Table 6. Mutant frequency (MF) of *cII* gene in forestomach of transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Number of animals	Number of plaque forming units	Number of mutant plaques	Mutant frequency ($\times 10^{-6}$)	P-value
Commercial diet a)	0	5	1,648,800	34	20.6	-
Agaritine	62.5 b)	5	1,771,200	41	23.1	0.3514
	250 c)	5	1,705,500	34	19.9	0.6030
	750 d)	5	1,687,500	36	21.3	0.4912
Product B	5 (%)	5	1,724,400	30	17.4	0.7894
ENU e)	50 (mg/kg)	3	918,900	147	160.0 *	<0.0001

* : $p \leq 0.05$, significant difference from control (Kastenbaum and Bowman method, upper-tailed)
a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

Appendix 1. Induction of mutation in kidney of transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Number of plaque forming units	Number of mutants	Mutant frequency ($\times 10^{-6}$)	Group Mean ± S.D. ($\times 10^{-6}$)
Commercial diet a)	0	1001	470,700	11	23.4	
		1002	415,800	7	16.8	
		1003	378,900	9	23.8	
		1004	405,900	7	17.2	
		1005	582,300	9	15.5	19.3 ± 3.9
Agaritine	62.5 b)	1101	302,400	7	23.1	
		1102	379,800	7	18.4	
		1103	495,900	16	32.3	
		1104	475,200	8	16.8	
		1105	554,400	9	16.2	21.4 ± 6.7
	250 c)	1201	661,500	16	24.2	
		1202	376,200	10	26.6	
		1203	619,200	9	14.5	
		1204	496,800	11	22.1	
		1205	768,600	15	19.5	21.4 ± 4.7
	750 d)	1301	399,600	6	15.0	
		1302	371,700	7	18.8	
		1303	365,400	9	24.6	
		1304	302,400	8	26.5	
		1305	421,200	7	16.6	20.3 ± 5.0
Product B	5 (%)	1401	485,100	7	14.4	
		1402	409,500	5	12.2	
		1403	461,700	11	23.8	
		1404	451,800	11	24.3	
		1405	531,900	10	18.8	18.7 ± 5.4
ENU e)	50 (mg/kg)	1501	422,100	71	168.2	
		1502	398,700	42	105.3	
		1503	329,400	85	258.0	177.2 ± 76.7

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

Appendix 2. Induction of mutation in liver of transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Number of plaque forming units	Number of mutants	Mutant frequency ($\times10^{-6}$)	Group Mean ± S.D. ($\times10^{-6}$)
Commercial diet a)	0	1001	306,000	10	32.7	
		1002	377,100	9	23.9	
		1003	504,900	10	19.8	
		1004	450,900	13	28.8	
		1005	304,200	7	23.0	25.6 ± 5.1
Agaritine	62.5 b)	1101	332,100	9	27.1	
		1102	303,300	7	23.1	
		1103	375,300	9	24.0	
		1104	337,500	10	29.6	
		1105	333,000	7	21.0	25.0 ± 3.4
	250 c)	1201	330,300	7	21.2	
		1202	330,300	11	33.3	
		1203	324,000	8	24.7	
		1204	327,600	6	18.3	
		1205	407,700	9	22.1	23.9 ± 5.7
	750 d)	1301	306,900	5	16.3	
		1302	438,300	9	20.5	
		1303	332,100	6	18.1	
		1304	462,600	10	21.6	
		1305	360,900	7	19.4	19.2 ± 2.1
Product B	5 (%)	1401	363,600	7	19.3	
		1402	381,600	10	26.2	
		1403	386,100	8	20.7	
		1404	302,400	6	19.8	
		1405	421,200	11	26.1	22.4 ± 3.4
ENU e)	50 (mg/kg)	1501	304,200	57	187.4	
		1502	305,100	46	150.8	
		1503	309,600	41	132.4	156.9 ± 28.0

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

Appendix 3. Induction of mutation in bone marrow of transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Number of plaque forming units	Number of mutants	Mutant frequency ($\times10^{-6}$)	Group Mean ± S.D. ($\times10^{-6}$)
Commercial diet a)	0	1001	482,400	8	16.6	
		1002	681,300	11	16.1	
		1003	302,400	2	6.6	
		1004	355,500	7	19.7	
		1005	591,300	9	15.2	14.8 ± 4.9
Agaritine	62.5 b)	1101	318,600	4	12.6	
		1102	702,000	20	28.5	
		1103	532,800	8	15.0	
		1104	332,100	2	6.0	
		1105	731,700	13	17.8	16.0 ± 8.2
	250 c)	1201	565,200	8	14.2	
		1202	552,600	7	12.7	
		1203	597,600	10	16.7	
		1204	594,900	9	15.1	
		1205	304,200	5	16.4	15.0 ± 1.6
	750 d)	1301	488,700	7	14.3	
		1302	349,200	3	8.6	
		1303	486,000	9	18.5	
		1304	351,900	5	14.2	
		1305	747,900	10	13.4	13.8 ± 3.5
Product B	5 (%)	1401	386,100	4	10.4	
		1402	386,100	7	18.1	
		1403	369,900	6	16.2	
		1404	471,600	5	10.6	
		1405	389,700	6	15.4	14.1 ± 3.5
ENU e)	50 (mg/kg)	1501	310,500	123	396.1	
		1502	496,800	167	336.2	
		1503	336,600	203	603.1	445.1 ± 140.0

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

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Appendix 4. Induction of mutation in thyroid gland of transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Number of plaque forming units	Number of mutants	Mutant frequency ($\times 10^{-6}$)	Group Mean ± S.D. ($\times 10^{-6}$)
Commercial diet a)	0	1001	379,800	6	15.8	
		1002	326,700	8	24.5	
		1003	347,400	8	23.0	
		1004	266,400	6	22.5	
		1005	304,200	6	19.7	21.1 ± 3.4
Agaritine	62.5 b)	1101	239,400	7	29.2	
		1102	356,400	8	22.4	
		1103	268,200	4	14.9	
		1104	315,000	7	22.2	
		1105	263,700	5	19.0	21.5 ± 5.3
	250 c)	1201	228,600	3	13.1	
		1202	314,100	5	15.9	
		1203	312,300	7	22.4	
		1204	251,100	5	19.9	
		1205	290,700	7	24.1	19.1 ± 4.5
	750 d)	1301	233,100	4	17.2	
		1302	221,400	5	22.6	
		1303	180,900	4	22.1	
		1304	307,800	6	19.5	
		1305	324,000	6	18.5	20.0 ± 2.3
Product B	5 (%)	1401	276,300	5	18.1	
		1402	230,400	5	21.7	
		1403	234,000	6	25.6	
		1404	236,700	8	33.8	
		1405	260,100	5	19.2	23.7 ± 6.3
ENU e)	50 (mg/kg)	1501	207,900	16	77.0	
		1502	253,800	22	86.7	
		1503	251,100	16	63.7	75.8 ± 11.5

