English version of the final report

Final Report

An Acute Oral Toxicity Study of in Rats

Study No. H-10233

February 9, 2011

Nippon Experimental Medical Research Institute Co., Ltd.
1967-11 Arima, Shibukawa-shi, Gunma, Japan

STATEMENT OF COMPLIANCE

Title : An Acute Oral toxicity Study of in Rats

Study No. H-10233

I, the undersigned, hereby declare that this report is the English version of the original report that has been written in Japanese language. Further, I declare that the data are exactly reflected in this report and similar to that of the original (Japanese) report.

Takashi Sato Date: June 11, 2012

Takashi Sato, M.S.
Study Director (translator)
Nippon Experimental Medical Research Institute Co., Ltd.

PREPARATION OF THE FINAL REPORT

Title : An Acute Oral Toxicity Study of in Rats
Study No.: H-10233

The study listed above was performed in compliance with Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (as reversed in 1997), ENV/MC/CHEM(98)17.

This study was conducted according to the methods described herein, and the data are accurately reflected in this report.

Study director: Takashi Sato , M.S. (Impression of the seal)
Date: February 9, 2011
Nippon Experimental Medical Research Institute Co., Ltd.

OUTLINE OF THE STUDY CONDUCT

Title: An Acute Oral Toxicity Study of in Rats
Study No.: H-10233

1. Purpose

This study was performed as part of safety studies of , and a single dose of the test substance was administered to rats to evaluate the toxic signs, and to determine the approximate LD_{50} value.

2. GLP compliance

Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (as reversed in 1997), ENV/MC/CHEM(98)17.

3. Guidelines referred

OECD Guideline for the Testing of Chemicals, Updating Guideline #420 (Acute Oral Toxicity – Fixed Dose Procedure, Adopted on December 17, 2001) (hereinafter referred to as "OECD Guideline").

4. Animal welfare

This study was approved by the Animal Experimental Committee of Nippon Experimental Medical Research Institute Co., Ltd. (Approval No. 2006011).

5. Sponsor

Name:
Address:

6. Contract laboratory

Name: Nippon Experimental Medical Research Institute Co., Ltd.
Address: 1967-11 Arima, Shibukawa-shi, Gunma, Japan

7. Testing facility

Name: Shibukawa Laboratories
Nippon Experimental Medical Research Institute Co., Ltd.
Address: 1967-11 Arima, Shibukawa-shi, Gunma, Japan

8. Storage of records, data and specimens

(1) Storage period

The items are stored for 10 years after completion of the study.

(2) Storage items and storing location

The study protocol (original), documents relating to the study, raw data obtained in the course of the study, final report (original) and other materials are stored in Shibukawa Laboratories, Nippon Experimental Medical Research Institute Co., Ltd.

(3) Address of the storing institute

1967-11 Arima, Shibukawa-shi, Gunma, Japan

9. Schedule of the study

Study initiation	: December 22, 2010
Animal arrival	: December 24, 2010
End of the quarantine period	: December 28, 2010
Identity determination	: December 28, 2010
Start of the experiment	: December 28, 2010
Administration (first sighting study)	: January 5, 2011
Necropsy (first sighting study)	: January 5, 2011
Administration (main study)	: January 11, 2011
Necropsy (main study)	: January 25, 2011
Completion of the experiment	: January 25, 2011
Submission of the draft final report	: February 3, 2011
Preparation of the final report	: February 9, 2011
Completion of the study	: February 9, 2011

10. Study personnel and work responsibility

Study director, protocol preparation, work supervising/management and preparation of the final report : Takashi Sato Note)

Clinical observation and body weight measurement during the quarantine/acclimation period : Masaaki Shirai, Yoko Wakana, Naoko Matsumura, Takashi Sato

Animal health assessment : Yoko Wakana

Control/preparation of the test substance : Takashi Sato

Test substance administration : Masaaki Shirai, Yoko Wakana

Clinical observation and body weight measurement : Masaaki Shirai, Yoko Wakana, Moeko Masuyama, Takashi Sato

Necropsy : Masaaki Shirai, Yoko Wakana, Takashi Sato

Note): Affiliation: Safety Department, Nippon Experimental Medical Research Institute Co., Ltd.
Address: 1967-11 Arima, Shibukawa-shi, Gunma, Japan

UNPREDICTED EVENTS CONSIDERED TO HAVE AFFECTED THE RELIABILITY OF THE STUDY AND DEVIATION FROM THE PROTOCOL

Title: An Acute Oral Toxicity Study of in Rats
Study No.: H-10233

1. Unpredicted events

The temperature in Animal Room 2 deviated from the lower limit (19℃) between 10:05:44 a.m. and 10:15:46 a.m. (for 10min and 2sec) on January 7, 2011, recording the lowest value of 18.2℃, and the humidity deviated from the lower limit (30%) between 9:53:42 a.m. and 10:16:17 a.m. (for 22min and 35sec), recording the lowest value of 19.9%. The deviations occurred due to vapor-supply disruption caused by an error of the maintainer shutting the vapor valve of the boiler.

