English version of the final report

FINAL REPORT

Acute Oral Toxicity Study of NAT-7051 in Rats

Study No. H-13157

February 24, 2014

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

STATEMENT OF COMPLIANCE

Study Title : Acute Oral Toxicity Study of NAT-7051 in Rats

Study No. H-13157

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: April 8, 2014

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

QUALITY ASSURANCE STATEMENT

Study Title: Acute Oral Toxicity Study of NAT-7051 in Rats

Study No.: H-13157

Inspection items	Performed on	Reported to	
		Study director	Management
Study protocol	Jan. 9, 2014	Jan 9, 2014	Jan 9, 2014
Amendment to the study protocol (1)	Feb. 13, 2014	Feb. 13, 2014	Feb. 13, 2014
Animal receipt and body weight measurement at receipt	Jan. 10, 2014	Jan. 10, 2014	Jan. 10, 2014
Quarantine/acclimation, animal health assessment, and grouping	Jan. 14, 2014	Jan. 14, 2014	Jan. 14, 2014
Test substance preparation, administration, body weight measurement, and clinical observation (at 15 min after administration) (starting dose for the sighting study)	Jan. 15, 2014	Jan. 15, 2014	Jan. 15, 2014
Necropsy (main study)	Feb. 5, 2014	Feb. 5, 2014	Feb. 5, 2014
Raw data	Feb. 14, 2014	Feb. 14, 2014	Feb. 14, 2014
Final report			
(draft)	Feb. 14, 2014	Feb. 14, 2014	Feb. 14, 2014
(review)	Feb. 21, 2014	Feb. 21, 2014	Feb. 21, 2014
(final)	Feb. 24, 2014	Feb. 24, 2014	Feb. 24, 2014

Based on the above inspections, I certify that this study has been 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.

Miki Wakabayashi <Impression of seal>
Quality Assurance Unit Manager
Date: February 24, 2014
Nippon Experimental Medical Research Institute Co., Ltd

PREPARATION OF THE FINAL REPORT

Study Title: Acute Oral Toxicity Study of NAT-7051 in Rats
Study No.: H-13157

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

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

Date: February 24, 2014
Takashi Sato , M.S. (Impression of the seal)
Study director
Nippon Experimental Medical Research Institute Co., Ltd.

OUTLINE OF THE STUDY CONDUCT

Study Title: Acute Oral Toxicity Study of NAT-7051 in Rats
Study No.: H-13157

1. Purpose

This study was performed as a part of safety assessment of NAT-7051, and the test substance was administered once by gavage to rats to evaluate the toxic signs and determine the approximate LD_{50} value.

2. GLP compliance

This study was conducted in compliance with the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

3. Guidelines referred to

This study was conducted by reference to the OECD Guideline for 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: Toda Kogyo Corporation.
Address: 1-1-1 Shinoki, Sanyoonoda-shi, Yamaguchi, Japan

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. Archive of records, data, and specimens

(1) Archive period

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

(2) Archive 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 location

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

9. Schedule of the study

Study initiation:	January 8, 2014
Animal arrival:	January 10, 2014
End of the quarantine period:	January 14, 2014
End of acclimation (first sighting study):	January 14, 2014
Start of the experiment:	January 15, 2014
Administration (first sighting study):	January 15, 2014
Administration (main study):	January 22, 2014
Necropsy (sighting study):	January 29, 2014
Necropsy (main study):	February 5, 2014
Completion of the experiment:	February 5, 2014
Submission of the draft final report:	February 14, 2014
Preparation of the final report:	February 24, 2014
Completion of the study:	February 24, 2014

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, Moeko Masuyama, Shinya Motozawa, Takashi Sato

Animal health assessment: Moeko Masuyama

Control/preparation of the test substance: Takashi Sato

Test substance administration: Masaaki Shirai, Shinya Motozawa

Clinical observation and body weight measurement: Masaaki Shirai, Moeko Masuyama, Shinya Motozawa, Takashi Sato

Necropsy: Masaaki Shirai, Shinya Motozawa, Takashi Sato

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

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

Study Title: Acute Oral Toxicity Study of NAT-7051 in Rats
Study No.: H-13157

There were no unpredicted events considered to have affected the reliability of the study and no deviations from the protocol.

CONTENTS

I. Summary

The acute oral toxicity of NAT-7051 was studied by single oral gavage administration to 8-week-old female SD [Crl:CD(SD)] rats according to the OECD Guideline.