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

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Appendix 5. Induction of mutation in lung of transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Number of plaque forming units	Number of mutants	Mutant frequency ($\times 10^{-6}$)	Group Mean ± S.D. ($\times 10^{-6}$)
Commercial diet a)	0	1001	347,400	7	20.1	
		1002	309,600	6	19.4	
		1003	343,800	8	23.3	
		1004	400,500	9	22.5	
		1005	569,700	11	19.3	20.9 ± 1.9
Agaritine	62.5 b)	1101	329,400	6	18.2	
		1102	315,900	7	22.2	
		1103	355,500	10	28.1	
		1104	304,200	7	23.0	
		1105	345,600	6	17.4	21.8 ± 4.3
	250 c)	1201	311,400	5	16.1	
		1202	392,400	10	25.5	
		1203	313,200	9	28.7	
		1204	310,500	6	19.3	
		1205	332,100	7	21.1	22.1 ± 5.0
	750 d)	1301	320,400	7	21.8	
		1302	304,200	9	29.6	
		1303	488,700	9	18.4	
		1304	441,900	8	18.1	
		1305	309,600	5	16.1	20.8 ± 5.3
Product B	5 (%)	1401	375,300	7	18.7	
		1402	422,100	10	23.7	
		1403	340,200	6	17.6	
		1404	358,200	5	14.0	
		1405	344,700	6	17.4	18.3 ± 3.5
ENU e)	50 (mg/kg)	1501	432,000	35	81.0	
		1502	552,600	45	81.4	
		1503	305,100	44	144.2	102.2 ± 36.4

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

Appendix 6. Induction of mutation in forestomach of transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Number of plaque forming units	Number of mutants	Mutant frequency ($\times10^{-6}$)	Group Mean ± S.D. ($\times10^{-6}$)
Commercial diet a)	0	1001	306,000	7	22.9	
		1002	355,500	6	16.9	
		1003	354,600	7	19.7	
		1004	301,500	7	23.2	
		1005	331,200	7	21.1	20.8 ± 2.6
Agaritine	62.5 b)	1101	322,200	9	27.9	
		1102	318,600	8	25.1	
		1103	304,200	6	19.7	
		1104	342,000	8	23.4	
		1105	484,200	10	20.7	23.4 ± 3.3
	250 c)	1201	343,800	9	26.2	
		1202	322,200	5	15.5	
		1203	370,800	6	16.2	
		1204	323,100	6	18.6	
		1205	345,600	8	23.1	19.9 ± 4.6
	750 d)	1301	306,000	4	13.1	
		1302	352,800	6	17.0	
		1303	358,200	8	22.3	
		1304	306,900	6	19.6	
		1305	363,600	12	33.0	21.0 ± 7.5
Product B	5 (%)	1401	372,600	6	16.1	
		1402	330,300	7	21.2	
		1403	360,000	5	13.9	
		1404	315,900	7	22.2	
		1405	345,600	5	14.5	17.6 ± 3.9
ENU e)	50 (mg/kg)	1501	305,100	49	160.6	
		1502	304,200	54	177.5	
		1503	309,600	44	142.1	160.1 ± 17.7

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

Appendix 7. Body weight in the gene mutation assay using transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Body weight of each period (g)					
			Received	Day 1(Allocated)	Day 8	Day 15	Day 22	Day 29
Commercial diet a)	0	1001	108	144	178	207	231	254
		1002	105	145	178	210	235	256
		1003	100	137	168	200	225	249
		1004	105	136	162	190	213	236
		1005	101	135	161	188	214	233
		1006	114	156	192	233	258	281
		Mean±S.D.	106±5	142±8	173±12	205±16	229±17	252±17
Agaritine	62.5 b)	1101	107	147	178	210	230	252
		1102	101	145	173	205	230	249
		1103	116	148	179	206	227	249
		1104	113	148	175	209	233	253
		1105	113	152	181	215	237	253
		1106	108	146	174	199	224	244
		Mean±S.D.	110±5	148±2	177±3	207±5	230±5	250±3
	250 c)	1201	111	145	168	194	216	232
		1202	97	141	169	194	212	225
		1203	111	150	172	198	212	224
		1204	113	148	174	201	219	232
		1205	121	156	184	211	233	246
		1206	105	139	167	193	211	222
		Mean±S.D.	110±8	147±6	172±6	199±7	217±8	230±9
	750 d)	1301	109	143	144	152	161	171
		1302	102	140	144	148	153	160
		1303	102	143	145	151	170	179
		1304	108	139	138	150	156	168
		1305	96	138	136	145	153	154
		1306	97	134	136	149	158	168
		Mean±S.D.	102±5	140±3	141±4	149±2	159±6	167±9
Product B	5 (%)	1401	94	134	166	191	211	229
		1402	101	143	172	202	219	234
		1403	97	135	170	197	219	237
		1404	114	152	176	207	230	249
		1405	111	148	170	195	216	233
		1406	96	136	164	192	218	232
		Mean±S.D.	102±8	141±8	170±4	197±6	219±6	236±7
ENU e)	50 (mg/kg)	1501	107	152	-	-	-	-
		1502	114	154	-	-	-	-
		1503	106	138	-	-	-	-
		1504	105	143	-	-	-	-
		Mean±S.D.	108±4	147±8	-	-	-	-

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 ml/kg, expression time:3 days)
-: Not measured

Appendix 7. Continued

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Compound	Dose (ppm)	Animal ID No.	Body weight of each period (g) Day 36	Day 43	Day 50	Day 57	Day 64	Day 71
Commercial diet a)	0	1001	271	292	306	322	340	351
		1002	274	294	310	325	337	344
		1003	265	281	295	301	316	329
		1004	256	271	290	299	317	330
		1005	253	269	285	294	303	313
		1006	300	316	316	338	352	358
		Mean±S.D.	270±17	287±17	300±12	313±18	328±18	338±17
Agaritine	62.5 b)	1101	274	289	290	317	332	339
		1102	269	286	297	315	329	335
		1103	264	278	293	309	318	330
		1104	268	280	288	298	312	320
		1105	272	286	296	310	321	334
		1106	262	280	290	306	320	329
		Mean±S.D.	268±5	283±4	292±4	309±7	322±7	331±7
	250 c)	1201	245	255	261	269	279	285
		1202	232	243	249	259	266	270
		1203	234	245	250	264	270	268
		1204	242	255	261	271	276	279
		1205	251	263	272	278	290	296
		1206	232	244	252	257	262	267
		Mean±S.D.	239±8	251±8	258±9	266±8	274±10	278±11
	750 d)	1301	175	178	179	174	174	178
		1302	168	172	172	171	175	180
		1303	187	192	184	181	187	181
		1304	171	178	181	185	191	193
		1305	161	163	162	165	169	172
		1306	174	176	177	181	180	179
		Mean±S.D.	173±9	177±9	176±8	176±7	179±8	181±7
Product B	5 (%)	1401	241	251	261	269	282	287
		1402	242	258	264	273	282	290
		1403	247	262	277	285	297	303
		1404	264	276	286	295	304	312
		1405	245	260	271	279	293	305
		1406	243	253	261	272	285	293
		Mean±S.D.	247±9	260±9	270±10	279±10	291±9	298±10

