4.6)
39.1#	91, 96, 97 (95 \pm 3.2)	9, 9, 12 (10 \pm 1.7)	19, 27, 37 (28 \pm 9.0)	/	/
78.1#	98, 103, 108 (103 \pm 5.0)	13, 13, 13 (13 \pm 0.0)	23, 27, 30 (27 \pm 3.5)	/	/
156.3#	97*, 98*, 116* (104 \pm 10.7)	10, 17, 20 (16 \pm 5.1)	25, 34, 34 (31 \pm 5.2)	/	/
312.5#	80*, 95*, 96* (90 \pm 9.0)	10, 10, 22 (14 \pm 6.9)	24, 30, 37 (30 \pm 6.5)	/	/
625#	/	13*, 16*, 17* (15 \pm 2.1)	26, 29, 37 (31 \pm 5.7)	/	/
1250#	/	6*, 7*, 13* (9 \pm 3.8)	20*, 28*, 32* (27 \pm 6.1)	/	/
Positive control					
Name	AF-2	NaN ₃	AF-2	AF-2	9AA
Concentration ($\mu\text{g}/\text{plate}$)	0.01	0.5	0.01	0.1	80
Number of colonies/plate	414, 459, 474 (449 \pm 31.2)	573, 635, 646 (618 \pm 39.4)	103, 132, 137 (124 \pm 18.4)	365, 374, 375 (371 \pm 5.5)	391, 413, 427 (410 \pm 18.1)

Negative control : Dimethylsulfoxide.

AF-2 : 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; NaN₃ : sodium azide; 9AA : 9-aminoacridine hydrochloride.

() : Mean \pm S.D.

* : Bacterial growth inhibition was observed.

: White oily membrane-like precipitations and white fine precipitations were observed on the surface of agar plate.

Table 2-2. Reverse mutation test of bumetrizole in bacteria (mutagenicity test I : +S9 mix)

Compound concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (number of colonies/plate)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 <i>uvrA</i>	TA98	TA1537
Negative control	99, 121, 129 (116 \pm 15.5)	11, 12, 15 (13 \pm 2.1)	33, 34, 34 (34 \pm 0.6)	26, 28, 34 (29 \pm 4.2)	17, 18, 20 (18 \pm 1.5)
9.77	107, 108, 121 (112 \pm 7.8)	9, 14, 26 (16 \pm 8.7)	/	23, 28, 32 (28 \pm 4.5)	22, 22, 24 (23 \pm 1.2)
19.5	108, 116, 130 (118 \pm 11.1)	5, 12, 17 (11 \pm 6.0)	/	20, 24, 32 (25 \pm 6.1)	16, 17, 20 (18 \pm 2.1)
39.1	94, 95, 123 (104 \pm 16.5)	7, 11, 11 (10 \pm 2.3)	29, 35, 35 (33 \pm 3.5)	22, 27, 29 (26 \pm 3.6)	12, 14, 20 (15 \pm 4.2)
78.1##	77, 96, 104 (92 \pm 13.9)	13, 14, 18 (15 \pm 2.6)	20, 29, 30 (26 \pm 5.5)	24, 28, 29 (27 \pm 2.6)	12, 13, 16 (14 \pm 2.1)
156.3##	91, 114, 120 (108 \pm 15.3)	12, 16, 17 (15 \pm 2.6)	27, 29, 30 (29 \pm 1.5)	20, 22, 29 (24 \pm 4.7)	13, 20, 21 (18 \pm 4.4)
312.5##	82*, 110*, 112* (101 \pm 16.8)	12*, 15*, 23* (17 \pm 5.7)	27, 29, 31 (29 \pm 2.0)	23*, 26*, 26* (25 \pm 1.7)	17*, 17*, 21* (18 \pm 2.3)
625##	/	/	17, 29, 30 (25 \pm 7.2)	/	/
1250##	/	/	20*, 26*, 35* (27 \pm 7.5)	/	/
Positive control					
Name	2AA				
Concentration ($\mu\text{g}/\text{plate}$)	1	2	10	0.5	2
Number of colonies/plate	861, 881, 920 (887 \pm 30.0)	315, 332, 346 (331 \pm 15.5)	737, 765, 778 (760 \pm 21.0)	389, 415, 436 (413 \pm 23.5)	128, 139, 140 (136 \pm 6.7)

Negative control : Dimethylsulfoxide.

2AA : 2-Aminoanthracene.

(): Mean \pm S.D.

* : Bacterial growth inhibition was observed.

: White fine precipitations were observed on the surface of agar plate.