Doses of 300 and 2000 mg/kg were tested in the sighting study using one animal. Since no death occurred at either dose, 2000 mg/kg was selected for the main study and administered to four additional animals to have a total of five animals assigned to the main study including the one used in the sighting study. The animals were observed for 14 days from the day of administration (Day 1) through Day 15 and necropsied at the end of the observation period.

As a result, no death occurred during the observation period. For general conditions, no significant signs of toxicity were observed except for compound-colored feces found in all animals between 6 h after administration and Day 5. There were no test substance-related changes in body weight or necropsy findings in any animals at either 300 or 2000 mg/kg.

Based on the above results, the approximate LD_{50} of NAT-7051 was estimated to be more than 2000 mg/kg under the conditions of the study, and the substance was classified as "not classified" under the GHS criteria.

II. Purpose of Study

This study was performed as a part of safety assessment of NAT-7051, and the test substance was administered once by gavage to rats to evaluate the toxic signs and determine the approximate LD_{50} value.

III. Materials and Methods

1. Test substance [1)]

(1) Name (abbr.):	NAT-7051
(2) Chemical name:	Lithium nickel cobalt aluminum boron oxide
(3) Molecular formula:	$LiNi_{0.8}Co_{0.15}Al_{0.05}O_{1.985}O_2(BO_3)_{0.01}$
(4) Molecular weight:	96.43
(5) Lot No.:	6730205
(6) CAS No.:	207803-51-8
(7) Purity:	>98%
(8) Property at ambient temperature:	Solid, black powder
(9) Melting point:	Not less than 1000°C
(10) pH:	11.5 (2%)

1) Characteristics and stability were based on the information (non-GLP) from the sponsor.

(11) Names of impurities and their concentration: Contains unknown impurities at a concentration of less than 2%
(12) Solubility: Suspendable in water
(13) Storing condition: At room temperature
(14) Date of manufacture: February 13, 2013
(15) Expiration date: Three years after the date of manufacture (February 12, 2016)
(16) Identity: The test substance was identified by checking the information provided and label descriptions on the delivered substance and visually by inspecting its appearance (black powder).
(17) Source:
Name: Toda Kogyo Corporation.
Address: 1-1-1 Shinoki, Sanyoonoda-shi, Yamaguchi, Japan
(18) Handling of the residual test substance: Returned to the following after the completion of related studies.
Name: Chemicals Compliance Division, Sumika Chemical Analysis Service, Ltd.
Address: Sumitomo Fudosan Hongo Building, 3-22-5 Hongo, Bunkyo-ku, Tokyo, Japan

2. Solvent

A 0.5% CMC-Na solution (prepared by dissolving carmellose sodium [Lot No. 2X261, Japanese Pharmacopoeia, Maruishi Pharmaceutical Co., Ltd.] in distilled water for injection [Lot No. 21001D, Japanese Pharmacopoeia, Fuso Pharmaceutical Industries, Ltd.] at a concentration of 0.5%)

3. Rationale for the selection of the solvent

A 0.5% CMC-Na solution was selected because in a preliminary study the test substance was found to be suspendable in 0.5% CMC-Na solution by mixing in an agate mortar.

4. Preparation of the test substance solution

The test substance solution of the starting dose for the sighting study was prepared by the following procedure: weigh 0.301 g of the test substance on an electronic balance (E1200S, Sartorius Co., Ltd.), place in an agate mortar, grind and mix with gradual addition of the solvent, transfer to a 10-mL measuring cylinder, add the solvent to make 10 mL, and mix by inversion to achieve a final concentration of 30 mg/mL. The test substance solution of the second dose for the sighting study was prepared by the same procedure except weighing 2.001 g of the test substance to achieve a final concentration of 200 mg/mL. The test substance

solution for the main study was prepared by weighing 6.000 g of the test substance, adding the solvent, transferring to a 50-mL measuring cylinder, and adding the solvent to make 30 mL to achieve a final concentration of 200 mg/mL.

All the test substance solutions were prepared at each time of use and visually inspected for the absence of heat, bubble, or color generation at the time of preparation and at the end of administration. Concentration analysis for the test substance solutions was not performed.