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm

Appendix 7. Continued

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Compound	Dose (ppm)	Animal ID No.	Body weight of each period (g) Day 78	Day 85	Day 91	Sacrificed #	Gain (f)
Commercial diet a)	0	1001	363	370	381	387	243
		1002	356	369	376	384	239
		1003	337	346	354	360	223
		1004	342	353	360	366	230
		1005	323	332	340	347	212
		1006	371	384	392	397	241
		Mean±S.D.	349±18	359±19	367±19	374±19	231±12
Agaritine	62.5 b)	1101	348	356	370	375	228
		1102	348	356	365	370	225
		1103	336	348	360	372	224
		1104	328	341	344	352	204
		1105	346	354	361	369	217
		1106	342	350	359	368	222
		Mean±S.D.	341±8	351±6	360±9	368±8	220±9
	250 c)	1201	289	293	295	294	149
		1202	270	278	282	284	143
		1203	273	276	279	279	129
		1204	281	286	293	291	143
		1205	298	300	303	304	148
		1206	270	277	279	281	142
		Mean±S.D.	280±11	285±10	289±10	289±9	142±7
	750 d)	1301	177	149	143	136	-7
		1302	178	181	182	189	49
		1303	189	189	175	185	42
		1304	192	193	196	199	60
		1305	172	169	173	176	38
		1306	176	177	161	148	14
		Mean±S.D.	181±8	176±16	172±18	172±25	33±25
Product B	5 (%)	1401	293	300	303	306	172
		1402	295	297	300	302	159
		1403	312	315	319	320	185
		1404	319	323	329	330	178
		1405	311	318	316	319	171
		1406	297	304	308	310	174
		Mean±S.D.	305±11	310±11	313±11	315±10	173±9
ENU e)	50 (mg/kg)	1501	-	-	-	154	2
		1502	-	-	-	155	1
		1503	-	-	-	144	6
		1504	-	-	-	144	1
		Mean±S.D.	-	-	-	149±6	3±2

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days. 10 mL/kg, expression time:3 days)
f): Gain=Sacrificed-Day 1(Allocated)
-: Not measured
#: The positive control group was sacrificed on Day 8.

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Appendix 8. Food consumption in the gene mutation assay using transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Food consumption (g/week) Week 1	Week 2	Week 3	Week 4	Week 5
Commercial diet a)	0	1001	99	95	94	101	108
		1002	95	104	105	99	103
		1003	96	102	105	106	106
		1004	96	95	94	97	104
		1005	90	90	95	96	102
		1006	101	118	118	120	120
		Mean±S.D.	96±4	101±10	102±9	103±9	107±7
Agaritine	62.5 b)	1101	90	98	98	102	104
		1102	96	98	101	99	106
		1103	100	101	99	102	97
		1104	93	95	99	105	103
		1105	91	102	101	106	104
		1106	92	95	98	98	106
		Mean±S.D.	94±4	98±3	99±1	102±3	103±3
	250 c)	1201	85	86	88	84	91
		1202	87	93	90	88	80
		1203	91	89	86	89	90
		1204	85	92	92	90	90
		1205	93	95	100	96	90
		1206	91	94	96	91	93
		Mean±S.D.	89±3	92±3	92±5	90±4	89±5
	750 d)	1301	56	65	63	66	64
		1302	47	58	60	65	60
		1303	52	58	71	74	66
		1304	50	67	58	63	61
		1305	50	67	63	58	60
		1306	52	59	59	66	63
		Mean±S.D.	51±3	62±4	62±5	65±5	62±2
Product B	5 (%)	1401	90	96	94	98	92
		1402	89	97	95	94	89
		1403	86	93	96	90	96
		1404	91	96	100	99	102
		1405	90	87	95	93	94
		1406	81	92	104	90	91
		Mean±S.D.	88±4	94±4	97±4	94±4	94±5

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm

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Appendix 8. Continued

Compound	Dose (ppm)	Animal ID No.	Food consumption (g/week) Week 6	Week 7	Week 8	Week 9	Week 10
Commercial diet a)	0	1001	111	104	111	110	110
		1002	100	107	110	109	105
		1003	97	105	104	102	102
		1004	102	98	102	104	108
		1005	97	100	99	103	102
		1006	113	108	113	117	110
		Mean±S.D.	103±7	104±4	107±6	108±6	106±4
Agaritine	62.5 b)	1101	102	101	106	106	103
		1102	105	102	105	106	101
		1103	95	98	103	99	104
		1104	97	90	95	98	96
		1105	101	98	104	98	106
		1106	102	102	108	107	105
		Mean±S.D.	100±4	99±5	104±5	102±4	103±4
	250 c)	1201	85	86	92	94	94
		1202	87	83	88	89	86
		1203	85	83	92	85	78
		1204	89	88	91	86	88
		1205	91	90	95	95	97
		1206	87	90	86	86	86
		Mean±S.D.	87±2	87±3	91±3	89±4	88±7
	750 d)	1301	59	58	54	57	62
		1302	59	58	51	59	56
		1303	67	55	56	63	54
		1304	60	60	61	66	62
		1305	55	56	56	57	57
		1306	63	60	63	58	60
		Mean±S.D.	61±4	58±2	57±4	60±4	59±3
Product B	5 (%)	1401	89	86	96	94	93
		1402	92	89	93	89	92
		1403	90	91	93	94	95
		1404	97	94	92	95	91
		1405	94	90	90	99	96
		1406	87	89	90	95	95
		Mean±S.D.	92±4	90±3	92±2	94±3	94±2

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm

Appendix 8. Continued

Compound	Dose (ppm)	Animal ID No.	Food consumption (g/week) Week 11	Week 12	Week 13	Total
Commercial diet a)	0	1001	108	106	97	1354
		1002	107	110	91	1345
		1003	104	100	90	1319
		1004	107	107	93	1307
		1005	102	98	90	1264
		1006	125	124	104	1491
		Mean±S.D.	109±8	108±9	94±5	1347±78
Agaritine	62.5 b)	1101	102	101	90	1303
		1102	106	100	93	1318
		1103	98	102	90	1288
		1104	98	101	80	1250
		1105	104	104	89	1308
		1106	111	104	93	1321
		Mean±S.D.	103±5	102±2	89±5	1298±26
	250 c)	1201	90	89	72	1136
		1202	87	84	71	1113
		1203	85	86	73	1112
		1204	83	85	74	1133
		1205	90	90	79	1201
		1206	87	84	77	1148
		Mean±S.D.	87±3	86±3	74±3	1141±33
	750 d)	1301	56	31	34	725
		1302	56	58	51	738
		1303	61	57	46	780
		1304	59	62	51	780
		1305	57	53	48	737
		1306	54	55	36	748
		Mean±S.D.	57±2	53±11	44±8	751±23
Product B	5 (%)	1401	91	89	78	1186
		1402	89	88	72	1168
		1403	94	93	76	1187
		1404	97	96	82	1232
		1405	92	93	77	1190
		1406	91	90	77	1172
		Mean±S.D.	92±3	92±3	77±3	1189±23

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm

Appendix 9. Test substance intake in the gene mutation assay using transgenic rats treated with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Test substance intake at each period (mg/kg/day) Week 1	Week 2	Week 3	Week 4	Week 5
Agaritine	62.5 a)	1101	2.8	2.8	3.3	3.9	3.6
		1102	3.1	2.9	3.3	3.6	3.6
		1103	3.0	2.8	3.4	3.9	3.4
		1104	2.8	2.8	3.3	3.9	3.6
		1105	2.7	3.0	3.2	3.8	3.6
		1106	2.8	2.9	3.4	3.7	3.7
		Mean±S.D.	2.9±0.2	2.9±0.1	3.3±0.1	3.8±0.1	3.6±0.1
	250 b)	1201	17.7	17.0	15.9	13.4	13.6
		1202	17.9	18.4	16.0	14.8	12.0
		1203	18.7	18.1	14.6	14.9	14.2
		1204	17.2	17.8	15.5	14.4	13.7
		1205	17.7	18.2	15.8	14.6	13.1
		1206	19.6	18.6	17.3	15.0	14.3
		Mean±S.D.	18.1±0.9	18.0±0.6	15.9±0.9	14.5±0.6	13.5±0.8
	750 c)	1301	77.2	62.6	57.3	54.2	52.0
		1302	68.5	56.4	59.6	57.3	54.9
		1303	67.5	55.7	62.1	62.9	49.2
		1304	69.9	71.5	52.3	55.6	52.9
		1305	71.0	73.0	60.4	51.9	57.0
		1306	72.0	57.6	51.9	55.2	52.6
		Mean±S.D.	71.0±3.4	62.8±7.7	57.3±4.3	56.2±3.7	53.1±2.7
Product B	5 (%)	1401	4333.3	3910.6	3233.8	3181.8	2766.0
		1402	4113.9	3743.3	3317.5	2863.4	2731.1
		1403	3921.6	3532.6	3365.4	2850.9	2892.6
		1404	3963.4	3645.8	3196.3	2916.7	2918.3
		1405	4088.1	3278.7	3398.1	2888.9	2719.7
		1406	4000.0	3651.7	3658.5	2888.9	2731.1
		Mean±S.D.	4070.1±148.2	3627.1±212.2	3361.6±164.4	2931.8±124.6	2793.1±88.8

a): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
b): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
c): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm

