

表4-1 *lacZ* 遺伝子突然変異頻度解析結果 (1回目)

Treatment	Dose (mg/kg)	No./Tissue	Dilution Factor	Nonselective Plating		Selective Plating			Average $\pm$ SD
				Total Plaques /4 plate	Titer (Total Population sum)	Total Mutants sum	Mutant Frequency ( $\times 10^{-6}$ )		
Control		11/Colon	1250	1580	1975000	308	155.9		
Control		12/Colon	1250	1168	1460000	66	45.2		
Control		13/Colon	1250	1458	1822500	89	48.8		
Control		14/Colon	1250	1578	1972500	82	41.6		
Control		14/Colon	1250	1424	1780000	78	43.8	88.6 $\pm$ 44.5	
Amaranth	500	41/Colon	1250	274	342500	14	40.9		
Amaranth	500	42/Colon	1250	456	570000	22	38.6		
Amaranth	500	43/Colon	1250	1544	1930000	63	32.6		
Amaranth	500	44/Colon	1250	1138	1420000	48	33.8		
Amaranth	500	45/Colon	1250	1568	1960000	75	38.8	35.8 $\pm$ 3.16 40.5 $\pm$ 4.91 *	
Amaranth	1000	31/Colon	1250	1238	1547500	94	60.7		
Amaranth	1000	32/Colon	1250	2154	2692500	81	30.1		
Amaranth	1000	33/Colon	1250	1222	1527500	106	69.4		
Amaranth	1000	34/Colon	1250	1330	1662500	76	45.7		
Amaranth	1000	35/Colon	1250	484	605000	16	26.4	46.4 $\pm$ 16.7	
Amaranth	2000	21/Colon	1250	1368	1710000	61	35.7		
Amaranth	2000	22/Colon	1250	1844	2305000	115	49.9		
Amaranth	2000	23/Colon	1250	1152	1440000	86	59.7		
Amaranth	2000	24/Colon	1250	1428	1785000	106	59.4		
Amaranth	2000	25/Colon	1250	2152	2690000	136	50.6	50.8 $\pm$ 8.7	
Streptozotisin	100	71/Colon	1250	2280	2862500	95	33.2		
Streptozotisin	100	72/Colon	1250	1052	1315000	177	134.6	65.1 $\pm$ 50.7	

\* No.11 のマウスを除いた値

表4-2 c//遠伝子突然変異頻度解析結果 (1回目)

Treatment	Dose (mg/kg)	No./Tissue	Dilution Factor	Nonselective Plating		Titer (Total Population)	Selective Plating		sum	Mean $\pm$ SD	independent mutants sequenced	Corrected	
				Total Plaques /2 plate	Total Mutants		Mutant Frequency ( $\times 10^{-6}$ )	Mutant Frequency				Mean $\pm$ SD	Mutant Frequency
Control	750	11/Colon	750	2156	1617000	43	26.6	8	10	21.3			
Control	750	12/Colon	750	2412	1809000	57	31.5	9	10	28.4			
Control	750	13/Colon	750	3660	2745000	54	19.7	10	10	19.7			
Control	750	14/Colon	750	3288	2466000	60	24.3	9	10	21.9			
Control	750	15/Colon	750	3292	2469000	62	25.1	24.9	25.4 $\pm$ 3.8	8	10	20.1	22.3 $\pm$ 3.2
Amaranth	500	41/Colon	750	626	469500	6	12.8						
Amaranth	500	42/Colon	750	920	690000	12	17.4						
Amaranth	500	43/Colon	750	2168	1626000	36	22.1						
Amaranth	500	44/Colon	750	1194	895500	39	43.6						
Amaranth	500	45/Colon	750	2204	1653000	25	15.1	22.1	22.2 $\pm$ 11.1	NT	NT	NT	NT
Amaranth	1000	31/Colon	750	2744	2058000	83	40.3						
Amaranth	1000	32/Colon	750	3544	2658000	56	21.1						
Amaranth	1000	33/Colon	750	2640	1980000	32	16.2						
Amaranth	1000	34/Colon	750	3064	2298000	64	27.9						
Amaranth	1000	35/Colon	750	672	504000	8	15.9	25.6	24.3 $\pm$ 9.1	NT	NT	NT	NT
Amaranth	2000	21/Colon	750	2604	1953000	167	85.5						
Amaranth	2000	22/Colon	750	4488	3366000	96	28.5	3	10	25.7	10	10	28.5
Amaranth	2000	23/Colon	750	3364	2523000	101	40.0	10	10	28.5	7	9	31.1
Amaranth	2000	24/Colon	750	2824	2118000	60	28.3	7	9	31.1	4	7	16.2
Amaranth	2000	25/Colon	750	3056	2292000	85	37.1	41.5	43.9 $\pm$ 21.3	8	9	33.0	26.9 $\pm$ 5.9
Streptozotisin	100	71/Colon	750	2516	1887000	84	44.5						
Streptozotisin	100	71/Colon	750	2288	1716000	61	47.2	45.8	45.8 $\pm$ 1.3	11	14	35.0	37.5 $\pm$ 2.6