5. Experimental animals

Eight 7.5-week-old female Sprague-Dawley rats [Crl:CD(SD)] (body weight range: 176-190 g) were purchased from Charles River Japan Inc. (Atsugi Breeding Center, 795 Shimofurusawa, Atsugi-shi, Kanagawa, Japan). The animals were quarantined for five days after arrival and observed for general conditions once daily and weighed on an electronic balance (LC2200S, Sartorius Co., Ltd.) on the first and last days of the quarantine period. A health assessment performed at the end of the quarantine period revealed no abnormalities in any animals. They were acclimated through the quarantine period up to the day before administration. During the quarantine/acclimation period, the animals were individually identified with color labels (showing Study number, name of the test substance, temporary animal numbers, date of acquisition, etc.) attached to cages.

Animals were weighed on the day before each administration, and the heaviest was chosen for the sighting study. For the main study, four animals were selected from those weighing within ±20% of the body weight of the animal used in the sighting study measured on the day before administration. They were 8-9 weeks old on the day of administration and weighed 224-230 g at the time of grouping. The animals excluded from the study were euthanized at the time of necropsy in the main study. After grouping, animals were individually identified with abbreviated animal numbers written with an oil marker on the root of the tail, and with color labels attached to cages to show the study number route of administration, animal species, dose levels, animal numbers, etc.

6. Animal husbandry

(1) Environmental conditions

Animals were individually housed in stainless-steel bracket cages for rats (260W × 380D × 180H mm) in a conventional animal room (No.2) set to a temperature of 22±3°C (actual value: 21.1-24.9°C), humidity of 50±20% (actual value: 37.6-56.4%), ventilation frequency of 10 times/h or more (all-fresh-air system), and lighting of 12 h/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. Trays and watering bottles were changed twice a week, but 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

Animals were allowed to free access to pellet diet for laboratory animals CE-2 (Lot No. E2093, Clea Japan Inc., 1-2-7 Higashiyama, Meguro-ku, Tokyo, Japan) served in feeders.

A feed sample of the same lot was analyzed by Tokyo Kenbikyo-in foundation (5-1 Toyomi-cho, Chuo-ku, Tokyo, Japan) at the request of the provider. We obtained the analysis results and confirmed that the levels were within the acceptable ranges specified by our facility.

(3) Drinking water

Animals were allowed free access to Shibukawa-city tap water via polycarbonate watering bottles (500 mL). Water samples periodically collected from the locations specified by our facility were analyzed by Environmental Technical Co., Ltd. (1709-1 Kaneko-cho, Takasaki-shi, Gunma, Japan) according to the "Ministerial Ordinance Concerning Water Quality Standards (MHLW Ordinance No. 101, 2003)(final amendment: MHLW Ordinance No. 11, January 28, 2011)," "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)." The results of the analyses [common bacteria and *E.coli* (once per month), chloric acid, chloride ion, organic matters, pH, taste, odor, chromaticity and turbidity (four times per year) and the test of 50 items (once every three years)] showed that the levels were within the acceptable ranges described in the said water quality standards.

7. Group design and dose levels

(1) Group composition, dose levels, number of animals and animal numbers

Group No.	Test substance	Dose level (mg/kg)	No. of animals used	Animal No.
00	NAT-7051	300	1	00F01*
01		2000	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 no information was available on the acute toxicity of the test substance, 300 mg/kg was selected as the starting dose for the sighting study as recommended by the OECD Guideline. Since no evident signs of toxicity were observed until two days after administration, 2000 mg/kg was chosen as the second dose for the sighting study. Since no death occurred at this dose, 2000

mg/kg was selected to be tested in the main study.

8. Administration route, frequency and method

Oral route of administration was selected as an expected route of exposure in humans.

The dose volume (10 mL/kg) was calculated from the body weight measured just before administration. The prepared test substance solution was drawn in a 2.5-mL disposable syringe attached with a rat gastric tube and orally given in a single dose. Animals were fasted from about 18 h before administration until being fed again at about 3 h after administration.

9. Observation and measurement

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

(1) General conditions and mortality

Animals were observed for 14 days after administration up to Day 15. They were observed for mortality and changes in general conditions just before administration and at about 15 min, 30 min, 1 h, 3 h, and 6 h after administration on Day 1 and once daily for the rest of the observation period.

(2) Body weight

Animals were weighed on an electronic balance (LC2200C, Sartorius Co., Ltd.) on Days 1 (just before administration), 2, 3, 4, 8, and 15.