Appendix 9. Continued

Compound	Dose (ppm)	Animal ID No.	Test substance intake at each period (mg/kg/day) Week 6	Week 7	Week 8	Week 9	Week 10
Agaritine	62.5 a)	1101	3.3	3.0	3.1	2.9	2.8
		1102	3.4	3.2	3.1	2.9	2.6
		1103	3.2	3.1	3.1	2.8	2.9
		1104	3.2	2.9	3.0	2.9	2.8
		1105	3.1	3.0	3.1	2.8	2.9
		1106	3.5	3.3	3.1	3.0	2.9
		Mean±S.D.	3.3±0.1	3.1±0.1	3.1±0.0	2.9±0.1	2.8±0.1
	250 b)	1201	12.0	11.6	12.3	11.9	11.5
		1202	12.6	12.2	12.8	12.4	11.2
		1203	12.5	12.1	12.6	11.2	10.2
		1204	13.1	12.6	12.2	10.9	11.7
		1205	12.6	12.1	12.7	12.3	11.9
		1206	12.6	13.1	11.8	11.5	11.3
		Mean±S.D.	12.6±0.4	12.3±0.5	12.4±0.4	11.7±0.6	11.3±0.6
	750 c)	1301	45.2	44.7	45.2	34.5	38.4
		1302	47.1	46.5	40.7	34.7	33.7
		1303	52.6	42.6	43.7	36.7	32.6
		1304	51.4	50.0	49.2	35.9	35.2
		1305	49.4	49.1	48.8	35.9	35.1
		1306	51.4	50.8	50.3	33.1	37.5
		Mean±S.D.	49.5±2.9	47.3±3.2	46.3±3.7	35.1±1.3	35.4±2.2
Product B	5 (%)	1401	2642.3	2343.8	2641.5	2355.1	2280.7
		1402	2600.0	2490.4	2416.4	2338.1	2272.7
		1403	2549.0	2407.4	2313.2	2233.7	2333.3
		1404	2592.6	2313.2	2233.7	2333.3	2110.4
		1405	2569.2	2443.6	2363.6	2447.6	2341.1
		1406	2419.4	2529.2	2434.5	2509.0	2422.1
		Mean±S.D.	2562.1±76.7	2421.3±83.4	2400.5±138.8	2369.5±96.4	2293.4±104.4

a): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
b): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
c): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm

Appendix 9. Continued

Compound	Dose (ppm)	Animal ID No.	Test substance intake at each period (mg/kg/day) Week 11	Week 12	Week 13	Mean (1-13)
Agaritine	62.5 a)	1101	2.7	2.5	2.6	3.0
		1102	2.7	2.5	2.8	3.1
		1103	2.6	2.7	2.6	3.0
		1104	2.7	2.6	2.4	3.0
		1105	2.8	2.7	2.6	3.0
		1106	3.0	2.7	2.8	3.1
		Mean±S.D.	2.8±0.1	2.6±0.1	2.6±0.2	3.0±0.1
	250 b)	1201	11.3	11.2	10.2	13.0
		1202	11.1	10.9	10.7	13.3
		1203	11.1	10.9	10.8	13.2
		1204	10.7	10.6	10.3	13.1
		1205	10.9	10.9	10.8	13.4
		1206	11.2	10.9	11.7	13.8
		Mean±S.D.	11.1±0.2	10.9±0.2	10.8±0.5	13.3±0.3
	750 c)	1301	33.7	18.4	30.8	45.7
		1302	33.5	33.3	37.1	46.4
		1303	36.5	31.7	33.0	46.7
		1304	31.1	35.0	34.6	48.0
		1305	34.9	35.1	35.1	49.0
		1306	33.7	33.9	26.6	46.7
		Mean±S.D.	33.9±1.8	31.2±6.4	32.9±3.7	47.1±1.2
Product B	5 (%)	1401	2241.4	2188.6	2152.3	2790.1
		1402	2218.4	2195.9	2006.7	2716.0
		1403	2110.4	2070.1	2050.5	2663.9
		1404	2215.2	2180.7	2147.2	2674.4
		1405	2110.4	2063.5	2050.5	2674.1
		1406	2203.4	2159.5	2124.2	2748.6
		Mean±S.D.	2183.2±57.7	2143.1±60.3	2088.6±60.6	2711.2±50.2

a): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
b): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
c): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm

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Appendix 10. Clinical observations in the gene mutation assay with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Compound	Dose (ppm)	Animal ID No.	Day of experiment 1	2	3	4	5	6	7
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	N	N	N	N	N
		1302	N	N	N	N	N	N	N
		1303	N	N	N	N	N	N	N
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	N	N	N	N	N
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N
ENU e)	50 (mg/kg)	1501	N	N	N	N	N	N	N
		1502	N	N	N	N	N	N	N
		1503	N	N	N	N	N	N	N
		1504	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)
N : Normal

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Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 8	9	10	11	12	13	14
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	N	N	N	N	N
		1302	N	N	N	N	N	N	N
		1303	N	N	N	N	N	N	N
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	N	N	N	N	N
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N
ENU e)	50 (mg/kg)	1501	N	-	-	-	-	-	-
		1502	N	-	-	-	-	-	-
		1503	N	-	-	-	-	-	-
		1504	N	-	-	-	-	-	-

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)
- : Not observed
N : Normal

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 15	16	17	18	19	20	21
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	N	N	N	N	N
		1302	N	N	N	N	N	N	N
		1303	N	N	N	N	N	N	N
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	N	N	N	N	N
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 22	23	24	25	26	27	28
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	N	N	N	N	N
		1302	N	N	N	N	N	N	N
		1303	N	N	N	N	N	N	N
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	N	N	N	N	N
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 29	30	31	32	33	34	35
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	N	N	N	N	N
		1302	N	N	N	N	N	N	N
		1303	N	N	N	N	N	N	N
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	N	N	N	N	N
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 36	37	38	39	40	41	42
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	N	N	N	N	N
		1302	N	N	N	N	N	N	N
		1303	N	N	N	N	N	N	N
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	N	N	N	N	N
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 43	44	45	46	47	48	49
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	N	N	N	N	N
		1302	N	N	N	N	N	N	N
		1303	N	N	N	N	N	N	N
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	N	N	N	N	N
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 50	51	52	53	54	55	56
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	N	N	AG	AG	AG	AG	AG, SP
		1302	N	N	N	N	N	N	N
		1303	N	N	AG	AG	AG	AG	AG
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	N	N	AG	AG	AG	AG	AG, SP
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal , AG : Ataxic gait , SP : Smudge of perinasal area