NT: not tested

表4-3 *lacZ* 遺伝子突然変異頻度解析結果 (2回目)

Treatment	Dose (mg/kg)	No./Tissue	Dilution Factor	Nonselective Plating		Selective Plating		sum	Mean $\pm$ SD
				Total Plaques /4 plate	Titer (Total Populator)	Total Mutants	Mutant Frequency ( $\times 10^{-6}$ )		
Control		11/Colon	1250	1488	1860000	825	443.5		
Control		12/Colon	1250	766	957500	78	81.5		
Control		13/Colon	1250	1352	1690000	93	55.0		
Control		14/Colon	1250	1780	2225000	103	46.3		
Control		14/Colon	1250	950	1187500	84	70.7	139.4 $\pm$ 153	
					6060000	1183		63.4 $\pm$ 13.6 *	
Amaranth	2000	21/Colon	1250	1390	1737500	61	35.1		
Amaranth	2000	22/Colon	1250	2616	3270000	72	22.0		
Amaranth	2000	23/Colon	1250	1356	1695000	97	57.2		
Amaranth	2000	24/Colon	1250	2016	2520000	187	74.2		
Amaranth	2000	25/Colon	1250	1092	1365000	81	59.3	49.6 $\pm$ 18.6	
					10587500	498	47.0		

\* No.11 のマウスを除いた値

表4-4 c//選伝子突然変異頻度解析結果 (2回目)

Treatment	Dose (mg/kg)	No./Tissue	Dilution Factor	Nonselective Plating		Selective Plating		Mean $\pm$ SD	independent mutants	Total mutant sequenced
				Total Plaques /2 plate	Titer (Total Population)	Total Mutants: sum	Mutant Frequency ( $\times 10^{-6}$ )			
Control		11/Colon	750	3384	2538000	89	35.1		7	9
Control		12/Colon	750	1948	1461000	33	22.6		7	8
Control		13/Colon	750	2880	2160000	72	33.3		8	10
Control		14/Colon	750	2732	2049000	80	39.0		9	10
Control		15/Colon	750	2288	1716000	45	26.2	31.3 $\pm$ 6.0	10	10
Amaranth	2000	21/Colon	750	2576	1932000	194	100.4		3	10
Amaranth	2000	22/Colon	750	2724	2043000	95	46.5		8	9
Amaranth	2000	23/Colon	750	2668	2001000	120	60.0		9	9
Amaranth	2000	24/Colon	750	2968	2226000	91	40.9		5	8
Amaranth	2000	25/Colon	750	1516	1137000	47	41.3	57.8 $\pm$ 22.4	7	10
					9,924,000	319	32.1			
					9,339,000	547	58.6			