(3) Necropsy

After the observation on Day 15, animals were exsanguinated by cutting the abdominal aorta under ether anesthesia. The body surface and organs in the cranial, thoracic, and abdominal cavities were examined macroscopically.

10. Statistical analysis

Means and standard deviations were calculated for body weight measurements, but statistical analysis was not performed.

IV. Results

1. Mortality (Table 1)

300 mg/kg group: No death was observed throughout the observation period.

2000 mg/kg group: No death was observed in any animals throughout the observation period.

2. Clinical signs (Table 2)

300 mg/kg group: No abnormal signs were observed during the observation period.

2000 mg/kg group: Compound-colored feces were observed in two animals at 6 h after administration, in four on Day 2, in two on Day 3, and in

one on Days 4 and 5. No other significant signs were observed in any animals.

3. Body weight (Figure 1 and Table 3)

300 mg/kg group: The body weight increased throughout the observation period.

2000 mg/kg group: A decrease in body weight from the previous measurement was observed in one animal on Days 3 and 4 (about 2-5% decrease) and in another on Day 4 (about 2% decrease). However, the mean body weight of all animals increased throughout the observation period.

4. Necropsy (Table 4)

300 mg/kg group: No abnormal findings were observed in any organs.

2000 mg/kg group: No abnormal findings were observed in any organs of animals.

V. Discussion and Conclusion

In accordance with the OECD Guideline, NAT-7051 was administered once by gavage to female 8-week-old SD [Crl:CD(SD)] rats to investigate the acute oral toxicity.

Initially, 300 mg/kg was tested in the sighting study using one animal. Since no death or evident signs of toxicity were observed at this dose, 2000 mg/kg was further administered but did not cause death or any signs of toxicity, either. Based on these results, 2000 mg/kg was selected for the main study and administered to four additional animals to have a total of five animals assigned to the main study including the one used in the sighting study. The animals were observed for 14 days from the day of administration (Day 1) through Day 15 and necropsied at the end of the observation period.

At 300 mg/kg, no death occurred during the observation period, and the body weight favorably increased throughout the observation period. No abnormal findings were observed in general conditions or necropsy findings.

At 2000 mg/kg, no death occurred during the observation period. For general conditions, compound-colored feces were observed in all animals between 6 h after administration and Day 5. On and after Day 6, however, the animals resumed normal feces and did not show any other abnormal signs. The mean body weight of all animals increased throughout the observation period, but when viewed individually, a decreased body weight from the previous measurement was observed in one animal on Days 3 and 4 and in another on Day 4. Necropsy revealed no macroscopic abnormalities in any animals.

The said compound-colored feces were considered to have resulted from a portion of the test substance being excreted without being absorbed or decomposed in the body. The decreased body weight was considered incidental because it was

small in magnitude, because the body weight resumed gaining on Day 8 and thereafter, and because there were no abnormal findings in necropsy.

Based on the above results, the approximate LD_{50} of NAT-7051 was estimated to be more than 2000 mg/kg under the conditions of the study, and the substance was classified as "not classified" under the GHS criteria.