Table 3-1. Reverse mutation test of bumetrizole in bacteria (mutagenicity test II : -S9 mix)

Compound concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (number of colonies/plate)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 <i>uvrA</i>	TA98	TA1537
Negative control	99, 101, 111 (104 \pm 6.4)	5, 6, 7 (6 \pm 1.0)	28, 30, 34 (31 \pm 3.1)	17, 18, 22 (19 \pm 2.6)	5, 7, 9 (7 \pm 2.0)
0.61	/	/	/	21, 22, 26 (23 \pm 2.6)	6, 10, 10 (9 \pm 2.3)
1.22	/	/	/	13, 21, 27 (20 \pm 7.0)	10, 10, 12 (11 \pm 1.2)
2.44	/	/	/	19, 19, 23 (20 \pm 2.3)	7, 8, 11 (9 \pm 2.1)
4.88	/	/	/	13, 25, 25 (21 \pm 6.9)	7, 8, 10 (8 \pm 1.5)
9.77	110, 112, 121 (114 \pm 5.9)	/	/	17*, 24*, 27* (23 \pm 5.1)	6*, 7*, 7* (7 \pm 0.6)
19.5#	98, 110, 117 (108 \pm 9.6)	/	/	14*, 18*, 29* (20 \pm 7.8)	4*, 6*, 10* (7 \pm 3.1)
39.1#	102, 105, 118 (108 \pm 8.5)	5, 6, 12 (8 \pm 3.8)	20, 24, 27 (24 \pm 3.5)	/	/
78.1#	95, 96, 104 (98 \pm 4.9)	9, 10, 11 (10 \pm 1.0)	21, 21, 30 (24 \pm 5.2)	/	/
156.3#	109*, 116*, 125* (117 \pm 8.0)	4, 12, 14 (10 \pm 5.3)	24, 28, 29 (27 \pm 2.6)	/	/
312.5#	89*, 104*, 108* (100 \pm 10.0)	5, 10, 11 (9 \pm 3.2)	18, 18, 29 (22 \pm 6.4)	/	/
625#	/	7*, 12*, 13* (11 \pm 3.2)	17, 20, 36 (24 \pm 10.2)	/	/
1250#	/	10*, 10*, 11* (10 \pm 0.6)	19*, 25*, 26* (23 \pm 3.8)	/	/
Positive control					
Name	AF-2	NaN ₃	AF-2	AF-2	9AA
Concentration ($\mu\text{g}/\text{plate}$)	0.01	0.5	0.01	0.1	80
Number of colonies/plate	451, 457, 581 (496 \pm 73.4)	515, 536, 601 (551 \pm 44.8)	123, 127, 131 (127 \pm 4.0)	454, 464, 472 (463 \pm 9.0)	294, 411, 597 (434 \pm 152.8)

Negative control : Dimethylsulfoxide.

AF-2 : 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; NaN₃ : sodium azide; 9AA : 9-aminoacridine hydrochloride.

() : Mean \pm S.D.

* : Bacterial growth inhibition was observed.

: White oily membrane-like precipitations and white fine precipitations were observed on the surface of agar plate.

Table 3-2. Reverse mutation test of bumetrizole in bacteria (mutagenicity test II: +S9 mix)

Compound concentration ($\mu\text{g}/\text{plate}$)	Number of revertants (number of colonies/plate)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 <i>uvrA</i>	TA98	TA1537
Negative control	117, 118, 128 (121 \pm 6.1)	7, 11, 13 (10 \pm 3.1)	24, 26, 31 (27 \pm 3.6)	20, 30, 33 (28 \pm 6.8)	14, 14, 22 (17 \pm 4.6)
9.77	104, 106, 109 (106 \pm 2.5)	7, 10, 12 (10 \pm 2.5)	/	19, 22, 24 (22 \pm 2.5)	11, 13, 15 (13 \pm 2.0)
19.5	101, 106, 132 (113 \pm 16.6)	5, 7, 11 (8 \pm 3.1)	/	21, 21, 25 (22 \pm 2.3)	12, 16, 20 (16 \pm 4.0)
39.1	104, 111, 121 (112 \pm 8.5)	10, 10, 11 (10 \pm 0.6)	21, 23, 34 (26 \pm 7.0)	21, 26, 34 (27 \pm 6.6)	11, 19, 20 (17 \pm 4.9)
78.1##	104, 113, 120 (112 \pm 8.0)	3, 6, 8 (6 \pm 2.5)	26, 33, 38 (32 \pm 6.0)	25, 27, 32 (28 \pm 3.6)	16, 18, 25 (20 \pm 4.7)
156.3##	114, 115, 118 (116 \pm 2.1)	6, 8, 9 (8 \pm 1.5)	22, 30, 30 (27 \pm 4.6)	20, 26, 28 (25 \pm 4.2)	3, 17, 18 (13 \pm 8.4)
312.5##	97*, 112*, 112* (107 \pm 8.7)	6*, 6*, 7* (6 \pm 0.6)	21, 31, 35 (29 \pm 7.2)	20*, 22*, 37* (26 \pm 9.3)	7*, 13*, 13* (11 \pm 3.5)
625##	/	/	28, 29, 34 (30 \pm 3.2)	/	/
1250##	/	/	31*, 33*, 38* (34 \pm 3.6)	/	/
Positive control					
Name	2AA				
Concentration ($\mu\text{g}/\text{plate}$)	1	2	10	0.5	2
Number of colonies/plate	898, 944, 958 (933 \pm 31.4)	347, 350, 419 (372 \pm 40.7)	769, 790, 795 (785 \pm 13.8)	431, 450, 475 (452 \pm 22.1)	115, 141, 141 (132 \pm 15.0)

Negative control : Dimethylsulfoxide.

2AA : 2-Aminoanthracene.

() : Mean \pm S.D.

* : Bacterial growth inhibition was observed.

: White fine precipitations were observed on the surface of agar plate.

