

Key to Tables 5 and 6 - Methods of detailed clinical observations, parameters, and scoring criteria

1. Preparations for the observations

The following preparations for the detailed clinical observations were made by the staffs who did not conduct observations so that the observations were performed by an observer unaware of the group assignment of the animals.

- 1) Order of animals for the observation was determined through a computer-generated randomization procedure.
- 2) Animals were housed in wire-mesh stainless steel cages (width 160 mm x depth 230 mm x height 180 mm: Tokiwa Kagakukikai Co., Ltd.) in observation order. Each cage was labeled with the order and carried to functional observation room.
- 3) The animals were acclimatized to the functional observation room at least for 30 minutes.

2. Recording of the observations

Recording of the data was performed by the staffs who aware of the group assignment of animals.

3. Observation methods

Detailed clinical observations were performed inside the home cage, with handling, and in an open field (a standard arena surrounded by clear acrylic plates, width 500 mm x depth 500 mm x height 400 mm: Muromachi Kikai Co. Ltd.). The animals were observed in the open field at least for 1 minute.

4. Observation parameters and scoring criteria

Observation parameters and scoring criteria in the detailed clinical observations are described as follows:

In principle, the normal condition is defined as score 0.

Key to Tables 5 and 6 - Methods of detailed clinical observations, parameters, and scoring criteria (continued)

Handling

Parameters	Scoring criteria	Parameters	Scoring criteria
Salivation	0: No abnormalities 1: Slight 2: Moderate 3: Marked	Muscle tone	-2: Marked decrease -1: Slight decrease 0: Normal tone 1: Slight increase 2: Marked increase
Pupillary reflex	-2: Marked decrease -1: Slight decrease 0: Normal size 1: Slight increase 2: Marked increase N: No assessment possible		

Open field

Parameters	Scoring criteria	Parameters	Scoring criteria
Exploration	-2: No exploration -1: Decrease 0: Intermittent 1: Frequent 2: Continuous	Activity	Counts / 1 minute Measured with automated device*
Rearing	Counts / 1 minute	Count of defecation	Counts / 1 minute

*: Photo beam-type activity monitoring system (PAS-1, Muromachi Kikai Co., Ltd.)
Animals showed score 'N' were eliminated from the statistical evaluation.

Remarks:

Week -1, Before initiation of treatment.

S.D., Standard deviation.

Table 5 - 1

Detailed clinical observations - Summary data in male rats

Open field: Exploration

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	-2	0	0	0	0
	-1	0	1	1	0
	0	2	4	6	5
	1	8	5	3	5
	2	0	0	0	0
1	-2	0	0	0	0
	-1	0	0	0	1
	0	0	3	6	2
	1	10	7	4	7
	2	0	0	0	0
8	-2	0	0	0	0
	-1	0	0	2	3
	0	2	5	6	7
	1	8	5	2	0
	2	0	0	0	0
				*	
80	-2	0	0	0	0
	-1	0	1	3	3
	0	4	8	5	6
	1	6	1	2	1
	2	0	0	0	0

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$.

Table 5 - 2

Detailed clinical observations - Summary data in male rats

Open field: Count of defecation

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	0	9	8	10	10
	1	1	0	0	0
	2	0	2	0	0
	3	0	0	0	0
1	0	9	9	10	10
	1	0	1	0	0
	2	0	0	0	0
	3	1	0	0	0
8	0	9	9	9	9
	1	1	1	0	1
	2	0	0	1	0
	3	0	0	0	0
80	0	7	7	10	10
	1	2	2	0	0
	2	1	1	0	0
	3	0	0	0	0

Table 5 - 3

Detailed clinical observations - Summary data in male rats

Handling: Salivation

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
1	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
8	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
80	0	10	9	9	10
	1	0	1	1	0
	2	0	0	0	0
	3	0	0	0	0

Table 5 - 4

Detailed clinical observations - Summary data in male rats

Handling: Muscle tone

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	-2	0	0	0	0
	-1	0	0	0	0
	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
1	-2	0	0	0	0
	-1	0	0	0	0
	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
8	-2	0	0	0	0
	-1	0	0	0	0
	0	10	10	9	9
	1	0	0	1	1
	2	0	0	0	0
80	-2	0	0	0	0
	-1	0	0	0	0
	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0

Table 5 - 5

Detailed clinical observations - Summary data in male rats

Handling: Pupillary reflex

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	N	0	0	0	0
1	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	N	0	0	0	0
8	0	10	10	10	9
	1	0	0	0	1
	2	0	0	0	0
	N	0	0	0	0
80	0	10	10	8	10
	1	0	0	2	0
	2	0	0	0	0
	N	0	0	0	0

Table 5 - 6 Detailed clinical observations - Summary data in male rats

Open field: Activity

(counts)

Dose (mg/kg/day)		Week			
		1	2	3	4
0	Mean	427	408	331	381
	S.D.	103	111	115	111
	Num.	10	10	10	10
1	Mean	493	406	358	364
	S.D.	60	104	78	142
	Num.	10	10	10	10
8	Mean	454	389	254	217 *
	S.D.	60	92	97	116
	Num.	10	10	10	10
80	Mean	387	287 *	217	212 *
	S.D.	115	107	121	135
	Num.	10	10	10	10

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$.