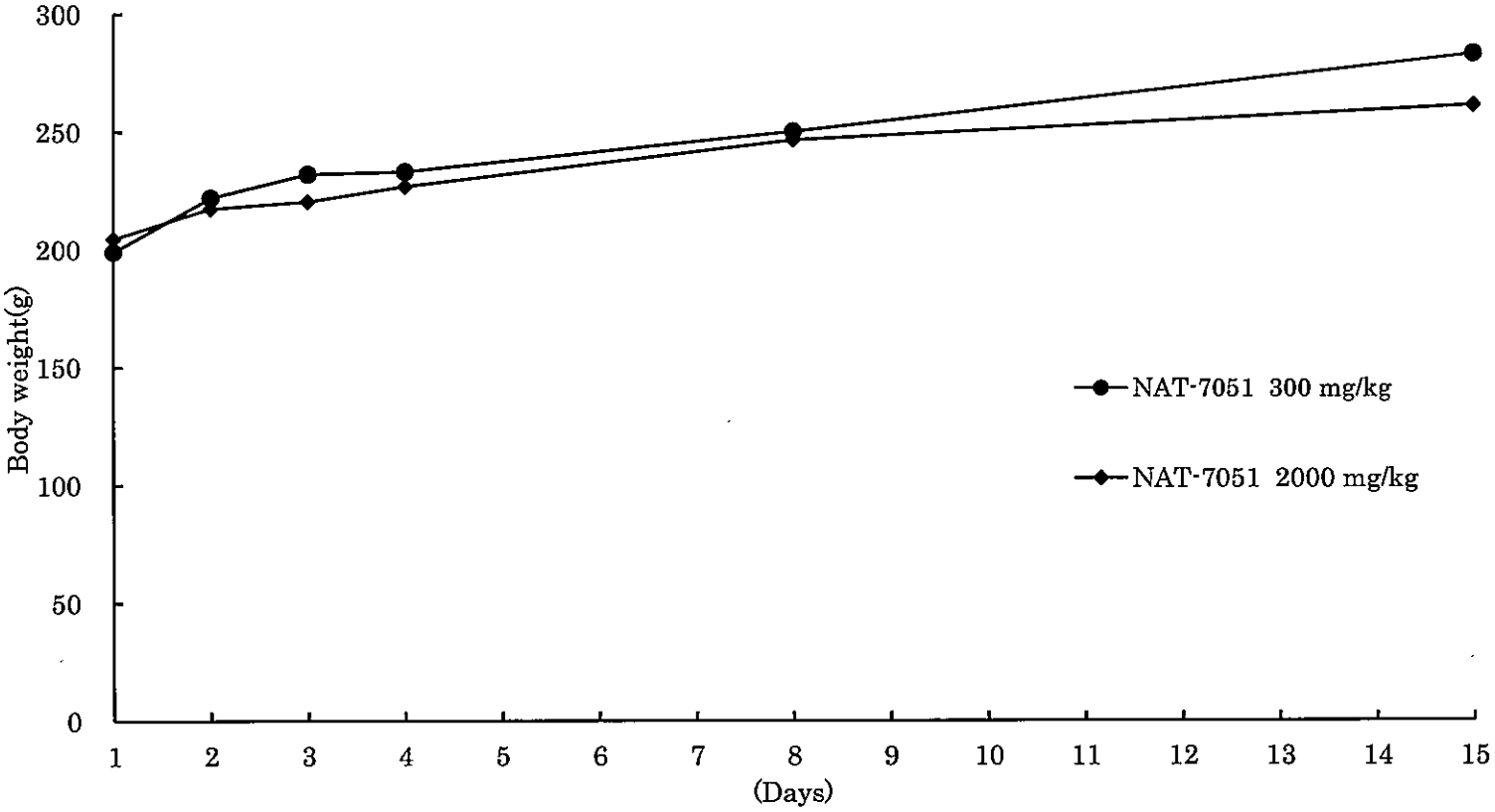


Figure 1 Body weight changes

Study No. H-13157

Table 1 Mortality

Group No. / Test substance / Dose(mg/kg)			< Days > 1 (min. 15, 30; hr. 1, 3, 6)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mortality (%) (death/treated)
00																		
NAT-7051	No. of treated	1																
	No. of death		0 0 0 0 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
300																		(0/1)
01																		
NAT-7051	No. of treated	5																
	No. of death		0 0 0 0 0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2000																		(0/5)

Study No. H-13157

Table 2 Clinical signs

Group No. / Test substance / Dose(mg/kg)	Animal No.	0※1	1 (min. 15)	1 (min. 30)	1 (hr. 1)	1 (hr. 3)	1 (hr. 6)	2	3	4	5	6	7	8	9	10	11	12	13	14	15
00 NAT-7051 300	00F01※2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
01 NAT-7051 2000	01F01※2	–	–	–	–	–	T30	T30	–	–	–	–	–	–	–	–	–	–	–	–	–
	01F02	–	–	–	–	–	–	T30	–	–	–	–	–	–	–	–	–	–	–	–	–
	01F03	–	–	–	–	–	–	–	T30	T30	T30	–	–	–	–	–	–	–	–	–	–
	01F04	–	–	–	–	–	–	T30	T30	–	–	–	–	–	–	–	–	–	–	–	–
	01F05	–	–	–	–	–	T30	T30	–	–	–	–	–	–	–	–	–	–	–	–	–

※1 : Just before administration 、※2 : Sighting study
– : no abnormality
T30 : Compound-colored feces

Table 3 Body weight changes

Group No. Test substance Dose(mg/kg)	Animal No.	< Days > 1	2	3	4	8	15
00 NAT-7051 300	00F01※1	199	222	232	233	250	283
	01F01※1	200	207	208	236	250	273
01	01F02	200	222	229	238	262	270
NAT-7051	01F03	207	215	211	201	234	248
2000	01F04	206	217	223	232	236	258
	01F05	210	226	231	227	250	257
	N	5	5	5	5	5	5
	Mean	205	217	220	227	246	261
	S.D.	4	7	10	15	12	10