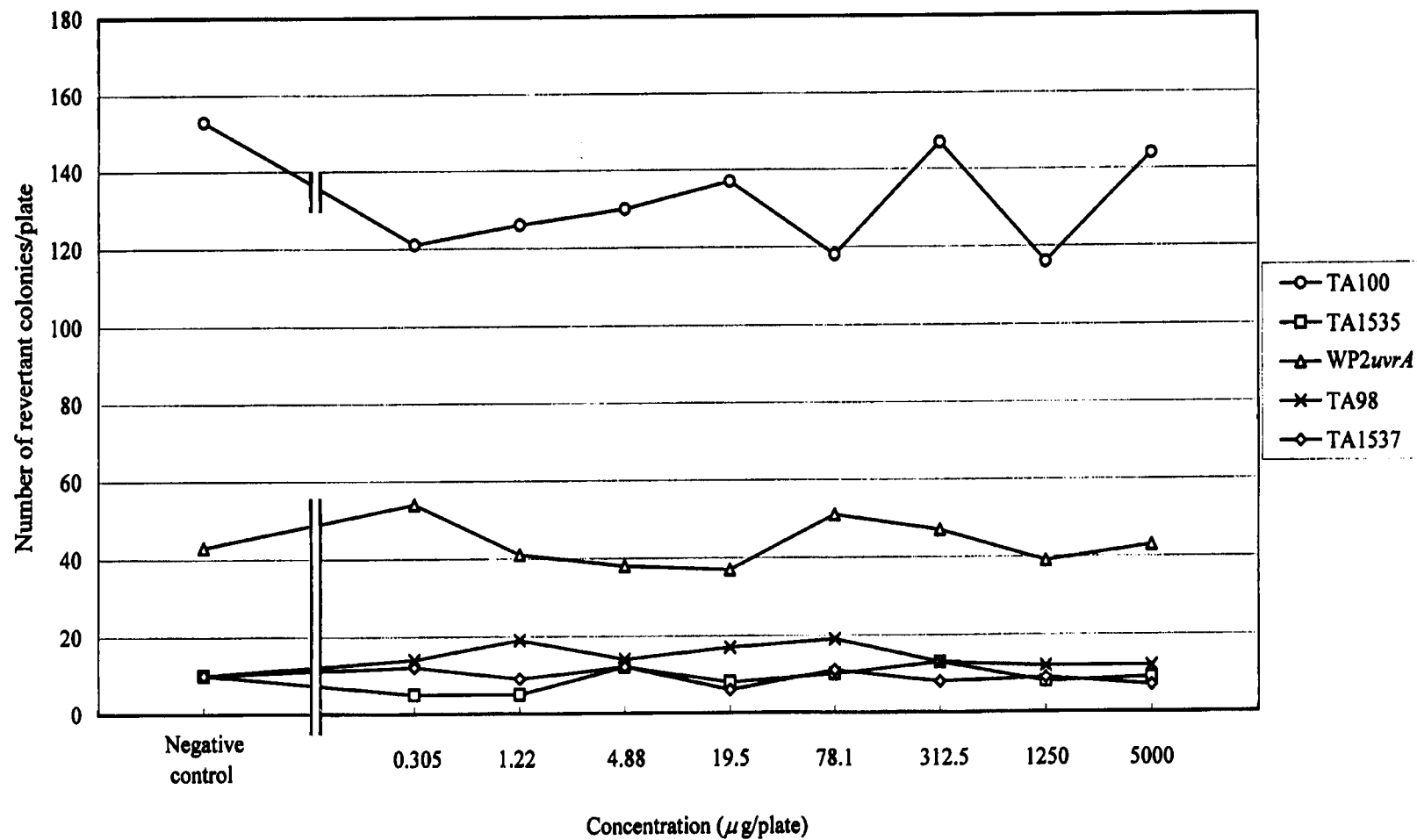


Figure 1-1. Reverse mutation test of bumetrizole in bacteria (dose-finding test: -S9 mix).