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 57	58	59	60	61	62	63
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1302	W	W	W	SP,W	SP,W	SP,W	SP,W
		1303	AG,W	AG,W	AG,W	AG,W	AG,W	AG,W	AG,W
		1304	N	N	N	N	N	N	N
		1305	N	N	N	N	N	N	N
		1306	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal , AG : Ataxic gait , SP : Smudge of perinasal area , W : Wasting

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 64	65	66	67	68	69	70
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1302	SP,W	SP,W	SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1303	AG,W	AG,W	AG,W	AG,W	AG,W	AG,W	AG,W
		1304	N	N	N	AG	AG	AG	AG
		1305	N	N	AG	AG	AG	AG	AG,SP
		1306	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal , AG : Ataxic gait , SP : Smudge of perinasal area , W : Wasting

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 71	72	73	74	75	76	77
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1302	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1303	AG,W	AG,W	AG,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1304	AG	AG	AG	AG	AG	AG	AG
		1305	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP
		1306	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal , AG : Ataxic gait , SP : Smudge of perinasal area , W : Wasting

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 78	79	80	81	82	83	84
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	SP,W,DH
		1302	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1303	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1304	AG	AG	AG	AG	AG	AG	AG
		1305	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP
		1306	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal , AG : Ataxic gait , SP : Smudge of perinasal area , W : Wasting , DH : Dragging of hindlimbs

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 85	86	87	88	89	90	91
Commercial diet a)	0	1001	N	N	N	N	N	N	N
		1002	N	N	N	N	N	N	N
		1003	N	N	N	N	N	N	N
		1004	N	N	N	N	N	N	N
		1005	N	N	N	N	N	N	N
		1006	N	N	N	N	N	N	N
Agaritine	62.5 b)	1101	N	N	N	N	N	N	N
		1102	N	N	N	N	N	N	N
		1103	N	N	N	N	N	N	N
		1104	N	N	N	N	N	N	N
		1105	N	N	N	N	N	N	N
		1106	N	N	N	N	N	N	N
	250 c)	1201	N	N	N	N	N	N	N
		1202	N	N	N	N	N	N	N
		1203	N	N	N	N	N	N	N
		1204	N	N	N	N	N	N	N
		1205	N	N	N	N	N	N	N
		1206	N	N	N	N	N	N	N
	750 d)	1301	SP,W,DH	SP,W,DH	SP,W,DH,SF	SP,W,DH,SF	SP,W,DH,SF	SP,W,DH,SF	SP,W,DH,SF
		1302	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1303	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
		1304	AG	AG	AG	AG	AG,SP	AG,SP	AG,SP
		1305	AG,SP	AG,SP	AG,SP	AG,SP	AG,SP,W	AG,SP,W	AG,SP,W
		1306	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W	AG,SP,W
Product B	5 (%)	1401	N	N	N	N	N	N	N
		1402	N	N	N	N	N	N	N
		1403	N	N	N	N	N	N	N
		1404	N	N	N	N	N	N	N
		1405	N	N	N	N	N	N	N
		1406	N	N	N	N	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal , AG : Ataxic gait , SP : Smudge of perinasal area , W : Wasting
DH : Dragging of hindlimbs , SF : Soiled fur (Back)

Appendix 10. Continued

Compound	Dose (ppm)	Animal ID No.	Day of experiment 92	93	94
Commercial diet a)	0	1001	N	N	N
		1002	N	N	N
		1003	N	N	N
		1004	N	N	N
		1005	N	N	N
		1006	N	N	N
Agaritine	62.5 b)	1101	N	N	N
		1102	N	N	N
		1103	N	N	N
		1104	N	N	N
		1105	N	N	N
		1106	N	N	N
	250 c)	1201	N	N	N
		1202	N	N	N
		1203	N	N	N
		1204	N	N	N
		1205	N	N	N
		1206	N	N	N
	750 d)	1301	SP,W,DH,SF	SP,W,DH,SF	SP,W,DH,SF
		1302	AG,SP,W	AG,SP,W	AG,SP,W
		1303	AG,SP,W	AG,SP,W	AG,SP,W
		1304	AG,SP	AG,SP	AG,SP
		1305	AG,SP,W	AG,SP,W	AG,SP,W
		1306	AG,SP,W	SP,W,DH	SP,W,DH
Product B	5 (%)	1401	N	N	N
		1402	N	N	N
		1403	N	N	N
		1404	N	N	N
		1405	N	N	N
		1406	N	N	N

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
N : Normal , AG : Ataxic gait , SP : Smudge of perinasal area , W : Wasting
DH : Dragging of hindlimbs , SF : Soiled fur (Back)

Appendix 11. Organ weight in the gene mutation assay with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Exp. No. A260(079-388)

Compound	Dose (ppm)	Animal ID No.	Liver (g)	Kidneys (g)	Lungs (g)	Heart (g)	Testes (g)
Commercial diet a)	0	1001	12.322	2.077	1.261	0.923	2.800
		1002	12.480	2.000	1.167	0.977	2.808
		1003	11.460	1.954	1.118	0.890	2.702
		1004	12.358	1.958	1.166	0.898	2.630
		1005	11.109	1.893	1.109	0.883	2.849
		1006	13.524	2.213	1.149	0.983	3.033
		Mean±S.D.	12.209±0.850	2.016±0.114	1.162±0.054	0.926±0.044	2.804±0.138
Agaritine	62.5 b)	1101	12.455	2.131	1.141	0.922	2.900
		1102	12.158	2.014	1.271	0.935	2.761
		1103	12.368	2.121	1.157	0.914	2.860
		1104	11.340	1.964	1.157	0.930	2.912
		1105	12.100	2.104	1.155	0.913	2.883
		1106	12.184	2.152	1.211	0.908	2.653
		Mean±S.D.	12.101±0.396	2.081±0.075	1.182±0.050	0.920±0.011	2.828±0.101
	250 c)	1201	9.261	1.910	1.004	0.827	2.842
		1202	9.705	2.015	0.898	0.859	2.714
		1203	9.490	1.973	0.891	0.774	2.549
		1204	9.378	1.990	0.946	0.794	2.862
		1205	10.088	2.046	0.958	0.842	2.728
		1206	8.908	1.874	0.932	0.793	2.479
		Mean±S.D.	9.472±0.401	1.968±0.065	0.938±0.042	0.815±0.033	2.696±0.154
	750 d)	1301	3.885	1.370	0.587	0.580	1.244
		1302	6.312	1.476	0.672	0.589	2.167
		1303	6.045	1.507	0.709	0.599	2.282
		1304	6.841	1.466	0.714	0.722	2.336
		1305	5.395	1.447	0.613	0.573	2.146
		1306	4.530	1.378	0.572	0.579	1.886
		Mean±S.D.	5.501±1.124	1.441±0.055	0.645±0.062	0.607±0.057	2.010±0.406
Product B	5 (%)	1401	9.822	2.002	0.987	0.844	2.479
		1402	9.971	1.968	1.018	0.836	2.485
		1403	10.240	2.049	1.046	0.826	2.679
		1404	11.136	2.013	1.065	0.874	2.612
		1405	9.711	1.839	1.047	0.822	2.544
		1406	9.837	1.935	1.074	0.855	2.752
		Mean±S.D.	10.120±0.530	1.968±0.074	1.040±0.032	0.843±0.019	2.592±0.110
ENU e)	50 (mg/kg)	1501	6.805	1.210	0.710	0.548	2.150
		1502	6.752	1.144	0.735	0.554	2.170
		1503	6.140	1.089	0.726	0.484	1.583
		1504	5.819	1.082	0.684	0.497	1.908
		Mean±S.D.	6.379±0.480	1.131±0.059	0.714±0.022	0.521±0.035	1.953±0.274