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

Study No. H-13157

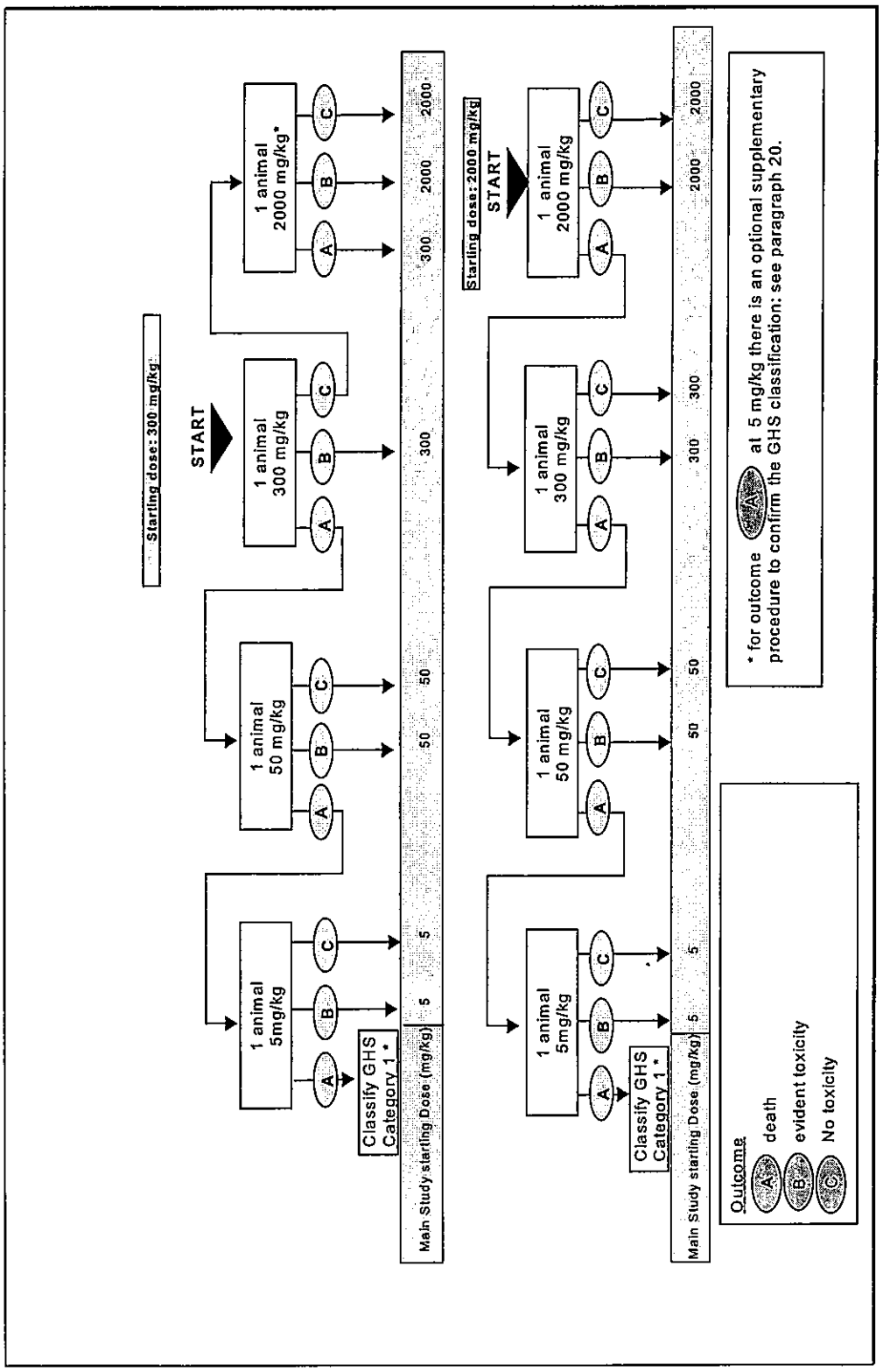
Table 4 Necropsy findings

Group No. Test substance Dose (mg/kg)	Animal No.	Organ / Findings
00 NAT-7051 300	00F01※1	No abnormality
01 NAT-7051 2000	01F01※1	No abnormality
	01F02	No abnormality
	01F03	No abnormality
	01F04	No abnormality
	01F05	No abnormality