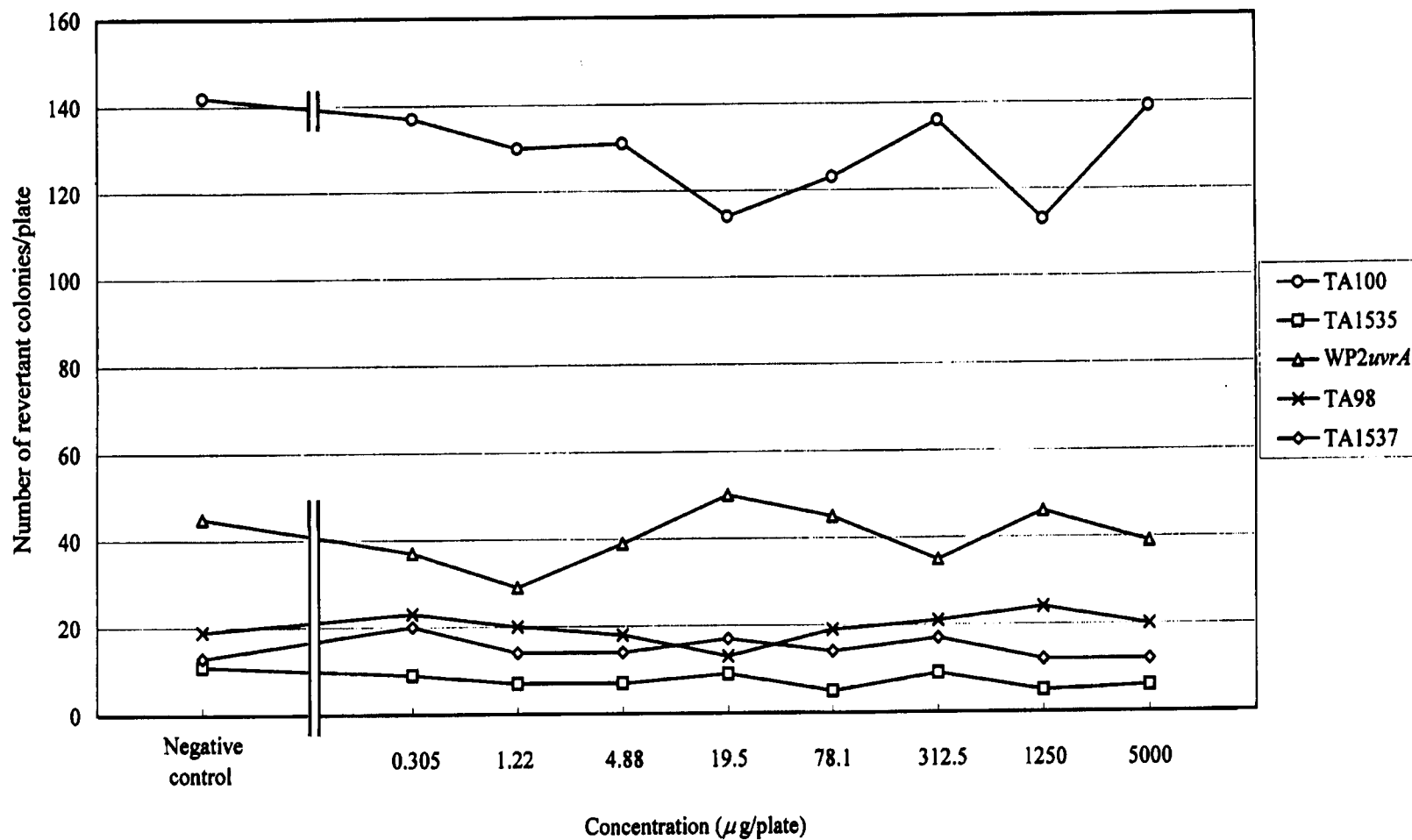


Figure 1-2. Reverse mutation test of bumetrizole in bacteria (dose-finding test: +S9 mix).