2. Deviation from the protocol

The protocol defined the producer of animals as Atsugi Breeding Center, Charles River Japan Inc.; however, the animals actually used in this study had been produced at Hino Breeding Center, Charles River Japan Inc.

3. Effects on the reliability of the study

The deviation of the temperature and humidity were considered to have no effect on the reliability of the study, because no abnormal finding was observed in general conditions of animals after the temperature and humidity returned to the normal range.

The difference of the producer was considered to have no effect on the reliability of the study, because of equality of the species and strain.

CONTENTS

Page

- PREPARATION OF THE FINAL REPORT 1/5
- OUTLINE OF THE STUDY CONDUCT 2/5
- UNPREDICTED EVENTS CONSIDERED TO HAVE AFFECTED THE RELIABILITY OF THE STUDY AND DEVIATION FROM THE PROTOCOL ... 5/5

I. Summary 1
II. Purpose of study 1
III. Materials and methods 1
IV. Results 6
V. Discussion and conclusion 6

- Figure
  1. Body weight changes of female rats treated orally with

- Table
  1. Mortality of female rats treated orally with
  2. Clinical signs of female rats treated orally with
  3. Body weights of female rats treated orally with
  4. Necropsy of female rats treated orally with

- Attached sheet
  1. Procedure of the sighting study
  2. Procedure of the main study

I. Summary

In accordance with the OECD Guideline, a single dose of was orally administered to female 8-9 weeks old SD [Crl:CD(SD)] rats to investigate the acute oral toxicity and approximate LD_{50}.

Initially, a sighting study was performed at the dose level of 300mg/kg using 1 animal. As a result, toxic signs were observed from 15min after administration, and death was observed at 30min after administration. Then, a sighting study was performed at the dose level of 50mg/kg using 1 animal. As a result, no toxic sign was observed.

Accordingly, the main study was performed at the dose level of 50mg/kg using 4 animals. As a result, no toxic sign was observed, and satisfactory body weight growth were noted throughout the observation period.

In necropsy, light red speck, dark red macula and erosion were observed in the thymus, lung and glandular stomach, respectively, in the dead animal receiving 300mg/kg of the test substance. No abnormal finding was observed in the animal receiving 50mg/kg of the test substance.

Based on the above-mentioned results, the approximate LD_{50} value of was estimated to be more than 50mg/kg and equal to or less than 300mg/kg under the conditions of this study.

II. Purpose of Study

This study was performed as part of safety studies of , and a single dose of the test substance was administered to rats to evaluate the toxic signs, and to determine the approximate LD_{50} value.

III. Materials and Methods

1. Test substance [Note 1)]

(1) Name (abbr. name) :
(2) Chemical name :
(3) Rational formula :
(4) Molecular formula :
(5) Molecular weight :
(6) CAS No. :
(7) Lot No. :
(8) Purity :
(9) Property at ambient temperature
:
(10) Name of impurities and the concentration
: Unknown, 0.5%

(11) Solubility : Soluble in water, dimethyl sulfoxide (DMSO) and acetone
(12) Storing conditions : Light shielding at room temperature
(13) Expiration date : May 27, 2011
(14) Identity (GLP) : Before the start of experiment, the infrared absorption spectrum of the test substance was measured with an infrared spectrophotometer (Fourier transform infrared spectrophotometer, FT-720, HORIBA, Ltd) (December 28, 2010). Comparing with the infrared absorption spectrum of the lot provided from the sponsor, confirmed the identity of the test substance based on the similarity of main peak shapes.
(15) Source
Name :

Address :
(16) Handling of the residual test substance
:Residual test substance is returned to the department given below after the end of the relevant experiments.
Name

Address

2. Solvent
Water for injection (Lot No.00610D, JP, Fuso Pharmaceutical Industries, Ltd.)

3. Rationale for selection of the solvent
Water for injection was preliminarily examined as solvent. As a result, the test substance was readily resolved to prepare 400mg/mL solution without bubble release, heat generation or coloration. Therefore we selected water for injection.

4. Preparation of the test substance solution
In the primary sighting study, 0.300g of the test substance weighed with an electric balance (E1200S, Sartorius Co., Ltd.) was placed in a 5mL measuring cylinder, to which water for injection was added bit by bit and measured up to a total volume of 5mL, and mixed by turning upside down to prepare the test substance solution at a concentration of 60mg/mL. In the second sighting study, 0.050g of the test substance was weighed, and the test substance solution at a concentration of 10mg/mL was prepared in a similar way. In the main study,

Note 1: Characteristics and the stability were based on information (Non-GLP) from the sponsor

0.100g of the test substance was weighed, and measured up to 10mL to prepare the test substance solution at a concentration of 10mg/mL.