表4-5 cII遺伝子突然変異体の変異スペクトル

Mutation Class	control	CpG (%)	Amaranth	CpG (%)	Streptozotosin	CpG (%)
Total	84	100	63	100	28	100
Base Substitution	76	90	52	83	26	93
Transitions	45	54	37	59	20	71
GC → AT	38	(34) 45	33	(28) 52	18	(8) 64
AT → GC	7	8	4	6	2	7
Transversions	31	37	15	24	6	21
AT → TA	5	6	4	6	3	11
AT → CG	6	7	0	0	1	4
GC → TA	17	20	10	16	2	7
GC → CG	3	4	1	2	0	0
-1 frameshifts	4	5	4	6	1	4
+1 frameshifts	3	4	6	10	1	4
Deletion	0	0	1	2	0	0
Insertion	0	0	0	0	0	0
Complex	1	1	0	0	0	0



表4-7 その他の臓器におけるc//遺伝子突然変異頻度解析結果

## 前胃

Date/Treatment	No./Tissue *	Dilution Factor	Nonselective Plating			Selective Plating		Mutant		
			Total Plaques /2 plate	Titer (Total Population)	sum	Total Mutant	sum	Frequency (x 10 <sup>-6</sup> )	sum	Mean ± SD
020510/Control	11/Forestomach	750	2592	1944000		27		13.9		
020510/Control	12/Forestomach	750	1576	1182000		19		16.1		
020510/Control	13/Forestomach	750	2580	1935000		28		13.4		
020510/Control	14/Forestomach	750	3176	2382000	7,443,000	34	106	14.3	14.2	14.4 ± 1.0
020510/Amaranth2000	21/Forestomach	750	1362	1021500		13		12.7		
020510/Amaranth2000	24/Forestomach	750	1608	1206000		20		16.6		
020510/Amaranth2000	25/Forestomach	750	2224	1668000	3,895,500	41	74	24.6	19.0	18.0 ± 4.9
020510/Streptozotocin	71/Forestomach	750	1416	1062000		31		29.2		
020510/Streptozotocin	72/Forestomach	750	1616	1212000	2,274,000	96	127	79.2	55.8	54.2 ± 25.0

\* No. 15, 22, 23はDNAが回収できず

## 脾臓

Date/Treatment	No./Tissue	Dilution Factor	Nonselective Plating			Selective Plating		Mutant		
			Total Plaques /2 plate	Titer (Total Population)	sum	Total Mutant	sum	Frequency (x 10 <sup>-6</sup> )	sum	Mean ± SD
020507/Control	11/GS	750	1696	1272000		103		81.0		
020507/Control	12/GS	750	2208	1656000		80		48.3		
020507/Control	13/GS	750	2304	1728000		122		70.6		
020507/Control	14/GS	750	1752	1314000		122		92.8		
020507/Control	15/GS	750	2520	1890000	7860000	71	498	37.6	63.4	66.1 ± 20.4
020507/Amaranth2000	21/GS	750	1984	1488000		97		65.2		
020507/Amaranth2000	22/GS	750	2640	1980000		106		53.5		
020507/Amaranth2000	23/GS	750	1984	1488000		78		52.4		
020507/Amaranth2000	24/GS	750	1508	1131000		43		38.0		
020507/Amaranth2000	25/GS	750	1888	1416000	7503000	66	390	46.6	52.0	51.2 ± 8.9
020507/Streptozotocin	71/GS	750	1776	1332000		65		48.8		
020507/Streptozotocin	72/GS	750	1120	840000	2172000	52	117	61.9	53.9	55.4 ± 6.6
020510/Streptozotocin	72/GS	750	956	717000	2889000	25	142	34.9	49.2	48.5 ± 11.0