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

Appendix 12. Organ weight per body weight in the gene mutation assay with agaritine
[Male rats (Dietary administration for 91 days, expression period; 3 days)]

Exp. No. A260(079-388)

Compound	Dose (ppm)	Animal ID No.	Body weight (g)	Liver (%)	Kidneys (%)	Lungs (%)	Heart (%)	Testes (%)
Commercial diet a)	0	1001	387	3.184	0.537	0.326	0.239	0.724
		1002	384	3.250	0.521	0.304	0.254	0.731
		1003	360	3.183	0.543	0.311	0.247	0.751
		1004	366	3.377	0.535	0.319	0.245	0.719
		1005	347	3.201	0.546	0.320	0.254	0.821
		1006	397	3.407	0.557	0.289	0.248	0.764
		Mean±S.D.	374±19	3.267±0.100	0.540±0.012	0.312±0.013	0.248±0.006	0.752±0.038
Agaritine	62.5 b)	1101	375	3.321	0.568	0.304	0.246	0.773
		1102	370	3.286	0.544	0.344	0.253	0.746
		1103	372	3.325	0.570	0.311	0.246	0.769
		1104	352	3.222	0.558	0.329	0.264	0.827
		1105	369	3.279	0.570	0.313	0.247	0.781
		1106	368	3.311	0.585	0.329	0.247	0.721
		Mean±S.D.	368±8	3.291±0.038	0.566±0.014	0.322±0.015	0.251±0.007	0.770±0.036
	250 c)	1201	294	3.150	0.650	0.341	0.281	0.967
		1202	284	3.417	0.710	0.316	0.302	0.956
		1203	279	3.401	0.707	0.319	0.277	0.914
		1204	291	3.223	0.684	0.325	0.273	0.984
		1205	304	3.318	0.673	0.315	0.277	0.897
		1206	281	3.170	0.667	0.332	0.282	0.882
		Mean±S.D.	289±9	3.280±0.116	0.682±0.023	0.325±0.010	0.282±0.010	0.933±0.041
	750 d)	1301	136	2.857	1.007	0.432	0.426	0.915
		1302	189	3.340	0.781	0.356	0.312	1.147
		1303	185	3.268	0.815	0.383	0.324	1.234
		1304	199	3.438	0.737	0.359	0.363	1.174
		1305	176	3.065	0.822	0.348	0.326	1.219
		1306	148	3.061	0.931	0.386	0.391	1.274
		Mean±S.D.	172±25	3.172±0.215	0.849±0.101	0.377±0.031	0.357±0.045	1.161±0.128
Product B	5 (%)	1401	306	3.210	0.654	0.323	0.276	0.810
		1402	302	3.302	0.652	0.337	0.277	0.823
		1403	320	3.200	0.640	0.327	0.258	0.837
		1404	330	3.375	0.610	0.323	0.265	0.792
		1405	319	3.044	0.576	0.328	0.258	0.797
		1406	310	3.173	0.624	0.346	0.276	0.888
		Mean±S.D.	315±10	3.217±0.113	0.626±0.030	0.331±0.009	0.268±0.009	0.825±0.035
ENU e)	50 (mg/kg)	1501	154	4.419	0.786	0.461	0.356	1.396
		1502	155	4.356	0.738	0.474	0.357	1.400
		1503	144	4.264	0.756	0.504	0.336	1.099
		1504	144	4.041	0.751	0.475	0.345	1.325
		Mean±S.D.	149±6	4.270±0.165	0.758±0.020	0.479±0.018	0.349±0.010	1.305±0.142

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

Appendix 13. Individual gross findings in the gene mutation assay with agaritine [Male rats (Dietary administration for 91 days, expression period; 3 days)]

Exp. No. A260(079-388)

Compound	Dose (ppm)	Animal ID No.	Day of experiment	Findings and comments
Commercial diet a)	0	1001	94	Non remarkable
		1002	94	Non remarkable
		1003	94	Non remarkable
		1004	94	Non remarkable
		1005	94	Non remarkable
		1006	94	Non remarkable
Agaritine	62.5 b)	1101	94	Non remarkable
		1102	94	Non remarkable
		1103	94	Non remarkable
		1104	94	Non remarkable
		1105	94	Non remarkable
		1106	94	Non remarkable
	250 c)	1201	94	Sternum:deformed
		1202	94	Sternum:deformed
		1203	94	Non remarkable
		1204	94	Sternum:deformed
		1205	94	Sternum:deformed
		1206	94	Sternum:deformed
	750 d)	1301	94	Thymus:atrophy Sternum:deformed
		1302	94	Thymus:atrophy Sternum:deformed
		1303	94	Thymus:atrophy Sternum:deformed
		1304	94	Thymus:atrophy Sternum:deformed
		1305	94	Thymus:atrophy Sternum:deformed
		1306	94	Thymus:atrophy Sternum:deformed Lung:red patch/zone,right,a few,5x4 mm
Product B	5 (%)	1401	94	Non remarkable
		1402	94	Sternum:deformed
		1403	94	Non remarkable
		1404	94	Non remarkable
		1405	94	Sternum:deformed
		1406	94	Non remarkable
ENU e)	50 (mg/kg)	1501	8	Non remarkable
		1502	8	Non remarkable
		1503	8	Non remarkable
		1504	8	Non remarkable

a): Negative control (CRF-1 powder, Oriental Yeast)
b): Week 1; 35 ppm, Week 2; 39 ppm, Week 3; 52 ppm, Week 4-13; 62.5 ppm
c): Week 1; 231 ppm, Week 2; 257 ppm, Week 3-13; 250 ppm
d): Week 1; 1389 ppm, Week 2; 1030 ppm, Week 3-8; 1000 ppm, Week 9-13; 750 ppm
e): Positive control (N-nitroso-N-ethylurea, i.p. once a day for 5 days, 10 mL/kg, expression time:3 days)