The test substance solution was prepared at the time of use. Adjustment to the purity of the test substance was not taken into account. Test substance solution at each concentration was visually confirmed to have no bubble release, heat generation or coloration. Concentration analysis of the test substance in the solvent was not performed.

5. Experimental animals

Eight female Sprague-Dawley [Crj:CD(SD)IGS] rats at 7.0 weeks of age (body weight range: 165 to 179g) were purchased from Charles River Japan Inc. (Hino Breeding Center, 735 Shimokomazuki, Hino-cho, Gamou-gun, Shiga, Japan). The animals were quarantined for 5 days after arrival, observed for general conditions once daily, and their body weights were measured with an electric balance (LC2200S, Sartorius Co., Ltd.) on the first day and last day of the quarantine period. The animals were evaluated for health conditions on the last day of the quarantine period, confirming the absence of abnormality. Animals were acclimatized to housing conditions until the day before administration including the quarantine period. Each animals was identified with a color label attached to the cage (study No, name of the test substance, temporary animal no., date of arrival etc. were entered).

Animals were weighed on the day before administration. One animal with the highest body weight was selected for the sighting study, and 4 healthy animals with body weight within the body weight of the animal used in the sighting study on the day before administration ± 20%. Animals were 8-9 weeks old on the day of administration, and the body weight range was 233-253g. Animals excluded from the study were euthanized at the time of necropsy in the main study. After grouping animals were identified with brevity codes of animal numbers marked with an oil marker on the root of the tail, and cages were identified with attached color labels showing the study No., route of administration, animal species, dose level, animal no. etc.

6. Housing and environmental conditions

(1) Environmental conditions

Animals were individually housed in stainless-steel bracket cages for rats (260W × 380D × 180H mm) in a conventional system animal room No.2, where the environmental conditions were maintained as follows. Temperature: 22±3°C (actual values: 18.2 to 23.2°C; humidity: 50±20% (actual values: 19.9 to 60.6%); ventilation frequency: 10 times/hr or more (all-fresh-air system); and lighting: 12 hr/day (7:00 a.m. to 7:00 p.m., 150-300 lux).

Cages, feeders, trays and watering bottles were sterilized with 500-fold aqueous dilution of sodium hypochlorite prior to use, and trays and watering

bottles were changed for new ones 2 times per week. Cages and feeders were not changed. The animal room was cleaned after work every day, and the floor was sterilized by wiping with 200-fold aqueous dilution of benzethonium chloride (Hyamine solution; Daiichi Sankyo Co., Ltd.).

(2) Feed

Pellet diet for experimental animals CE-2 (Lot Nos. E2090 and E2100, Clea Japan Inc., 1-2-7 Higashiyama, Meguro-ku, Tokyo, Japan) was provided *ad libitum* via feeders.

Feed analysis for impurities and contaminants in the lot used in the study was entrusted by the maker to Tokyo Kenbikyo-in foundation (44-1 Nihonbashi Hakozaki-cho, Chuo-ku, Tokyo, Japan). Based on the results (obtained as a copy), the conformity to the standards specified by Nippon Experimental Medical Research Institute Co., Ltd. was confirmed before feeding.

(3) Drinking water

Shibukawa-city tap water was provided *ad libitum* via polycarbonate watering bottles (500mL).

Water analysis in the samples periodically collected at sites specified by this facility was entrusted to Environmental Technical Co., Ltd. (1709-1 Kaneko-cho, Takasaki-shi, Gunma, Japan) based on the "Ministerial Ordinance Concerning Water Quality Standards (MHLW Ordinance No. 101, 2003)", "Partial Amendment of Ministerial Ordinance Concerning Water Quality Standards (MHLW Ordinance No. 174, 2008)", and "Partial Amendment of the Ordinance for Enforcement of the Water Law (MHLW Ordinance No.175, 2008)". Based on the results [common bacteria (once per month), chloric acid, chloride ion, organic matters, pH, taste, odor, chromaticity, turbidity (4 times per year) and the test of 50 items (once per 3 years)], the conformity to the above-mentioned water quality standards was confirmed.

7. Group design and dose level

(1) Dose level, number of animals and animal numbers

Group No.	Test substance	Dose level (mg/kg)	No. of animals used	Animal No.
00		300	1	00F01*
01		50	5	01F01* - 01F05
		Total	6	

*: Sighting study

(2) Justification for dose selection

Dose levels were set according to the procedure described in Attached sheets 1 and 2.

Since there was no information concerning acute toxicity of the test substance, we selected 300mg/kg as the dose level in the sighting study, based on

recommendation of the OECD Guideline.

In the primary sighting study, the test substance was administered at a dose level of 300mg/kg to one animal. As a result, toxic signs were observed at 15min after administration, and death was noted at 30min after administration. Therefore, in the second sighting study, the test substance was administered at a dose level of 50mg/kg to one animal. As a result, no toxic sign was observed. Based on these results, we supposed that the test substance at the dose level having caused death in the sighting study should cause at least 2 deaths in the main study; therefore, in the main study, excepting the said dose level, the test substance was administered at a dose level of 50mg/kg to 4 animals.