Table 5 - 7

Detailed clinical observations - Summary data in male rats

Open field: Rearing

(counts)

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	0	0	1	0	1
	1-5	3	2	4	1
	6-10	3	3	3	4
	11-15	3	3	2	3
	16-20	1	1	1	1
	21-25	0	0	0	0
1	0	0	1	0	1
	1-5	0	2	3	1
	6-10	4	2	3	1
	11-15	4	3	3	6
	16-20	2	1	1	1
	21-25	0	1	0	0
8	0	0	0	2	3
	1-5	0	5	4	3
	6-10	7	2	4	4
	11-15	3	3	0	0
	16-20	0	0	0	0
	21-25	0	0	0	0
80	0	0	0	1	2
	1-5	2	8	6	5
	6-10	5	0	3	2
	11-15	3	1	0	1
	16-20	0	1	0	0
	21-25	0	0	0	0

Table 6 - 1

Detailed clinical observations - Summary data in female rats

Open field: Exploration

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	-2	0	0	0	0
	-1	0	1	0	0
	0	2	0	0	0
	1	8	5	6	8
	2	0	4	4	2
1	-2	0	0	0	0
	-1	0	0	0	0
	0	3	1	1	0
	1	7	6	6	10
	2	0	3	3	0
8	-2	0	0	0	0
	-1	0	1	0	0
	0	3	1	2	0
	1	7	5	7	8
	2	0	3	1	2
80	-2	0	0	0	0
	-1	0	1	1	0
	0	6	6	4	5
	1	4	2	4	4
	2	0	1	1	1
			*	*	

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$.

Table 6 - 2

Detailed clinical observations - Summary data in female rats

Open field: Count of defecation

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
1	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
8	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
80	0	10	8	9	10
	1	0	1	1	0
	2	0	0	0	0
	3	0	1	0	0

Table 6 - 3

Detailed clinical observations - Summary data in female rats

Handling: Salivation

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
1	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
8	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
80	0	10	9	9	9
	1	0	1	1	1
	2	0	0	0	0
	3	0	0	0	0

Table 6 - 4

Detailed clinical observations - Summary data in female rats

Handling: Muscle tone

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	-2	0	0	0	0
	-1	0	0	0	0
	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
1	-2	0	0	0	0
	-1	0	0	0	0
	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
8	-2	0	0	0	0
	-1	0	0	0	0
	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
80	-2	0	0	0	0
	-1	0	1	1	0
	0	10	9	9	10
	1	0	0	0	0
	2	0	0	0	0

Table 6 - 5

Detailed clinical observations - Summary data in female rats

Handling: Pupillary reflex

Dose (mg/kg/day)	Score	Week			
		1	2	3	4
0	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	N	0	0	0	0
1	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	N	0	0	0	0
8	0	10	10	10	10
	1	0	0	0	0
	2	0	0	0	0
	N	0	0	0	0
80	0	10	10	9	7
	1	0	0	1	3
	2	0	0	0	0
	N	0	0	0	0

*

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$.

Table 6 - 6 Detailed clinical observations - Summary data in female rats

Open field: Activity

Dose (mg/kg/day)		Week				(counts)
		1	2	3	4	
0	Mean	411	443	470	464	
	S.D.	112	121	49	31	
	Num.	10	10	10	10	
1	Mean	423	442	467	475	
	S.D.	78	97	84	45	
	Num.	10	10	10	10	
8	Mean	382	390	419	464	
	S.D.	92	137	65	63	
	Num.	10	10	10	10	
80	Mean	292 *	261 **	297 **	318 *	
	S.D.	115	123	141	131	
	Num.	10	10	10	10	

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$.