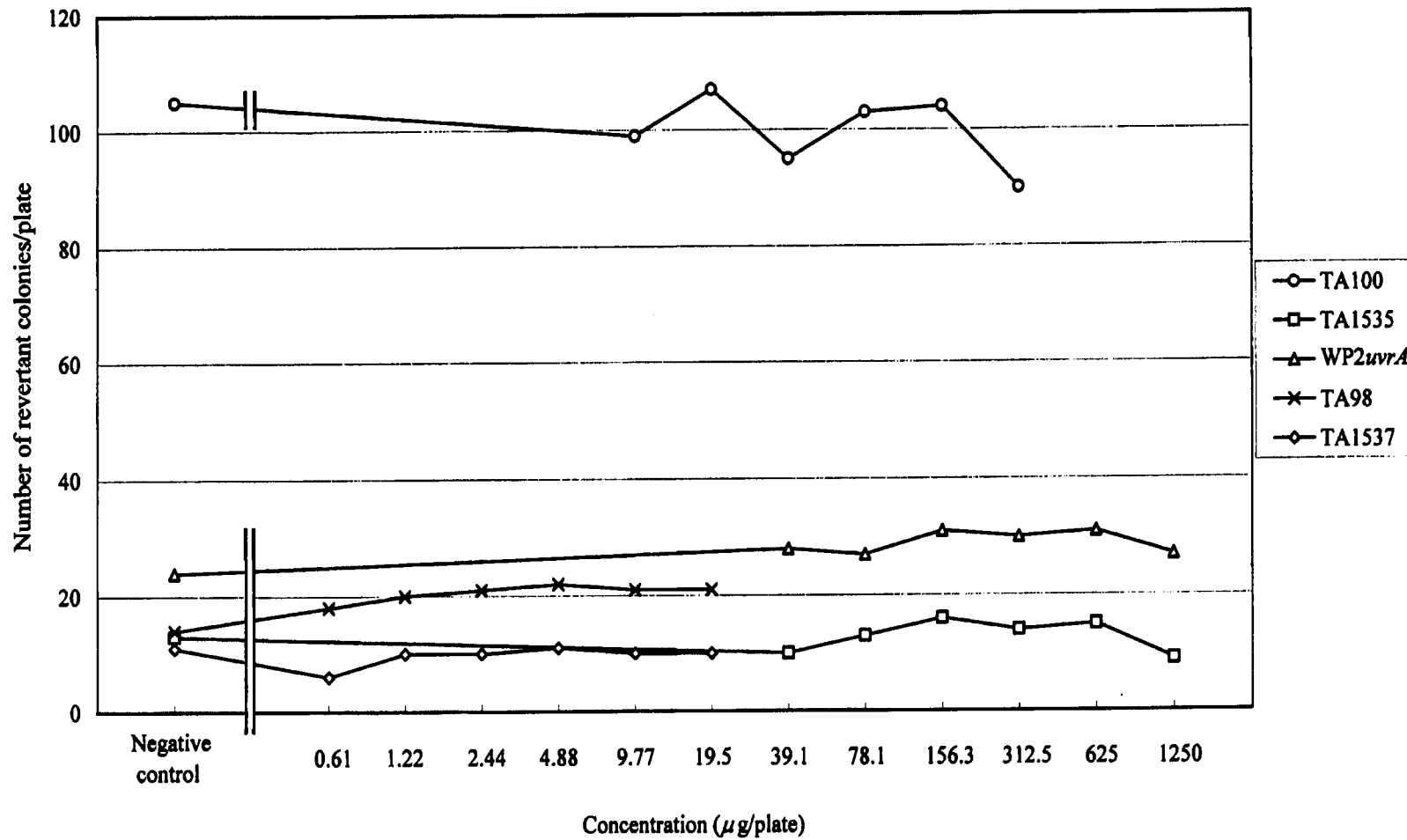


Figure 2-1. Reverse mutation test of bumetrizole in bacteria (mutagenicity test I: -S9 mix).

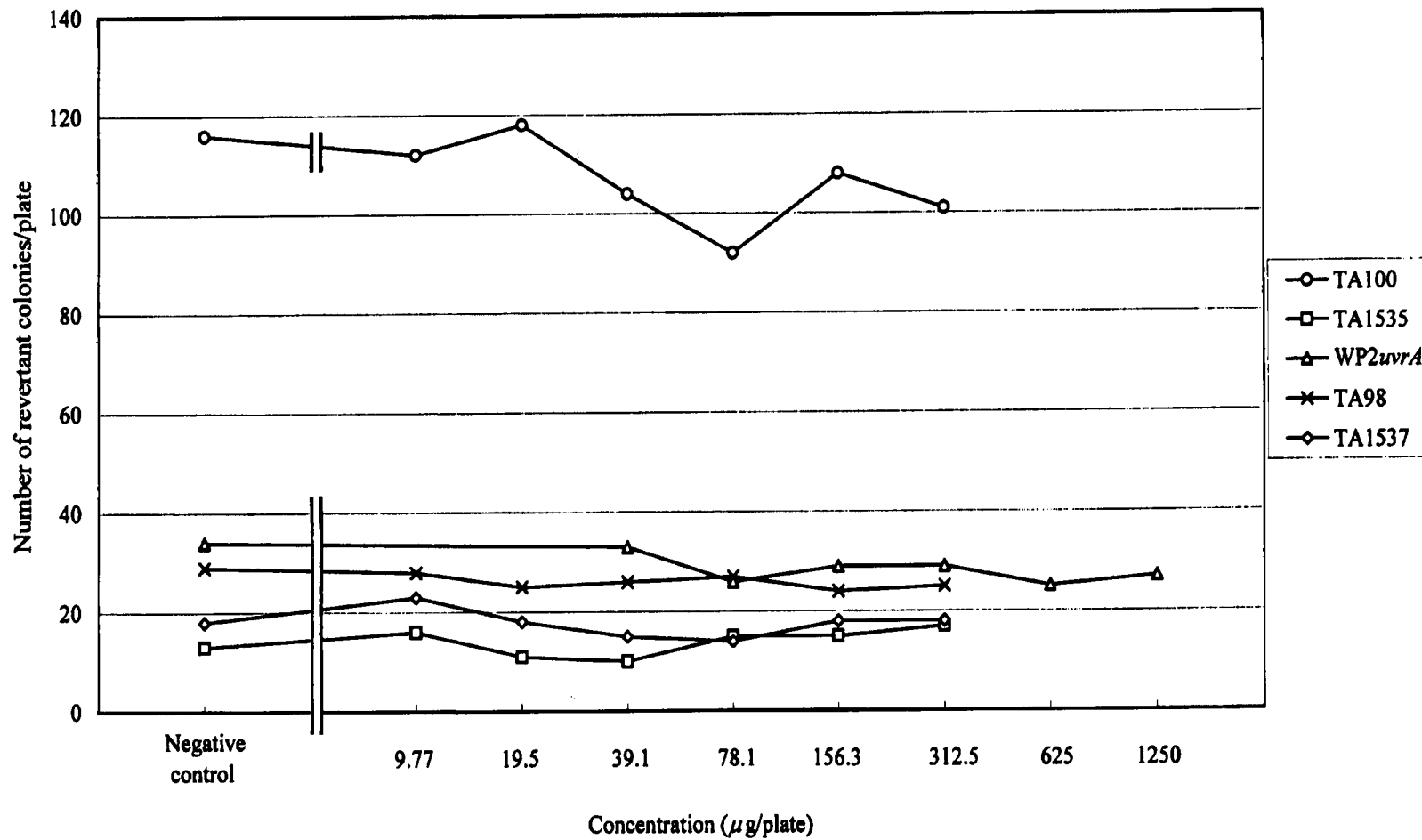


Figure 2-2. Reverse mutation test of bumetizole in bacteria (mutagenicity test I: +S9 mix).