8. Administration route, frequency and method

As possible human exposure, oral route was selected.

The dose volume (5mL/kg) was calculated based on the body weight just before administration. Single dose of the prepared test solution suctioned in a 2.5mL disposable syringe attached with a gastric sonde for rats was orally administered to rats. Animals were fasted from approximately 18hr before administration, and feeding was restarted approximately 3hr after administration.

9. Observation and measurement

The day of administration was designated as Day 1, and the following observations and measurements were performed on all animals.

(1) Mortality and general conditions

The observation period was 14 days (Day 1 to Day 15). Animals were observed for changes in general conditions including mortality, just before treatment and at 15 and 30min, 1, 3 and 6hr after treatment on Day 1, and once daily thereafter.

(2) Body weights

Body weights of animals were measured with an electric balance (LC2200S, Sartorius Co., Ltd.) on Days 1 (just before treatment), 2, 3, 4, 8 and 15.

(3) Necropsy

The animal found dead was immediately necropsied. After observation on Day 15, surviving animals were exsanguinated by cutting the abdominal aorta under ether anesthesia, and the body surface and organs/tissues in the cranial, thoracic and abdominal cavities were macroscopically examined.

10. Statistical analysis

The mean values and standard deviations were calculated for the body weight measurements obtained in the study; however, statistical analyses were not performed.

IV. Results

1. Mortality (Table 1)

300mg/kg group: Death was observed at 30min after administration.

50mg/kg group : No death was observed in any animals throughout the observation period in the sighting study or main study.

2. Clinical signs (Table 2)

300mg/kg group : Ataxic gate, hypersensitivity, twitch and tonic convulsion were observed at 15min after administration. Hypersensitivity, twitch, tonic convulsion, side position and irregular respiration were observed at 30min after administration and resulted in a death.

50mg/kg group :No abnormal finding was observed throughout the observation period.

3. Body weights (Figure 1 and Table 3)

300mg/kg group: The body weight was 215g at the time of administration and at the time of death, respectively, indicating no changes.

50mg/kg group: Comparing with the values on previous day, no decreased body weight was observed; body weights were increased in all animals throughout the observation period.

4. Necropsy (Table 4)

300mg/kg group: Light red speck, dark red macula and erosion were observed in the thymus, lung and glandular stomach, respectively.

50mg/kg group: No abnormal finding was observed in any animals.

V. Discussion and Conclusion

In accordance with the OECD Guideline, a single dose of was orally administered to female 8-9 weeks old SD [Crl:CD(SD)] rats to investigate the acute oral toxicity and approximate LD_{50} value.

Initially, a sighting study was performed at the dose level of 300mg/kg using 1 animal. As a result, toxic signs were observed from 15min after administration, and death was observed at 30min after administration. Then, a sighting study was performed at the dose level of 50mg/kg using 1 animal. As a result, no toxic sign was observed.

Based on these results, we supposed that the test substance at the dose level having caused death in the sighting study should cause at least 2 deaths in the main study; therefore, in the main study, excepting the said dose level, the test substance was administered at a dose level of 50mg/kg to 4 animals. Designating

the day of administration as Day 1, animals were observed for clinical signs from Day 1 to Day 15 (14 days), and necropsied on the last day of the observation period.

In general conditions, ataxic gate, hypersensitivity, twitch and tonic convulsion were observed at 15min after administration in the 300mg/kg group. Adding to these findings, at 30min after administration, side position and irregular respiration were observed and resulted in a death. No remarkable toxic sign was observed in the 50mg/kg group throughout the observation period.

In body weight measurements, no difference was observed between body weights at the time of administration and at the time of death in the 300mg/kg group, because the animal early died. In the 50mg/kg group, comparing with the values on previous day, no decreased body weight was observed; body weights were increased in all animals throughout the observation period.

In necropsy, light red speck, dark red macula and erosion were observed in the thymus, lung and glandular stomach, respectively, in the dead animal in the 300mg/kg group. No abnormal finding was observed in animals in the 50mg/kg group.

Effects of the test substance on the respiratory system and digestive system were suggested by toxic signs observed in general conditions and abnormal findings noted in necropsy.

Based on the above-mentioned results, the approximate LD_{50} value of was estimated to be more than 50mg/kg and equal to or less than 300mg/kg under the conditions of this study.