Table 6 - 7

Detailed clinical observations - Summary data in female rats

		Open field: Rearing				(counts)
Dose (mg/kg/day)	Score	Week				
		1	2	3	4	
0	0	0	0	0	0	
	1-5	2	1	0	0	
	6-10	6	0	1	1	
	11-15	2	3	0	1	
	16-20	0	5	3	3	
	21-25	0	1	6	3	
	26-30	0	0	0	2	
1	0	0	0	0	0	
	1-5	0	1	0	0	
	6-10	7	1	1	1	
	11-15	3	5	4	3	
	16-20	0	2	2	2	
	21-25	0	0	3	4	
	26-30	0	1	0	0	
8	0	0	0	0	0	
	1-5	2	2	1	0	
	6-10	3	2	2	0	
	11-15	4	3	0	4	
	16-20	1	1	4	4	
	21-25	0	2	3	2	
	26-30	0	0	0	0	
80	0	0	1	0	0	
	1-5	3	4	6	4	
	6-10	3	3	1	3	
	11-15	3	1	1	2	
	16-20	1	0	1	0	
	21-25	0	1	1	1	
	26-30	0	0	0	0	
				**	**	

Significantly different from control: *, $p \leq 0.05$; **, $p \leq 0.01$.

Key to Tables 7 and 8 - Assessment methods and scoring criteria of sensorimotor responses

Parameters assessed, methods and scoring criteria of sensorimotor responses in the functional tests are described as follows:

In principle, the normal condition is defined as score 0.

Sensorimotor responses

Parameters	Method	Scoring criteria
Visual placing	The reaction of the animal when it was held on the tail and moved slowly toward the flat plate was scored.	0: Normal response 1: Slight decrease 2: Marked decrease N: No assessment possible
Approach response	The reaction of the animal when a stick was approached slowly to the head was scored.	-2: Marked decrease -1: Slight decrease 0: Normal response 1: Slight increase 2: Marked increase
Touch response	The reaction of the animal when touched with forceps on the back was scored.	-2: Marked decrease -1: Slight decrease 0: Normal response 1: Slight increase 2: Marked increase
Pain response	The reaction of the animal when the tail was pinched with forceps was scored.	-2: Marked decrease -1: Slight decrease 0: Normal response 1: Slight increase 2: Marked increase
Auditory response	The reaction of the animal when exposed to a sound of a clicker from behind was scored.	-2: Marked decrease -1: Slight decrease 0: Normal response 1: Slight increase 2: Marked increase
Aerial righting reflex	The animal was held supine and dropped from approximately 20 cm above the bench. The reaction of the animal at landing was scored.	0: Normal reflex 1: Slight decrease 2: Marked decrease 3: No reflex

Remarks:

Week -1, Before initiation of treatment.

S.D., Standard deviation.

Table 7 - 1 Functional tests - Summary data in male rats

Sensorimotor responses at 4 weeks

Dose (mg/kg/day)	Score	Visual placing	Approach response	Touch response	Pain response	Auditory response	Aerial righting reflex
0	-2		0	0	0	0	
	-1		0	0	0	0	
	0	10	10	10	10	10	10
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3						0
1	-2		0	0	0	0	
	-1		0	0	0	0	
	0	10	10	10	10	10	10
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3						0
8	-2		0	0	0	0	
	-1		0	0	0	0	
	0	10	10	10	10	10	10
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3						0
80	-2		0	0	0	0	
	-1		0	0	0	0	
	0	10	10	10	10	10	10
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3						0

Table 7 - 2 Functional tests - Summary data in male rats

Data of quantitative measurements at 4 weeks

Dose (mg/kg/day)		Body temperature (°C)	Landing foot splay (mm)	Grip strength	
				Forelimb (g)	Hindlimb (g)
0	Mean	38.0	94	447	227
	S.D.	0.5	10	59	37
	Num.	10	10	10	10
1	Mean	38.0	101	413	249
	S.D.	0.4	12	66	44
	Num.	10	10	10	10
8	Mean	37.7	97	465	233
	S.D.	0.4	10	91	36
	Num.	10	10	10	10
80	Mean	38.2	95	405	224
	S.D.	0.5	11	84	22
	Num.	10	10	10	10

Table 7 - 3

Functional tests - Summary data in male rats

		Motor activity							
		(counts)							
Dose (mg/kg/day)	No. of animals examined	Week							
		min	4						Total
			0-10	10-20	20-30	30-40	40-50	50-60	
0	10	Mean	1864	1199	757	693	211	112	4836
		S.D.	194	430	505	495	327	177	1392
1	10	Mean	1866	1197	785	517	209	72	4646
		S.D.	190	400	317	376	291	115	1210
8	10	Mean	1772	1089	817	448	185	62	4373
		S.D.	211	278	377	389	269	77	934
80	10	Mean	1852	1416	785	731	278	142	5203
		S.D.	316	427	351	484	350	285	1571