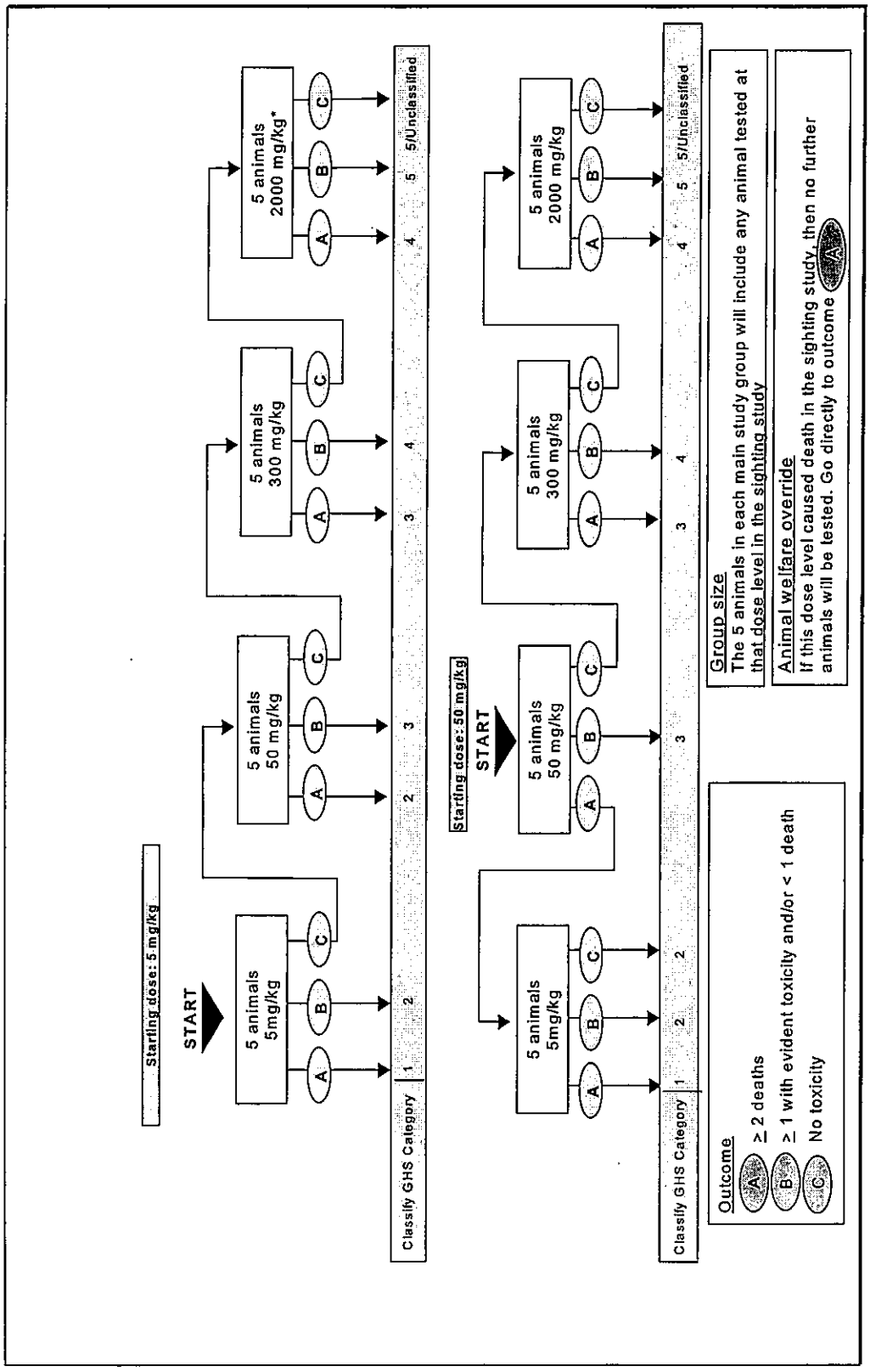
※1 : Sighting study

Study No. H-13157

Attached sheet 1 Procedure of the sighting study



Attached sheet 2-1 Procedure of the main study



Attached sheet 2-2 Procedure of the main study