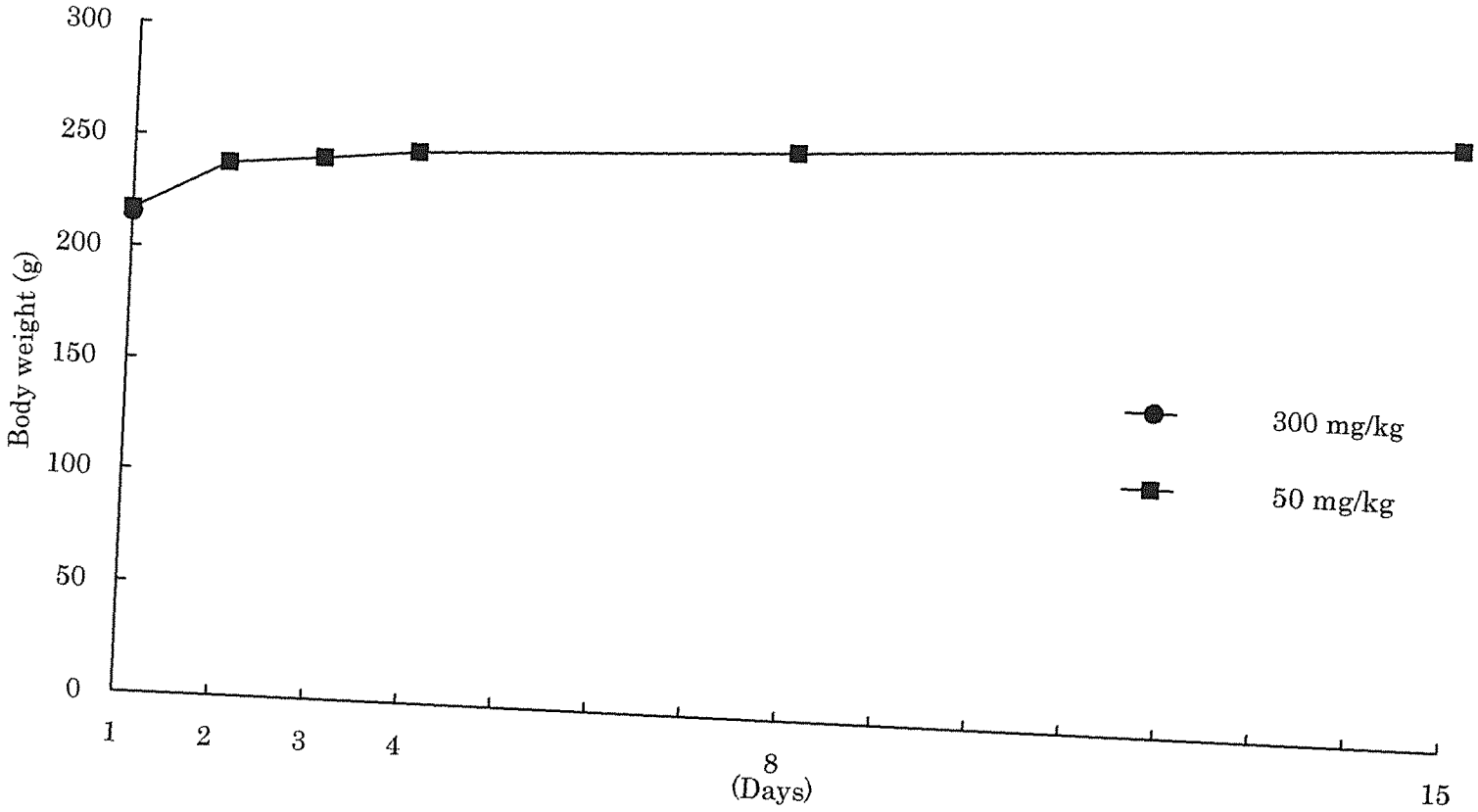


Figure 1 Body weight changes of female rats treated orally with

Study No.H-10233

Table 1 Mortality of female rats treated orally with

Group No. Test substance Dose(mg/kg)			< Days >																			Mortality
			1					2	3	4	5	6	7	8	9	10	11	12	13	14	15	
			min.		hr.																	
			15	30	1	3	6															
00																						
	No. of treated	1																				
300	No. of death		0	1																		1/1
01																						
	No. of treated	5																				
50	No. of death		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5

Study No.H-10233

Table 3 Body weights of female rats treated orally with

Group No. Test substance Dose(mg/kg)	Animal No.	< Days > 1	2	3	4	8	15
00							
	00F01[1)]	215					
300		(215) (at death)					
01	01F01[1)]	214	240	240	242	245	269
	01F02	215	233	238	243	246	263
50	01F03	207	234	242	242	255	268
	01F04	228	250	250	262	268	282
	01F05	219	236	242	245	257	266
	N	5	5	5	5	5	5
	Mean	217	239	242	247	254	270
	S.D.	8	7	5	9	9	7

1) : Sighting study Unit : g N : Number of animals

Study No.H-10233

Table 4 Necropsy of female rats treated orally with

Group No. Test substance Dose (mg/kg)	Animal No.	Organs / Findings
00 300	00F01 [1)]	Glandular stomach / Erosion, Thymus / Light red speck, Lung / Dark red macula
01 50	01F01 [1)]	No abnormality
	01F02	No abnormality
	01F03	No abnormality
	01F04	No abnormality
	01F05	No abnormality