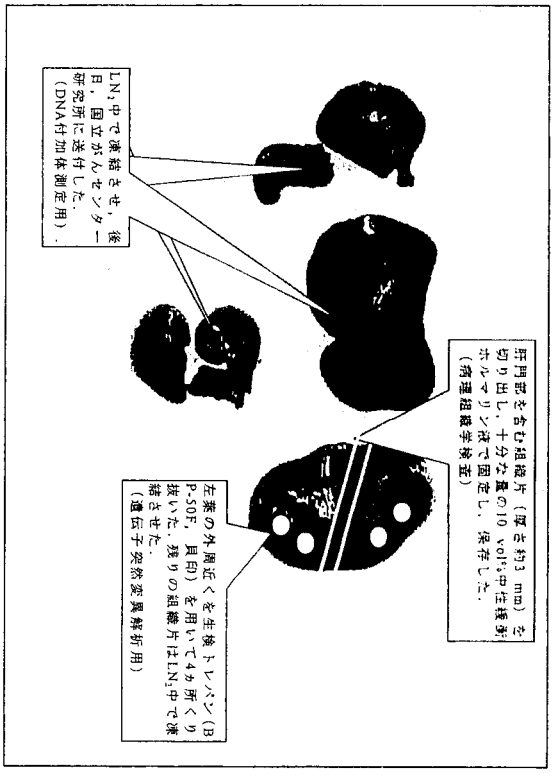


図1 肝臓のサンプリング

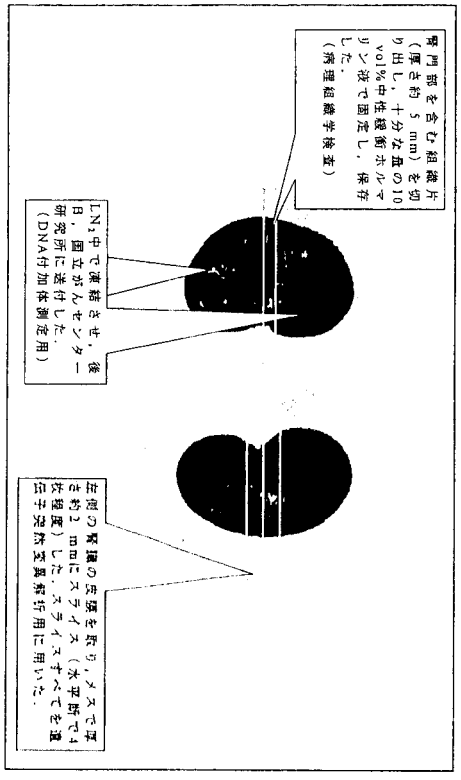


図2 腎臓のサンプリング

添付資料2

アガリチンのDNA付加体解析（試験計画書）

試料：アガリチンを投与したBig Blue Ratより，肝臓，腎臓等の組織を摘出後，ゲノムDNAを抽出し，試料とする．DNA付加体の解析は肝臓および腎臓を優先し，その他の臓器に関しては関係者と協議の上，実施の有無を決定する．

試験群：コントロール（n=3）

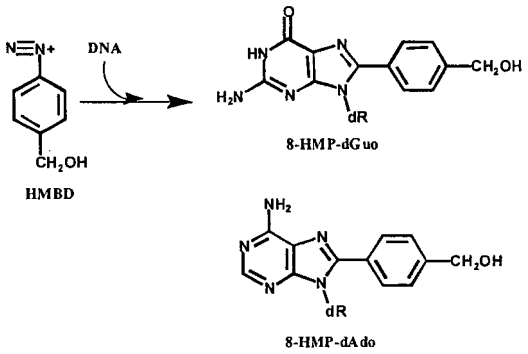
アガリチン高用量群（120 mg/kg）（n=3-5）

キリン製品投与群（n=3-5）

DNA付加体の解析方法

1) まずはアガリチンの代謝産物と考えられる4-(hydroxymethyl)phenylhydrazine (HMBO)から生成される既知のDNA付加体である，8-[4-(hydroxymethyl)phenyl]dGuo (8-HMP-dGuo)および8-[4-(hydroxymethyl)phenyl]dAdo (8-HMP-dAdo)の形成の有無について，HPLC，LC/MS/MSまたは^{32}P-ポストラベル法（注1）等を用いて解析する．

2) 試料中から8-HMP-dGuoおよび8-HMP-dAdoが検出されない場合は，その他のアガリチン由来のDNA付加体の生成についてさらにLC/MS/MSおよび^{32}P-ポストラベル法等を用いて検討を行う予定である．



（注1）^{32}P-ポストラベル法

^{32}P-ポストラベル法とはDNA付加体を好感度に検出する方法で，具体的には，DNAを分解酵素で2'-deoxynucleoside 3'-monophosphateに分解した後，5'-末端を[γ-^{32}P]ATPで標識し，2次元薄層クロマトグラフィー等で正常ヌクレオチドとDNA付加体を分離し，解析する．

添付資料3

アガリチンのDNA付加体解析（試験報告書）

国立がんセンター研究所
がん予防基礎研究プロジェクト
戸塚ゆ加里

[試料]アガリチンを投与したBig Blue Ratの肝臓，腎臓からゲノムDNAを抽出し，試料とした．

試験群：コントロール（n=3）

アガリチン低用量群（n=3）

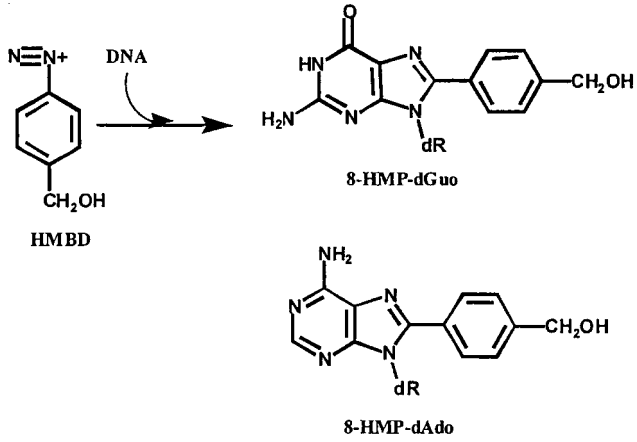
アガリチン中用量群（n=3）

アガリチン高用量群（n=3）

キリン製品投与群（n=3）

[方法]

アガリチンの代謝産物と考えられる4-(hydroxymethyl)phenylhydrazine (HMBD)から生成される既知のDNA付加体である，8-[4-(hydroxymethyl)phenyl]dGuo (8-HMP-dGuo)および8-[4-(hydroxymethyl)phenyl]dAdo (8-HMP-dAdo)の解析を，蛍光検出器を用いたHPLCにより行った．



HPLC 分析条件

検出波長：励起波長; 300 nm，蛍光波長; 400 nm

カラム：TSK-gel ODS 80Ts (5 μM, 4.6 x 250 mm)

流速：0.5 mL/min

溶離液：30%メタノール– 20mM ギ酸アンモニウム

高分子 DNA サンプル（ウシ胸腺 DNA およびラット組織のゲノム DNA）は，100 μg 相当を DNA 分解酵素（ミクロコッカルヌクレアーゼおよびホスホジエステラーゼ II）で分解した後，更にアルカリホスファターゼで脱リン酸化を行ない，モノヌクレオシドに分解して HPLC で解析した．

［結果］

まず始めに，既知濃度の標準品 (8-HMP-dGuoおよび8-HMP-dAdo) をHPLCで分析し，検量線の作成を行った（図 1）．その結果，この条件下におけるこれら付加体の検出限界は，絶対量で 25 pg，付加体レベルに換算すると，約 2 adducts / 10^7 nucleotideであることが分かった．次に，HMBDをウシ胸腺DNAと反応させて生成したHMP-DNAの解析を行った．その結果，8-HMP-dGuoおよび8-HMP-dAdoと同じ保持時間に溶出されるピークが観察された（図2）．これらのピークはHMBDと反応させていないウシ胸腺DNAを解析した場合には観察されないこと，また，付加体標準品（8-HMP-dGuo，8-HMP-dAdo）とのco-chromatographyでピークが一致したことから，HMBDと反応させたウシ胸腺DNAサンプル中に 8-HMP-dGuoおよび 8-HMP-dAdoが生成していることがわかった．この時の付加体レベルは，8-HMP-dGuoが 1 adduct / 10^4 nucleotidesであり，8-HMP-dAdoが 0.6 adduct / 10^4 nucleotidesであった．

一方，アガリチンおよびキリン製品を投与した Big Blue Rat の肝臓，腎臓からゲノム DNA を抽出し，同様に解析を行ったところ，8-HMP-dGuo および 8-HMP-dAdo に相当するピークは観察されなかった（図 3）．

［考察］

今回解析したアガリチン（低，中，高用量）およびキリン製品を投与した Big Blue Rat の肝臓および腎臓 DNA 中には，アガリチン由来の既知の DNA 付加体である 8-HMP-dGuo，8-HMP-dAdo の生成は観察されなかった．その理由として，以下の事が考えられる．