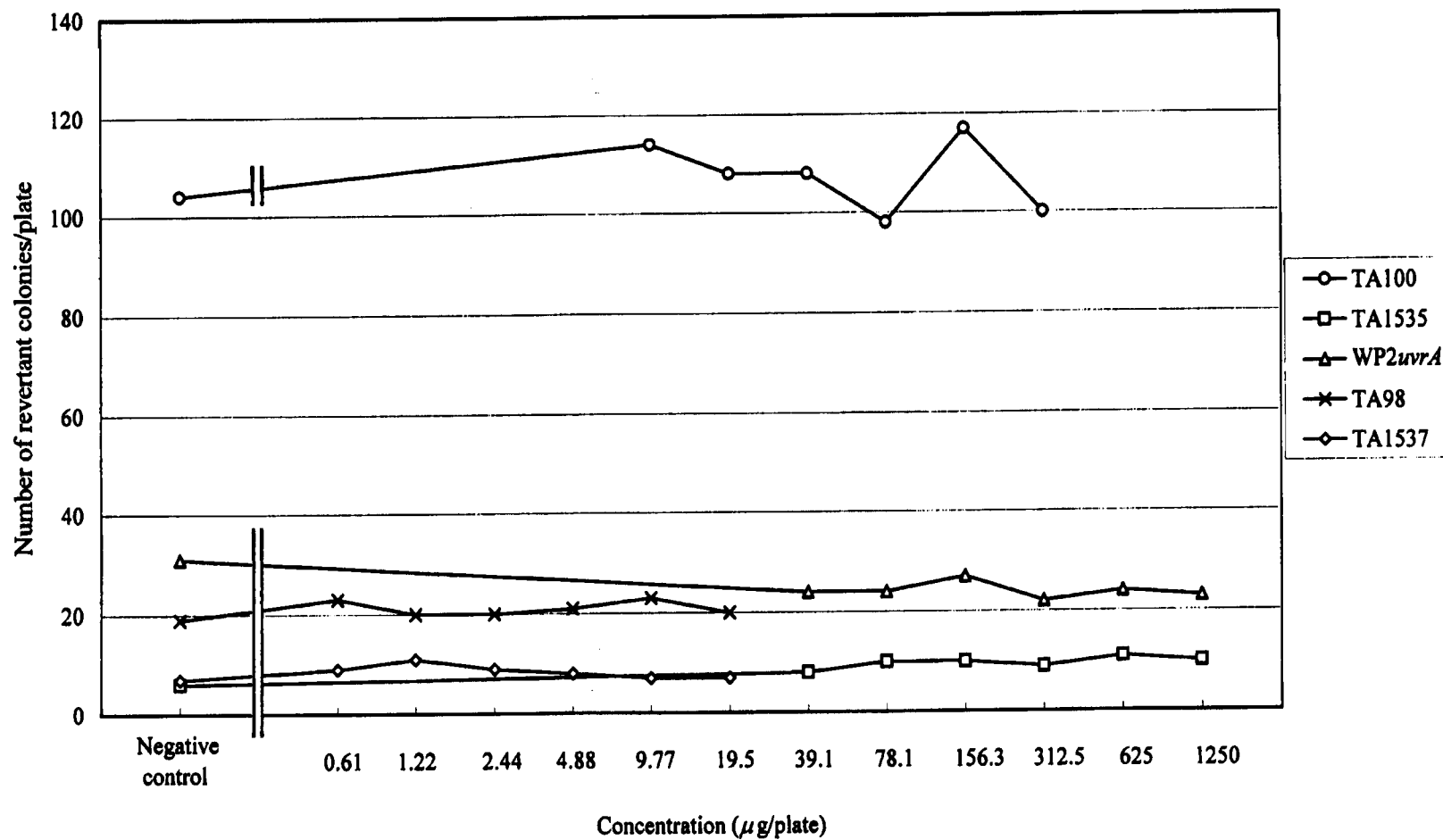


Figure 3-1. Reverse mutation test of bumetizole in bacteria (mutagenicity test II: -S9 mix).