1) : Sighting study

Study No. H-10233

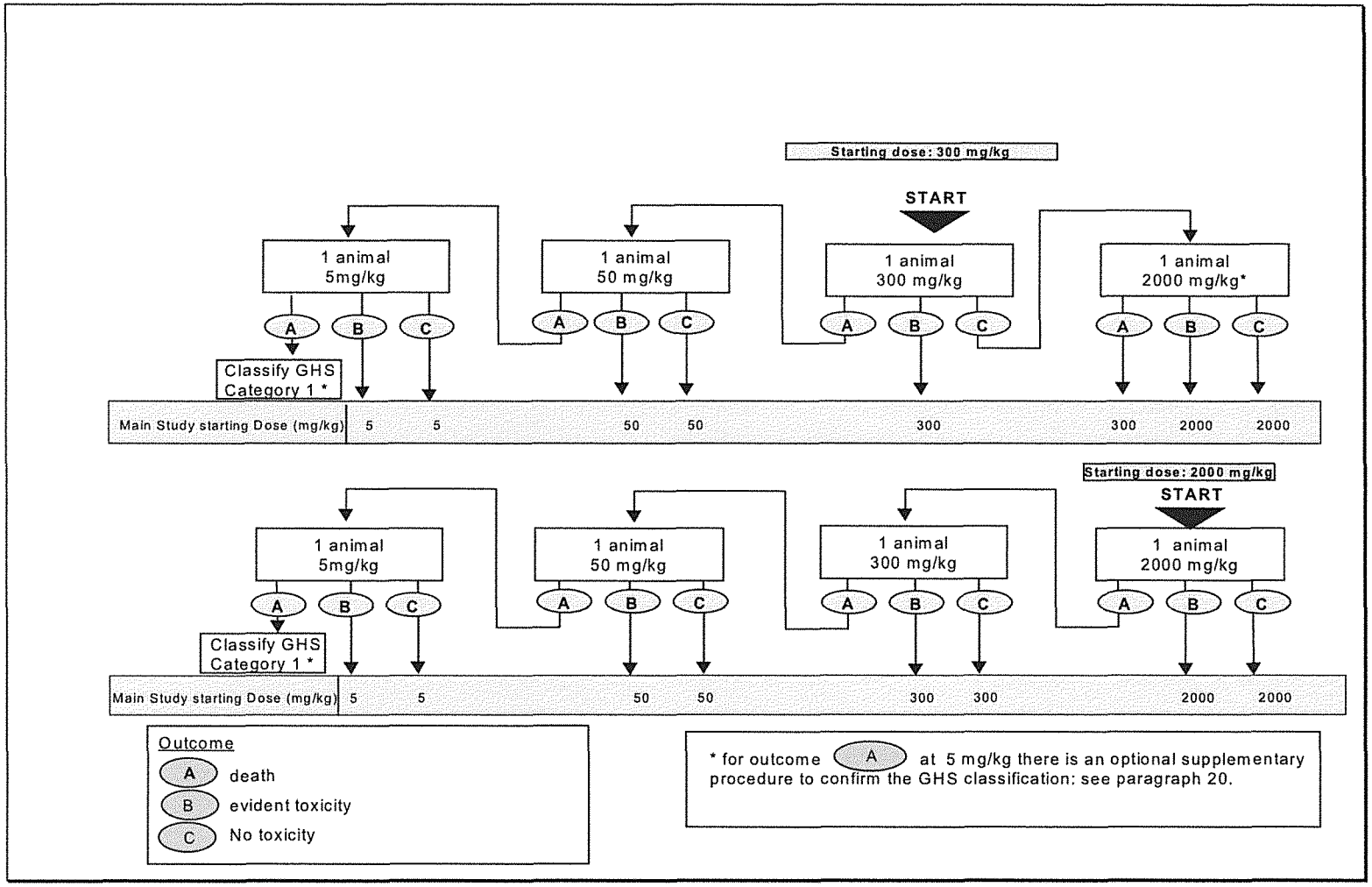
Table 2 Clinical signs of female rats treated orally with

Group No.								< Days >													
Test substance		JB	1					2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dose(mg/kg)	Animal No.		min.		hr.																
			15	30	1	3	6														
00																					
	00F01[1)]	–	a,b,c,d	b,c,d,e,f,D																	
300																					
	01F01[1)]	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
01	01F02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	01F03	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
50	01F04	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	01F05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

1) : Sighting study JB : Just before administration
a:Ataxic gait、b:Hypersensitivity、c:Twitch、d:Tonic convulsion、
e:Side position、f:Irregular respiration、D:Death