1) これら実験動物の生体内で生成する DNA 付加体のレベルが低く，検出限界以下であった．
2) サンプリングのタイミングが，アガリチンおよびキリン製品投与 3 日後であることから，8-HMP-dGuo，8-HMP-dAdo が速やかに生体内から排除された．
3) 8-HMP-dGuo，8-HMP-dAdo は試験管内では生成するが，生体内においてはこれら既知の付加体は生成せず，アガリチン由来の未知の付加体が生成している．

生体内におけるアガリチン由来の DNA 付加体の解析は，検出方法の高感度化や試料採取のタイミング等，更に検討する事が望ましいと思われる．

図 1

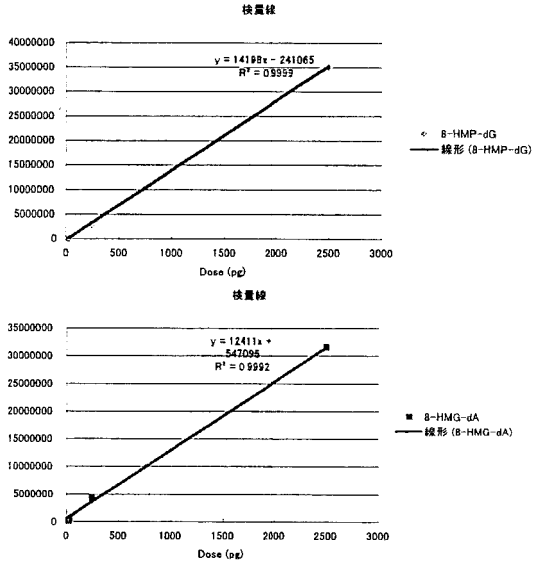


図2

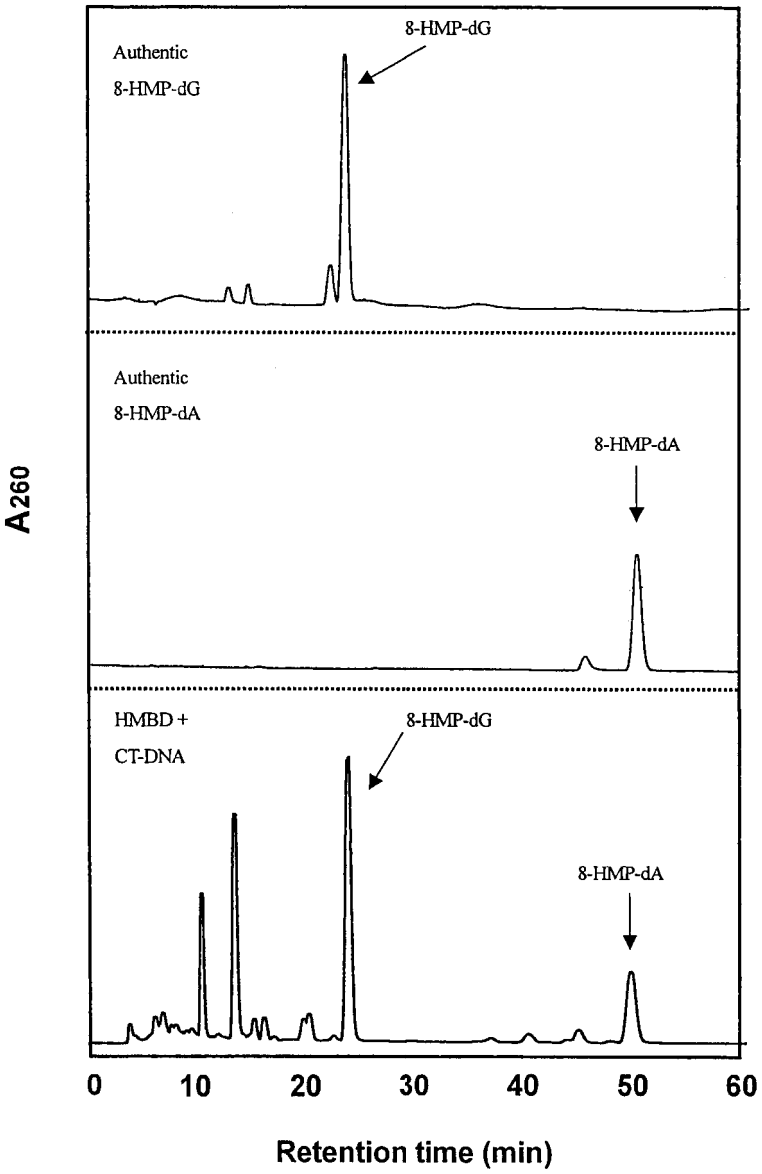
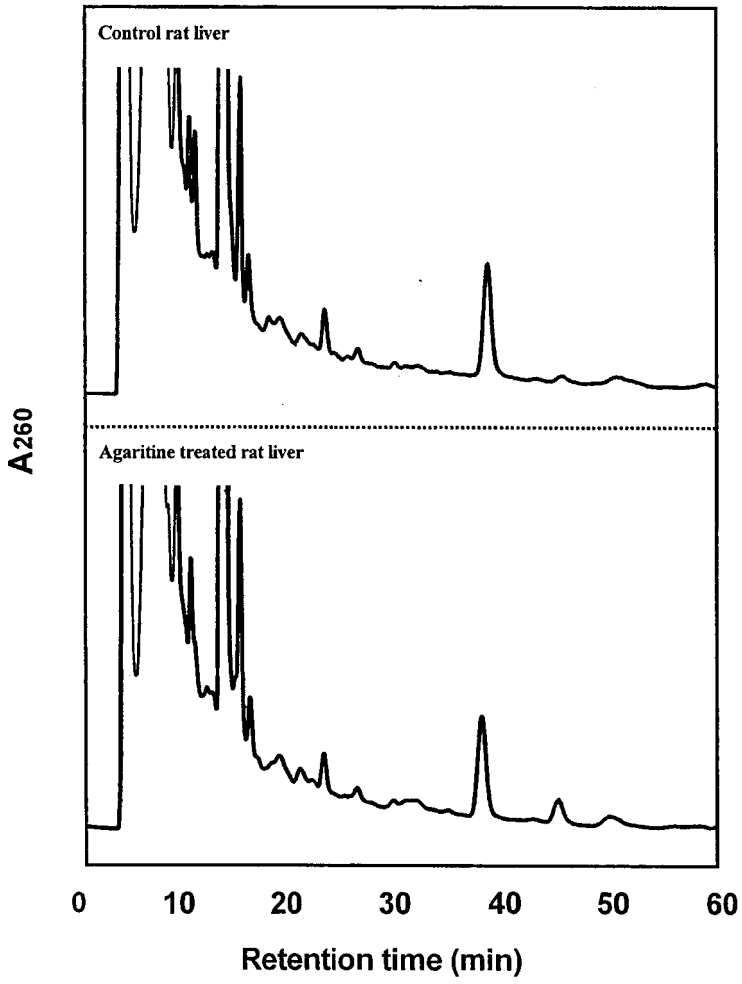

Authentic
8-HMP-dG
8-HMP-dG
Authentic
8-HMP-dA
8-HMP-dA
HMBD +
CT-DNA
8-HMP-dG
8-HMP-dA
A260
0
10
20
30
40
50
60
Retention time (min)

図3


Control rat liver
Agaritine treated rat liver
A260
0
10
20
30
40
50
60
Retention time (min)