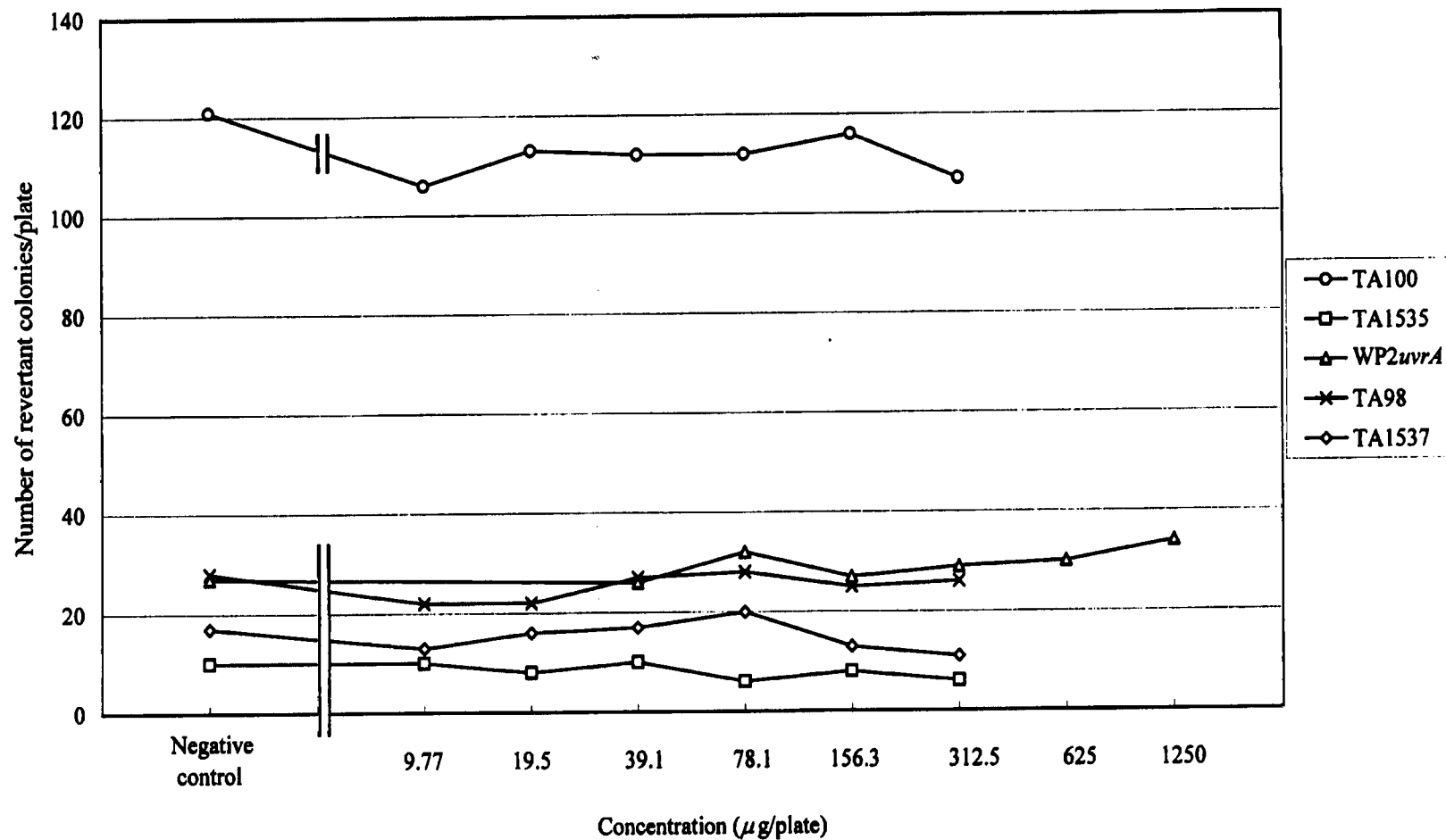


Figure 3-2. Reverse mutation test of bumetrizole in bacteria (mutagenicity test II: +S9 mix).