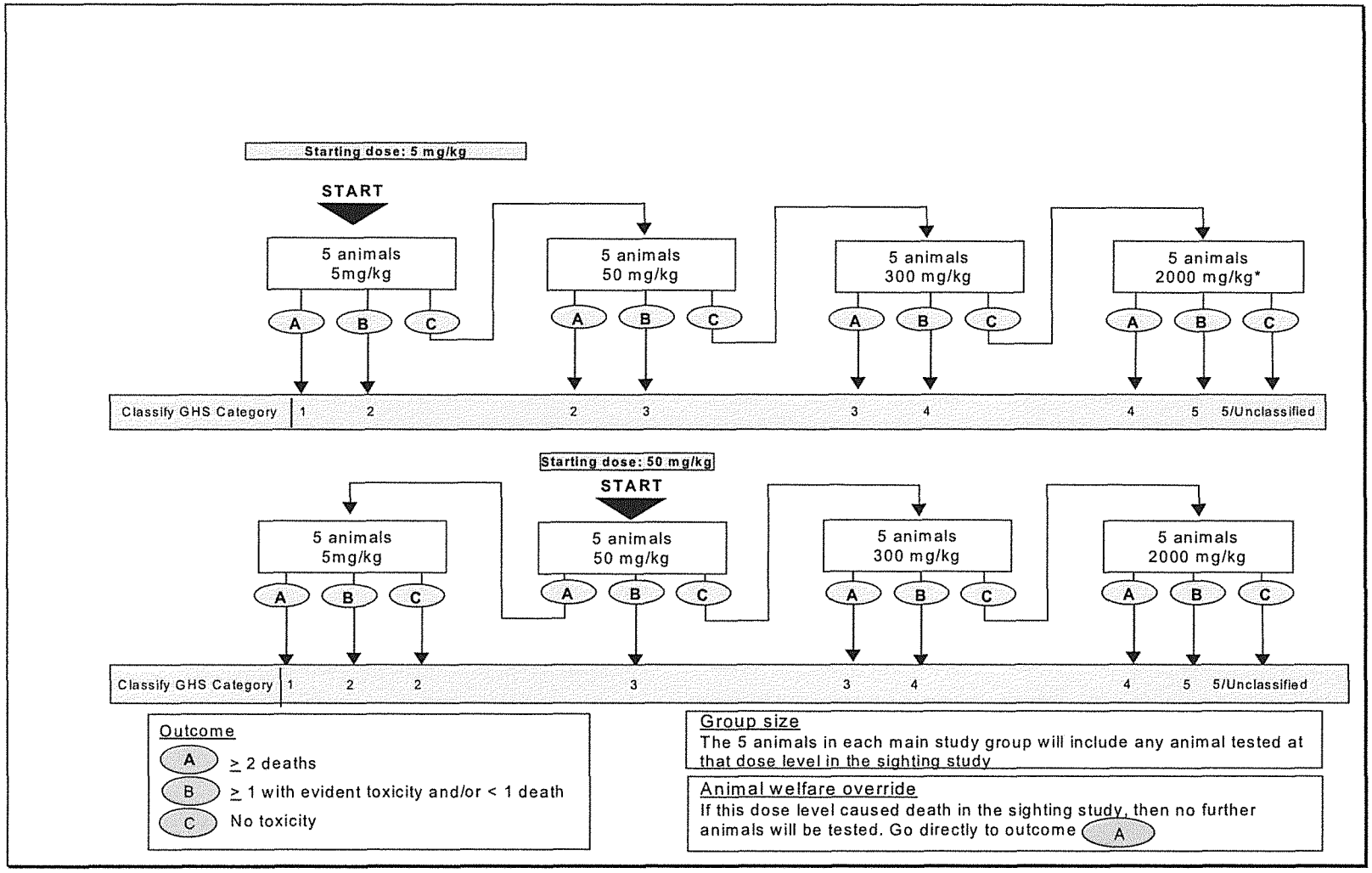
Study No.H-10233

Atached sheet 1 Procedure of the sighting study

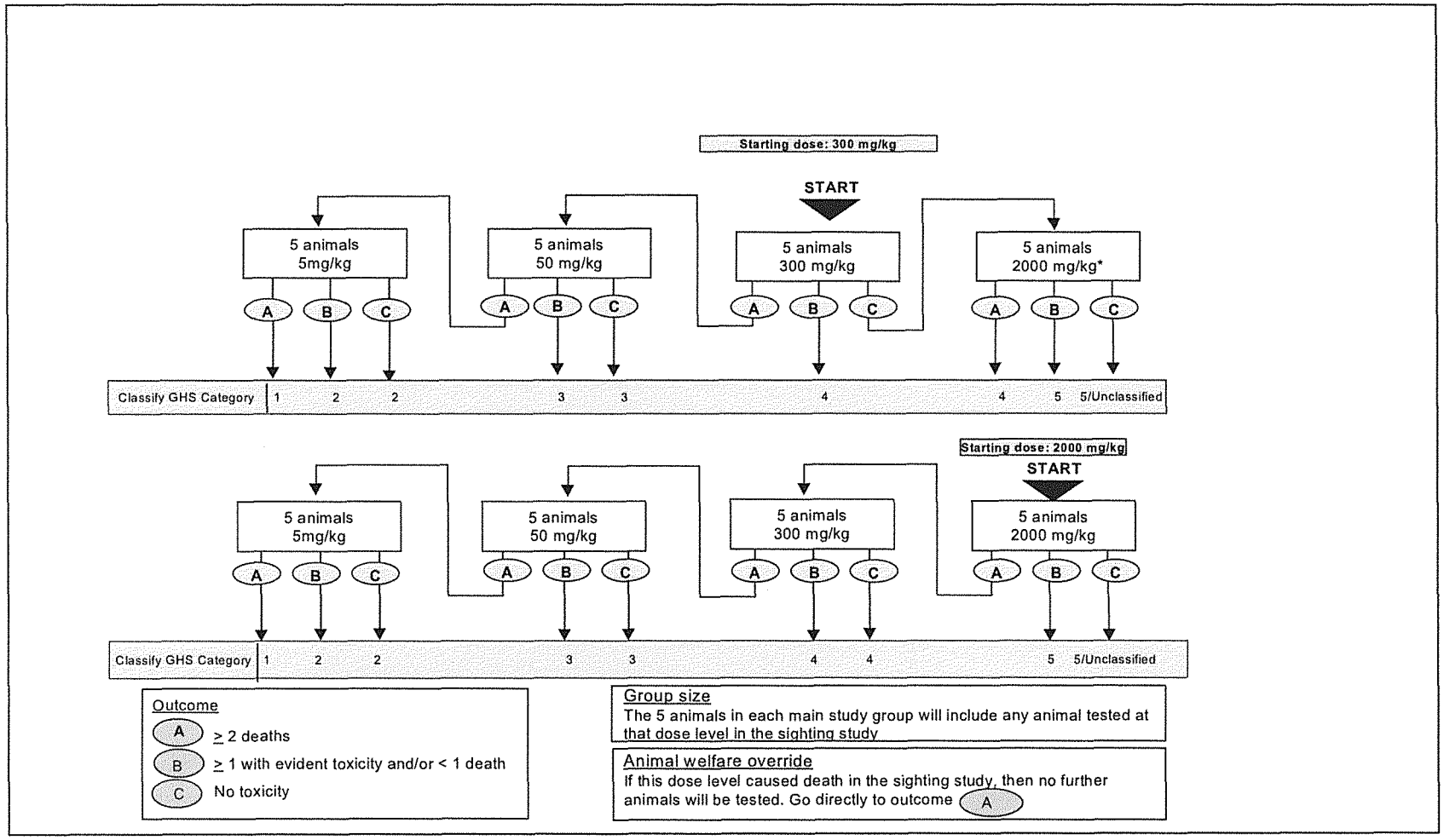


H-10233

Atached sheet 2-1 Procedure of the main study



Atached sheet 2-2 Procedure of the main study



QUALITY ASSURANCE STATEMENT

Title : An Acute Oral toxicity Study of in Rats
Study No. : H-10233

Inspection items	Performed	Reported to	
		Study director	Management
Study protocol	Dec. 24, 2010	Dec. 24, 2010	Dec. 24, 2010
Identity determination of the test substance (infrared absorption spectrum	Dec. 28, 2010	Dec. 28, 2010	Dec. 28, 2010
Animal receipt and body weight measurement at delivery *	Oct. 8, 2010	Oct. 8, 2010	Oct. 8, 2010
Quarantine/acclimatization, animal health assessment and grouping	Jan.4, 2011	Jan.4, 2011	Jan.4, 2011
Test substance preparation, administration, body weight measurement and clinical observation (at 15 and 30min after administration) (First sighting study)	Jan. 5, 2011	Jan. 5, 2011	Jan. 5, 2011
Necropsy (Main study)	Jan. 25, 2011	Jan. 25, 2011	Jan. 25, 2011
Raw data	Feb. 3-4, 2011	Feb. 4, 2011	Feb. 4, 2011
Final report			
(draft)	Feb. 3-4, 2011	Feb. 4, 2011	Feb. 4, 2011
(re-inspection)	Feb. 8, 2011	Feb. 8, 2011	Feb. 8, 2011
(final)	Feb. 9, 2011	Feb. 9, 2011	Feb. 9, 2011

Based on the above inspections, I have certified that this study was conducted in accordance with the GLP, protocol and standard operating procedures prescribed by Nippon Experimental Medical Research Institute Co., Ltd., and that this report precisely describes the methods and procedures employed in this study, and accurately reflects the raw data obtained by the conduct of the study. The item marked with * was inspected as a process inspection.

Quality Assurance Unit Manager : Miki Wakabayashi <Impression of seal>
Date : February 9, 2011
Nippon Experimental Medical Research Institute Co., Ltd