## 肝臓

Date/Treatment	No./Tissue	Dilution Factor	Nonselective Plating			Selective Plating		Mutant		
			Total Plaques /2 plate	Titer (Total Population)	sum	Total Mutant	sum	Frequency (x 10 <sup>-6</sup> )	sum	Mean ± SD
020321/Control	11/Liver	750	4652	3489000		71		20.3		
020321/Control	12/Liver	750	4788	3591000		66		18.4		
020321/Control	13/Liver	750	3256	2442000		75		30.7		
020321/Control	14/Liver	562.5	5020	2823750	12345750	119	331	42.1	26.8	49.9 ± 9.5
020321/Amaranth2000	21/Liver	750	2800	2100000		58		27.6		
020321/Amaranth2000	22/Liver	750	4032	3024000		58		19.2		
020321/Amaranth2000	23/Liver	750	2588	1941000		41		21.1		
020321/Amaranth2000	24/Liver	750	3472	2604000		47		18.0		
020321/Amaranth2000	24/Liver	750	2908	2181000	11850000	24	228	11.0	19.2	19.4 ± 5.3
020328/Streptozotocin	71/Liver	750	1176	882000		251		284.6		
020328/Streptozotocin	72/Liver	750	2740	2055000	2937000	526	777	256.0	264.6	270.3 ± 14.3

## 肺

Date/Treatment	No./Tissue	Dilution Factor	Nonselective Plating			Selective Plating		Mutant		
			Total Plaques /2 plate	Titer (Total Population)	sum	Total Mutant	sum	Frequency (x 10 <sup>-6</sup> )	sum	Mean ± SD
020321/Control	11/Lung	750	4132	3099000		60		19.4		
020321/Control	12/Lung	750	3524	2643000		54		20.4		
020321/Control	13/Lung	750	3040	2280000		166		72.8		
020321/Control	14/Lung	750	2324	1743000		38		21.8		
020321/Control	15/Lung	750	584	438000	10203000	8	326	18.3	32.0	30.5 ± 21.2
020321/Amaranth2000	21/Lung	750	3100	2325000		46		19.8		
020321/Amaranth2000	22/Lung	750	3800	2850000		90		31.6		
020321/Amaranth2000	23/Lung	750	5252	3939000		119		30.2		
020321/Amaranth2000	24/Lung	750	164	123000		3		24.4		
020321/Amaranth2000	25/Lung	750	33	24750	9261750	1	259	40.4	28.0	29.3 ± 7.0

表4-8 小核試験成績

被験物質	投与量 mg/kg/day	投与方法 投与回数	動物 数	幼若赤 血球観 察数	リング 時間 <sup>a)</sup>	小核含有幼若赤血球		
						検定 <sup>c)</sup>	% ± SD	( Min / Max )
赤色2号 (amaranth)	0	l.g.	5	10000	48	—	0.29 ± 0.07	( 0.20 / 0.40 )
	2000	l.g.	5	10000	48	N.S.	0.37 ± 0.09	( 0.25 / 0.50 )
	1000	l.g.	5	10000	48	N.S.	0.39 ± 0.15	( 0.15 / 0.60 )
	500	l.g.	5	10000	48	N.S.	0.29 ± 0.06	( 0.20 / 0.35 )
Streptozotocin	100	l.p.	2	4000	48	**	2.28 ± 1.18	( 1.10 / 3.45 )

  

被験物質	投与量 mg/kg/day	投与方法 投与回数	動物 数	幼若赤 血球観 察数	リング 時間 <sup>a)</sup>	小核含有幼若赤血球		
						検定 <sup>c)</sup>	% ± SD	( Min / Max )
赤色2号 (amaranth)	0	l.g.	5	10000	48	—	0.25 ± 0.10	( 0.10 / 0.40 )
	2000	l.g.	5	10000	48	N.S.	0.32 ± 0.08	( 0.20 / 0.40 )
	1000	l.g.	5	10000	48	N.S.	0.28 ± 0.15	( 0.15 / 0.55 )
	500	l.g.	5	10000	48	N.S.	0.33 ± 0.12	( 0.20 / 0.50 )
Streptozotocin	100	l.p.	2	4000	48	**	2.35 ± 0.35	( 2.00 / 2.70 )

a) : 投与後のリング時間

b) : Kastenbaum-Bowman法による検定

N.S. : Not significantly different from the vehicle control. (p &gt; 0.05)

\*\* p &lt; 0.01