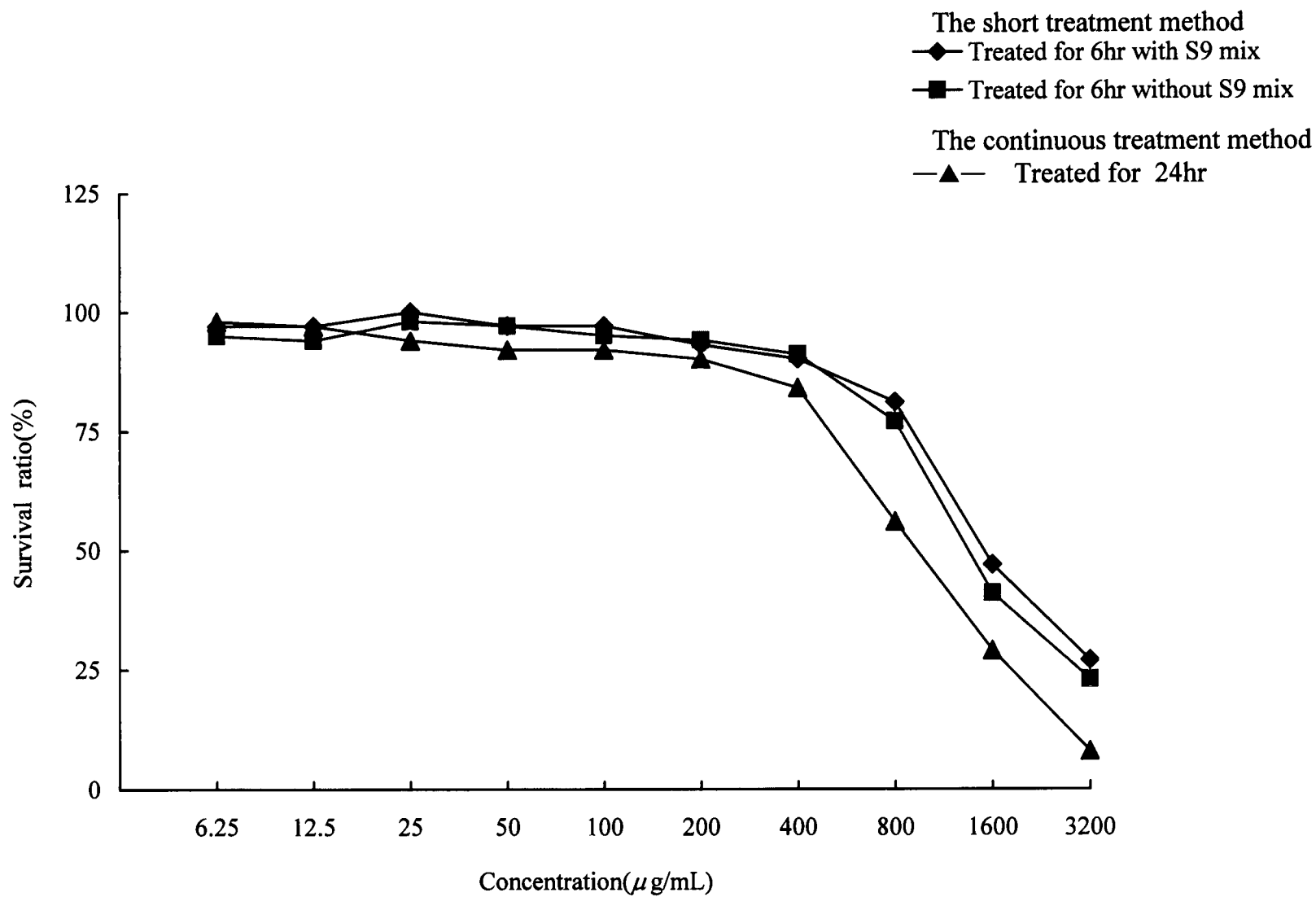


Figure 1. Cell growth inhibition test of bumetizole in cultured CHL cells.

Appendix 1-1. Chromosomal aberration test of bumetrizole 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		
bumetrizole	150*	+	100	0	0	0.5	-	0	0	0	0	0	0	0	0	0	0	0	-	96
			100	1	0			0	0	0	0	0	0	0	0	0	0	0		
	300*	+	100	0	0	0	-	0	1	0	1	0	0	2	2	1.0	1.0	-	91	
			100	0	0			0	0	0	0	0	0	0	0	0	0			
	600*	+	100	0	0	0	-	0	1	0	0	0	0	1	1	0.5	0.5	-	85	
			100	0	0			0	0	0	0	0	0	0	0	0	0			
	1200*	+	100	1	0	1.5	-	0	0	0	0	0	0	0	0	0	0	0	-	51
			100	2	0			0	0	0	0	0	0	0	0	0	0			
	2400*	+	100	2	0	1.5	-	0	0	0	0	0	0	0	0	0	0	0	-	34
			100	1	0			0	0	0	0	0	0	0	0	0	0			
	Dimethylnitrosamine	500	+	100	0	0	0	-	0	32	0	63	1	0	73	73	72.5	72.5	+	85
				100	0	0			0	34	0	61	1	0	72	72	72.5	72.5		

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): (bumetrizole treated group or positive control / negative control) \times 100.

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

*: White oily membrane-like precipitations and white fine precipitations were noted in culture fluid.

Appendix 1-2. Chromosomal aberration test of bumetizole 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	—	0	1	0	0	0	0	0	1	1	0.5	0.5	—	100		
			100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—	100	
585 -26- bumetizole	150*	—	100	1	0	0.5	—	0	0	0	0	0	0	0	0	0	0	0	—	95		
			100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—	95	
	300*	—	100	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0.5	0.5	—	95
			100	0	0	0	0	—	0	1	0	0	0	0	1	1	0	0	0	0	—	95
	600*	—	100	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0	—	89
			100	0	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0	—	89
	1200*	—	100	2	0	1.5	—	0	1	0	0	0	0	1	1	0.5	0.5	—	48			
			100	1	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0	—	48	
	2400*	—	100	0	0	1.0	—	0	0	0	1	0	0	1	1	0.5	0.5	—	34			
			100	2	0	0	—	0	0	0	0	0	0	0	0	0	0	0	0	—	34	
	Mitomycin C	0.1	—	100	0	0	0	—	0	32	0	39	0	0	55	55	52.5	52.5	+	88		
				100	0	0	0	—	0	21	0	36	0	0	50	50	52.5	52.5	+	88		

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): (bumetizole treated group or positive control / negative control) \times 100.

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

*: White oily membrane-like precipitations and white fine precipitations were noted in culture fluid.

Appendix 2. Chromosomal aberration test of bumetrizole 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	0.5	0.5	—	100
			100	0	0	0	0	0	0	0	1	0	0	1	1	0	0	—		
bumetrizole	75*	24	100	0	0	0	—	0	0	0	0	0	0	0	0	0	0	0	—	97
			100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	—		
	150*	24	100	1	0	1.0	—	0	0	0	0	1	0	1	1	1.0	1.0	—	92	
			100	1	0	1.0	—	0	1	0	0	0	0	1	1	1.0	1.0	—		
	300*	24	100	0	0	0.5	—	0	0	0	1	0	0	1	1	0.5	0.5	—	88	
			100	1	0	0.5	—	0	0	0	0	0	0	0	0	0.5	0.5	—		
	600*	24	100	1	0	1.5	—	0	0	0	0	0	0	0	0	0.5	0.5	—	62	
			100	2	0	1.5	—	0	1	0	0	0	0	1	1	0.5	0.5	—		
	1200*	24	100	2	0	1.0	—	0	0	0	0	0	0	0	0	0	0	0	—	34
			100	0	0	1.0	—	0	0	0	0	0	0	0	0	0	0	0	—	
	Mitomycin C	0.05	24	100	0	0	0	—	0	20	0	26	0	0	44	44	43.5	43.5	+	88
				100	0	0	0	—	0	20	0	30	0	0	43	43	43.5	43.5	+	

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): (bumetrizole treated group or positive control / negative control) \times 100.

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

*: White oily membrane-like precipitations and white fine precipitations were noted in culture fluid.